15/4-8-96 (1)

CONF-9509173
(DE96001220)

1995 CONFERENCE PROCEEDINGS

THE FIFTH INTERNATIONAL CONFERENCE ON MICROBIAL ENHANCED OIL RECOVERY AND RELATED BIOTECHNOLOGY FOR SOLVING ENVIRONMENTAL PROBLEMS

REBECCA BRYANT, CO-CHAIRMAN AND EDITOR
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THE UNIVERSITY OF TULSA

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ACKNOWLEDGMENTS

The conference chairs wish to gratefully acknowledge the U.S. Department of Energy (DOE) for sponsoring the 5th International Conference on Microbial Enhanced Oil Recovery and Related Biotechnology for Solving Environmental Problems. We specifically wish to thank George Stosur of the U.S. DOE, Fossil Energy Division, for his support and opening address at the conference. We wish to also acknowledge the co-sponsors of the conference, Phillips Petroleum Company, BDM-Oklahoma, the University of Tulsa Center for Environmental Research and Technology, Texaco, Conoco, Inc., Kerr-McGee Corp., and the University of Oklahoma Sarkeys Energy Center. We also acknowledge the assistance of Winnie Ho, manager of Technology Transfer for BDM-Oklahoma, and Dana Ridley and Helen Bresson for carrying out the many duties associated with administering the conference. The Information Service Department of BDM-Oklahoma is also acknowledged for the preparation of the proceedings.

Rebecca S. Bryant

Kerry L. Sublette

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Microbial Enhanced Oil Recovery: Entering the Log Phase

Rebecca S. Bryant
BDM-Oklahoma

Abstract

Microbial enhanced oil recovery (MEOR) technology has advanced internationally since 1980 from a laboratory-based evaluation of microbial processes to field applications. In order to adequately support the decline in oil production in certain areas, research on cost-effective technologies such as microbial enhanced oil recovery processes must focus on both near-term and long-term applications. Many marginal wells are desperately in need of an inexpensive improved oil recovery technology today that can assist producers in order to prevent their abandonment. Microbial enhanced waterflooding technology has also been shown to be an economically feasible technology in the United States. Complementary environmental research and development will also be required to address any potential environmental impacts of microbial processes. In 1995 at this conference, the goal is to further document and promote microbial processes for improved oil recovery and related technology for solving environmental problems.

Introduction

Oil production using enhanced oil recovery technology is increasing even with the lower cost of oil in the world. According to the September 26, 1994, *Oil and Gas Journal,* in the United States, oil produced from EOR projects now exceeds 10% of the domestically produced oil. Overall worldwide production from enhanced oil recovery projects at the start of 1994 represents 3.2% of the world's oil production.[1] The application of improved oil recovery (IOR) technology is being applied worldwide, and can only be expected to increase, due to the diminishing development of new fields and the decline of more mature ones.

The National Institute for Petroleum and Energy Research (NIPER) is operated by BDM-Oklahoma for the United States Department of Energy. An active laboratory and field research program in the area of microbial improved oil recovery (MIOR) has been ongoing at the facility for 12 years.

Microbial improved oil recovery (MIOR) has been recognized as a potentially cost-effective method, particularly for stripper well production. Stripper wells (wells that produce less than 10 bbl/D) are in need of cost-effective IOR because operators produce about 50% of the total oil recovered in the United States[2] Microbial methods for improving oil recovery are particularly well suited to be applied in today's economic climate. Adequate data exist to demonstrate both the viability and variety of options for using microbial technology for improved oil production. A significant number of producers and oil fields have been treated with microbial formulations in the United States and internationally.

Importance of Oil

The oil industry, particularly in the United States, is undergoing tremendous change. In the United States, the demand for oil is rising at a rate of three to four percent per year, while domestic production is decreasing. Environmental regulations continue to increase, adding additional liabilities for an already highly regulated industry. Domestic production by independents is expected to increase relative to the production of oil produced by the majors. Domestic refineries will continue to close due to environmental concerns. The U.S. known oil resource is 351 billion barrels, while the potential undiscovered oil is 25 to 60 billion barrels. The U.S. Department of Energy projects that advancements in technology can result in 40-billion-barrel growth in reserves. Advancements in technology that must contribute to this growth will have to include microbial processes. There is no other energy source that can replace crude oil in the next decade.

Potential Reservoirs for MIOR

The U.S. DOE Reservoir Data Base (public copy) was used to screen several oil-producing states for reservoirs with original oil in place greater than 20 million bbl that satisfy the following criteria: injected and connate water salinities less than 150,000 ppm, rock permeability greater than 50 md, depth less than 7,700 ft, and a bottomhole temperature limitation of about 80°C. Table 1 shows the number of reservoirs that satisfied these parameters. A graph of the percent of reservoirs in each state that satisfied these limiting criteria is shown in Figure 1. If microbial technology was applied in only 10% of these reservoirs, the domestic reserves could be greatly increased.

Microbial Processes for Oil Production Problems

Because of the diverse nature of MEOR technology, several different oil production problems have been addressed by microbial and/or nutrient injection. Some classification scheme is required to separate these different processes. To differentiate among field projects using microorganisms, the treatments are separated according to the classification in Table 2.

The processes listed in Table 2 will be used for classification only. In some instances no field projects using the process are being conducted, but field work has been planned based upon laboratory results.

Conference Papers

The papers presented at the 5th International Microbial Enhanced Oil Recovery and Related Biotechnology for Solving Environmental Problems Conference represented a very diversified suite of microbial technologies that can be used for improving oil production in large oil fields, mitigating microbially influenced corrosion, control of souring of crude oil, plus actual case studies of bioremediation. New and expanded areas of research in microbial technology included many permeability modification studies and microbial techniques for exploration.

Conclusions

In over a decade of microbial IOR research, it is important to recognize the progress that microbial IOR technology has made. In a microbial IOR meeting held in 1982, the audience was composed mainly of microbiologists who had almost no

knowledge of petroleum recovery processes. The proceedings of that conference contained results of laboratory research, and one field pilot conducted in the 1950s. In 1992 at the International MEOR Conference in Brookhaven, New York, researchers presented results from field projects conducted in nine countries and two commercial operations. In 1995 at this conference, the goal was to further document and promote microbial processes for improved oil recovery and related technology for solving environmental problems. Microbial IOR processes are now being designed by integrated disciplinary teams of microbiologists, geologists, and petroleum engineers. Certainly there are still unknown facets of the technology, but in less than 10 years, microbial technology has been shown to be a reality throughout the world.

The increasing number of microbial enhanced oil recovery field projects and the variety of different microbial processes that are applicable demonstrates the difficulty and complexity of placing reservoir limitations on the technology. Significant consideration must be given to what type of microbial process is desired, which means that some knowledge of the reservoir problem must first be obtained.

References

Moritis, G. 1994. EOR Dips in U.S. But Remains a Significant Factor. Oil & Gas Journal, v. 92, no. 39, p. 51–79.

Table 1 Number of Reservoirs by State with Potential for MIOR Technology

State	Total No. of Reservoirs	No. of Reservoirs That Fit the Criteria	%
Oklahoma	107	32	30
Texas	685	233	34
Louisiana	195	77	40
Kansas	44	22	50
California	197	122	62
Colorado	40	29	73
Mississippi	49	20	41
New Mexico	71	5	7
Wyoming	71	34	48
Illinois	46	34	74
			46 (Avg)

Table 2 A Classification of Different Microbial Reservoir Treatments

MEOR Process	Production Problem	Type of Microorganism Used
Well stimulation	Formation damage Low oil relative permeability	Generally surfactant, gas, acid, and alcohol producers
Waterflooding	Trapped oil due to capillary forces	Generally surfactant, gas, acid, and alcohol producers
Permeability modification	Poor sweep efficiency Channeling	Microorganisms that produce polymer and/or copious amounts of biomass
Wellbore cleanup	Paraffin problems Scaling	Microorganisms that produce emulsifiers, surfactants, and acids Microorganisms that degrade hydrocarbons
Polymer flooding	Unfavorable mobility ratio Low sweep efficiency	Microorganisms that produce polymer
Mitigation of coning	Water or gas coning	Microorganisms that produce polymer and/or copious amounts of biomass

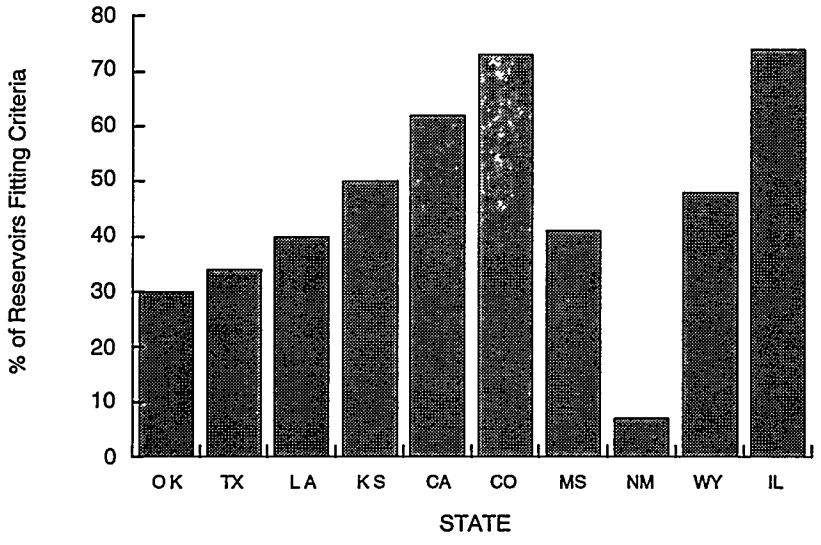

Figure 1 Graph Showing Percent of Reservoirs in Major Oil-Producing States That Have Potential for MEOR Processes

Development and Application of Microbial Selective Plugging Processes

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Abstract

Phillips Petroleum Company recently completed a microbial selective plugging (MSP) pilot at the North Burbank Unit (NBU), Shidler, Oklahoma. Nutrients were selected for the pilot that could stimulate indigenous microflora in the reservoir brine to grow and produce exopolymer. It was found that soluble corn starch polymers (e.g., maltodextrins) stimulated the indigenous bacteria to produce exopolymer, whereas simple sugars (e.g., glucose and sucrose), as well as complex media (e.g., molasses and Nutrient Broth), did not. Injection of maltodextrin into rock cores in the presence of indigenous NBU bacteria resulted in stable permeability reductions (> 90%) across the entire length, while injection of glucose resulted only in face plugging. In addition, it was found that organic phosphate esters (OPE) served as a preferable source of phosphorus for the indigenous bacteria, since orthophosphates and condensed phosphates precipitated in NBU brine at reservoir temperature (45°C). Injection of maltodextrin and ethyl acid phosphate into a producing well stimulated an increase in maltodextrin utilizing bacteria (MUB) in the back-flowed, produced fluid. Additional screens of indigenous and nonindigenous bacteria yielded several nonindigenous isolates that could synthesize polymer when growing in brine containing 6% NaCl at 45°C.

Introduction

Conventional technologies for applying selective plugging or profile modification to reservoirs have primarily involved the use of chemical-based blocking agents (e.g., gels, polymers, foams). Typically, the blocking agent is generated in situ through the injection of polyacrylamides or biopolymers, along with a chemical cross-linker (e.g., chromium, aluminum) for gels, or gas in the case of foams. Many potential problems can arise from the use of these chemicals, such as shearing of the polymers, microgel formation, injection of high viscosity solutions with poor selectivity, and injection of expensive and sometimes toxic chemicals. Microbial selective plugging (MSP) processes can resolve many of these problems through the use of inexpensive, environmentally friendly, low viscosity nutrients that stimulate indigenous bacteria to produce bioblocking agents (i.e., cells and polymer) in situ.

Recently, Phillips Petroleum Company completed an MSP project at the North Burbank Unit (NBU) in Osage County, Oklahoma, where nutrients were injected to stimulate indigenous microorganisms to generate cells and polymer in situ.[1] It was felt that the use of indigenous microorganisms would circumvent some of the problems that would otherwise be encountered with the use of nonindigenous microorganisms, for example: above-ground fermentations, injection of bacteria, bacterial transport, bacterial competition, and survival. Microbial enrichment tests using reservoir brine collected at the NBU revealed that a fermentable carbon and phosphorus source were the only two nutrients limiting for good biomass production by indigenous bacteria. Ample nitrogen for biomass production, in the form of ammonia (approximately 33 ppm), was already present in the brine. Typically, nutrients used in MEOR field tests have been products containing simple sugars such as glucose, sucrose, or molasses.[2–5] Molasses has been widely used because it is a waste product that is inexpensive and easy to obtain in large quantities. However, simple sugars such as glucose and sucrose, while supporting good growth of indigenous bacteria found in NBU brine, did not stimulate the production of significant amounts of extracellular polymer.

Significant polymer production was stimulated, however, by the addition of higher molecular weight, soluble, corn starch polymers (e.g., maltodextrin).[6] In addition, it was found that orthophosphates, such as ammonium phosphate and potassium phosphates, were not good phosphorus sources for use in the NBU brine because of their propensity to precipitate with the calcium and magnesium ions contained in the brine. Organic phosphate esters (OPE) were found to provide a superior phosphorus source for use in NBU brine due to increased solubility and stability, which improved transport properties in the reservoir.[7] The purpose of this paper is to describe the selection of the nutrients for the pilot and their effect on the stimulation of polymer and indigenous, polymer-producing microorganisms during

both laboratory and field tests. Information is also presented on the screening of nonindigenous, polymer-producing bacteria with potential application at the NBU.

Field and Site Description

Detailed descriptions of the NBU pilot area have been given in previous publications.[8,9] The NBU is located near Shidler, Oklahoma, in Osage County. The unit is about 12 miles long (i.e., north to south) by 5 miles wide. The wells produce out of the Burbank sandstone, which is approximately 3,000 ft deep with a bottomhole temperature of around 45°C. The NBU brine at the site of the pilot contains 10 to 12% total dissolved solids of the following composition (all mg/l): ammonium 33; chloride 66,000; sulfate 20; calcium 6,290; barium 755; magnesium 1,250; sodium 31,000; iron 17. The pilot site consisted of four producing wells (16-1, 16-2, 16-9, and 16-10) surrounding a single injector (16W21) (see Fig. 1).

Materials and Methods

Indigenous Screens

Enrichments. The brine used as the source of indigenous microorganisms was collected at the North Burbank Unit from either the bottom of the oil/water separator at the Tract 5 battery or from the injection header at injector 16W21. The procedures for collection of samples under low oxygen tensions have been previously described.[10]

Serum bottles, 120 cc, containing 0.2 g of carbohydrate were placed in an anaerobic glove box containing an atmosphere of 10% hydrogen, 5% carbon dioxide, and 85% nitrogen. These bottles were incubated in the glove box for at least 12 hr to ensure anaerobic conditions. To each bottle was added 100 ml of brine, along with either sodium trimetaphosphate or ethyl acid phosphate at a final concentration of 100 μM. The bottles were stoppered with butyl rubber stoppers, removed from the glove box, and sealed with aluminum crimp seals. The carbohydrate was dissolved by vigorous shaking of the bottles. Enrichments were incubated without shaking at 45°C for at least 10 days or until turbidity, indicative of bacterial growth, was observed.

Polymer Screen. Following the incubation period, the bottles were held by the neck between the thumb and forefinger and gently swirled in a unidirectional motion. If the bottle contained a large amount of insoluble material or viscous film that detached from the bottom of the bottle, but remained largely intact or cohesive

when swirled, it was rated a "++." However, if this material dispersed or broke up into separate strands or particles upon swirling, it was given only a "+." Those bottles that failed to show any significant cohesive film or insoluble material were given a "-."

Adsorption Screen. Serum bottles, 120 cc, were preincubated in an anaerobic glove box to remove traces of oxygen. Brine collected from the field was filtered through a 0.22 μm membrane filter and dispensed into the bottles. The bottles contained 1 g of crushed and sieved Burbank rock (i.e., reservoir rock) that had previously been cleaned to remove crude oil by repeated soxhlet extractions using alternating volumes of toluene and methanol. To each bottle was added either an organic phosphate ester (OPE) or sodium trimetaphosphate (STMP) (Monsanto Chemical Co. St Louis, Missouri) to a final phosphorus concentration of 100 mg/l. The bottles were stoppered with butyl rubber stoppers and crimped with aluminum seals, removed from the glove box, and placed on a platform shaker-incubator set approximately at 100 rpm and 40°C. After a 72-hr incubation, the samples were removed from the shaker, and a sample of the liquid taken through the stopper by means of a 20-gauge needle attached to a plastic syringe. The contents of the syringe were passed through a 0.45 μm syringe filter to remove any suspended rock grains and sent to the Phillips Analysis Branch for total phosphorus analysis by inductively coupled plasma (ICP) analysis.

An adsorption index (AI) was established to rank the various OPEs based upon their ability to be retained by the Burbank rock relative to the inorganic standard, STMP. The AI was defined as the mg of STMP-phosphorus adsorbed by 1.0 g of rock after 72 hr at 40°C, divided by the mg of OPE-phosphorus adsorbed per gram of rock after 72 hr at 40°C. Therefore, STMP had an AI of 1.0, and all compounds retained to a lesser degree by the rock had an AI greater than 1.0.

Precipitation Screen. Samples of brine and deionized water containing STMP or OPEs were prepared as described in the adsorption screens, except that no crushed rock was added to the bottles, and the samples were incubated for 23 days at 40°C instead of 3 days. The brine and water samples were filtered through a 0.45 μm syringe filter and dispensed into plastic bottles. The contents of the bottles were assayed by inductively coupled plasma for total phosphorus. The precipitation index (PI) was defined as the mg of dissolved phosphorus in the brine after 23 days, divided by the mg of dissolved phosphorus in the deionized water, after 3 days at room temperature. All phosphorus compounds listed in Table 3 were completely soluble in deionized water at the concentration tested; therefore, a PI of less than 1.0 indicated either lack of solubility in the brine or an inability to stay in solution.

Nonindigenous Isolates

Sources. Sources of material for isolation of bacteria capable of producing polymer under simulated reservoir conditions included both food and terrestrial samples, such as smoked salmon, pasteurized whole milk, raw cream, soy milk, olives, pickles, cabbage, ground beef, as well as marine samples, hot spring sediments, lagoon sediments, produced sand from oil wells, compost, and sediment from solar ponds.

Isolation and Identification. Primary isolations were performed by adding a small amount of source material collected from various locations to either liquid enrichments, as described above, or by plating directly onto molasses-containing agar (BPM) media of the following composition: 5% sucrose, 2% molasses, 50 mM KNO_3, 6% NaCl, 1.5% $CaCl_2 \cdot 2H_2O$, 0.4% $MgCl_2 \cdot 6H_2O$, and 1mM glycerol phosphate. Cysteine (0.01%) and sodium ascorbate (0.02%) were used as reductants. Incubations were performed at 45°C. Specific identification of microorganisms was performed by fatty acid analysis (Microchek Inc., Northfield, Vermont).

Polymer Assays. Polymer production by isolates was measured after aerobic growth of cultures on liquid molasses medium (BPM without agar, nitrate, and reductants). Cultures were incubated at 40°C for 36 hours with shaking. Soluble polymer was assayed by centrifuging the whole broth to remove the cells and precipitating the soluble polymer with an equal volume of cold ethanol. The resulting precipitate was pelleted by centrifugation, dissolved in water, and reprecipitated with cold ethanol. This material was then washed twice, dissolved, and assayed for total reducing sugars using the Anthrone assay with glucose as a standard.[19] Insoluble polymer was measured as viscous material that sedimented with the cells upon centrifugation.

Corefloods

Core Preparation. Cores used in the coreflood experiments were cleaned by soxhlet extraction, epoxied, fitted with pressure taps along their length, and flooded to residual oil saturation as described previously.[10]

Coreflood Apparatus. Details of the coreflood apparatus, as well as procedures for monitoring differential pressure and injection of nutrients, were previously described for cores used in coreflood 1 and coreflood 2.[9,10]

Resistance Factors (RF). Differential pressure readings were converted to resistance factors by the formula $RF = P/P_0$, where P is the differential pressure

recorded following nutrient injection, and P_0 is the differential pressure with brine prior to bacterial inoculation. Resistance factors (RF) are a measure of the increase in pressure along the length of the core during growth and polymer production. The abbreviations RF1, RF2, RF3, and RF4 refer to RFs measured over the first, second, third, and fourth segments of the core. Distances of the first, second, third, and fourth segments of the cores from the inlet end are approximately 0.5, 1.5, 2.5, and 3.0 in., respectively.

Residual Resistance Factors (RRF). The RRF is the residual resistance factor obtained when injecting filtered brine, following the buildup in pressure. RRF is related to the stability of the plug once growth and polymer production have ceased.

Nutrient Treatments. All chemical solutions injected into the core, with the exception of the unfiltered brine inoculum, were first filtered through a 0.22 μm filter.

Coreflood 1. The core was inoculated with 492 ml of unfiltered Tract 5 brine, followed by the injection of approximately 3 pore volumes (PV) of filtered Tract 5 brine and 50 PV of a 1.56 mM solution of sodium trimetaphosphate dissolved in filtered Tract 5 brine. The phosphate slug was followed by an additional 2 PV of filtered brine to flush out the nonadsorbed phosphate. A 0.2% solution of SD-1 dissolved in Tract 5 brine was injected at a velocity of approximately 1.6 ft/D for the next 23 days, for a total of 350 PV of SD-1 injected. During this time, pressure was observed to increase along the entire length of the core; however, large pressure fluctuations made it impossible to determine accurate resistance factors. Therefore, on the 16th day of SD-1 injection, a backpressure regulator was added to the effluent end of the core and the injection pressure set at 500 psig. The increase in injection pressure stabilized the pressure readings, allowing an accurate measurement of RF to be made along the core. Following SD-1 injection, the core was injected with filtered brine to determine the RRFs along the entire length of the core.

Coreflood 2. The core was inoculated by injection with 1,270 ml of unfiltered Tract 5 brine at a velocity of approximately 21 ft/D. Approximately 120 ml of filtered Tract 5 brine was injected, followed by 1.3 PV of a 1.56 mM solution of STMP dissolved in filtered Tract 5 brine, and an additional 13.7 ml of brine. A solution of 0.4% Clearsweet (CS) (Cargill, 99% glucose) dissolved in brine was then injected at a velocity of approximately 7.5 ft/D. A total of 333 PV of CS was injected through this core, during which time the maximum RFs along the length of the core were determined from the pressure profiles. The RRFs were then determined by flooding with brine for the indicated times.

Following the injection of CS, a solution containing a mixture of 0.2% SD-1 and 0.2% Clearsweet was injected into the core. Immediately prior to this, the core was injected with approximately 1,100 cc of filtered Tract 5 brine to raise the effluent pH from 4.4 to 6.1, approximately the pH prior to CS injection. The 50:50 mixture was injected for almost two days at the same velocity, at which time about 57 PV of the mixture had been injected.

The core was then switched over to injection with 0.4% SD-1, without any prior pH adjustment. As much as 188 PV of solution was injected at 8 ft/D. This was followed by brine injection to determine the RRFs along the core.

Field Test

Near Wellbore Test. A total of 420 barrels of a solution of SD-1 and ethyl acid phosphate (EAP) were injected down producer 12-9, which was then shut in for two weeks, after which time the well was restarted. Samples of produced fluid were back-flowed and collected at predetermined intervals until about 35% of the injected fluids had been produced.

Maltodextrin-Utilizing Bacteria (MUB). Enumeration of MUB was carried out by either bottle counts or most probable number (MPN). The MUB media consisted of the following: SD-1 (0.1%), yeast extract (0.01%), and 2.2 mg/l EAP, all added to filtered (0.22 μm) brine collected at injector 16W21. Media (9.0 cc) was dispensed into anaerobic, serum bottles (15 cc capacity) and stoppered with butyl rubber septa.

Bottle Counts. Ten-fold serial dilutions were performed on a single series of 8 to 10 serum vials using a 1.0 cc inoculum of either produced or injected brine. The highest dilution bottle showing visible growth after 14 days incubation at 40°C was read as the titer for that series of bottles. Therefore, a series of bottles with only the first bottle showing growth would be recorded as 10 bacteria/ml; two bottles would be 100 bacteria/ml; three bottles would be 1,000 bacteria/ml; and so on.

MPN. Ten-fold serial dilutions were performed using 1.0 ml of coproduced brine collected from well 12-9. Three tests were run with 10 tubes/test. MPN tables of de Man[11] were used to calculate results.

Acridine Orange Direct Counts (AODC). A modification of the Nucleopore Epicount technique was used, as previously described.[9]

Results

Nutrient Selection

Carbohydrates that could potentially serve as a carbon and energy source for the in-situ production of exopolymer by indigenous bacteria were screened using enrichment cultures made with NBU brine. It was found, after extensive testing of the carbohydrates, that only certain dextrins supported any exopolymer production. Simple sugars, such as glucose and sucrose, as well as corn syrups and molasses with high dextrose contents did not support significant exopolymer production (see Table 1). On the other hand, the dextrins, Star-Dri 1 (SD-1) and Koldex 60 (Staley Mfg., Decatur, Illinois), stimulated the best exopolymer production. SD-1 was found to be particularly well suited for use in NBU brine because of its good solubility and low retention by NBU rock.[12] Some properties of SD-1 are listed in Table 2.

The phosphorus source was selected based on its ability to support good growth and polymer production in the presence of SD-1 (results not shown), as well as by its ability to stay in solution (i.e., stability) in NBU brine and its low retention by Burbank sandstone. The results in Table 3 indicate that all OPE were adsorbed less and were more stable in NBU brine when compared to the inorganic phosphate compound, STMP. Based on modeling of the transport behavior of several OPE in Burbank rock, EAP was eventually chosen as the OPE for use in the NBU pilot. The EAP used in the pilot tests was manufactured by Albright and Wilson (Richmond, Virginia) and shipped as a liquid in 55-gallon drums of approximately 97% active chemical. Other properties of EAP are listed in Table 2.

Corefloods

Burbank sandstone cores flooded to residual oil saturation with Burbank crude oil were injected with either SD-1 or Clearsweet 95 (CS 95, Cargill Mfg., Memphis, Tennessee). The ability of these two carbon sources to reduce the permeability of a Burbank core in the presence of indigenous bacteria was demonstrated by measuring RF and RRF values along the length of the cores following nutrient injection (see Table 4). Although the CS 95 (Coreflood 2) was capable of reducing the overall permeability of the core by 99% (RF= 100), the plugging was confined to the front section of the core. A follow-up injection of a 50:50 mixture of CS 95 (0.2%) and SD-1(0.2%) into the core did not result in any additional plugging along the length of the core; however, following an injection of 0.4% SD-1, the RF in section 2 increased to 55, or a permeability reduction of greater than 98% (see Table 4). Likewise, initial treatment of Coreflood 1 with SD-1 resulted in an overall

permeability reduction of greater than 99% (RF > 100), and reductions along the entire length of the core of better than 90%. Stability of this plug was very good, as residual resistance factors along the entire length of the core were 10 or greater after injecting 65 PV of brine over a 10-day period.

Field Test

In the Fall of 1992, two near wellbore tests were run at two producers (well 12-9 and well 5-9) within the NBU located over a mile from the site of the pilot.[1] These tests followed the sequential nutrient injection tests, which were ineffective at reducing reservoir permeability.[1] The tests were designed to evaluate the effects of co-injected nutrients on in-situ biological activity to ensure that a lack of in-situ metabolism was not a cause of the poor results. After co-injection of nutrients and shut-in of the wells, analyses of the produced fluids that back-flowed from the producers indicated an increasing concentration of ethanol, as well as a decrease in the ammonia concentration of the brine, both indicative of in-situ microbial activity.[1] It was also shown that the injected nutrients (SD-1 and EAP) resulted in a significant increase in the total number of indigenous bacteria (AODC), and more importantly, an increase in maltodextrin-utilizing bacteria (MUB) in the produced brine (see Fig. 2). The number of bacteria in the produced fluids prior to nutrient injection was 100 to 1,000/cc of MUB, and 1.0×10^5/cc of total bacteria, as determined by AODC (see Fig. 2). Some of the MUB bottles, particularly those at the lower dilutions, contained insoluble polymer characteristic of the polymer observed in enrichment cultures. Results of the near wellbore test at producer 5-9 were similar to those obtained at 12-9 (data not shown).

Throughout both the sequential nutrient injection and co-injection phases of the pilot, the four pattern producers, and the single injector, 16W21, were monitored for changes in populations of MUB. Slightly elevated numbers of MUB were observed at producers 16-2 and 16-9, as well as at injector 16W21 during the co-injection tests, whereas prior to the nutrient co-injection and near wellbore tests, only background numbers of MUB were measured (see Fig. 3).

Follow-up Polymer Screens

Following the NBU field tests, a series of enrichments were carried out to isolate either indigenous or nonindigenous bacteria in an attempt to identify other nutritional conditions that could elicit the production of exopolymer under simulated reservoir conditions. These tests were designed to identify nutrients and microorganisms that could produce exopolymer superior to that of SD-1.

Indigenous Screens. Table 5 lists some of the complex media tested for the determination of growth and polymer production by microorganisms contained in Burbank brine. Good growth and metabolism were observed, as indicated by a decrease in pH and an increase in cell number; however, formation of significant amounts of extracellular polymer was not observed.

Crude oil from the Burbank field was tested as a potential source of nutrients and bacteria for exopolymer production. Crude oil (4% vol) was tested in the presence of either simple compounds or complex media (e.g., nutrient broth, brain and heart infusion, whey). Cultures were incubated for 3 weeks with no indication of significant exopolymer production (data not shown).

Nonindigenous Screens. A number of soil, sediment, and food sources were screened for the presence of bacteria which could produce polymer under simulated reservoir conditions. All sources yielded very good growth under the conditions tested, with the exception of olives. Four isolates were obtained that produced significant polymer (see Table 6). All four isolates produced a soluble polymer and were classified as gram-positive rods. Viscosity measurements performed on culture supernatants of both aerobic and anaerobic cultures from isolates 1 and 2 indicated increased viscosity (results not shown). Isolates 3 and 4, obtained from the air in the anaerobic chamber, produced soluble polymer along with some insoluble polymer that pelleted with the cells when centrifuged (see Table 6).

Discussion

The importance of exopolymer production in the establishment of in-depth, stable plugging was borne out by the results of the corefloods. SD-1, a polymer-stimulating carbohydrate, and not CS 95, supported good residual resistances along the entire length of a Burbank core. The importance of exopolymer production in the plugging of model cores has also been demonstrated by Jack, et al.[13] using a dextran-producing *Leuconostoc* sp. grown on beet molasses. Knapp et al.[14] successfully used molasses and nitrate to stimulate indigenous bacteria and thereby reduce permeability in the Vasser Vertz Unit pilot. Little information was given, however, on the ability of these indigenous microbes to form exopolymer under the conditions employed. This study marks the first time, to our knowledge, that the ability of a natural microbial community to form exopolymer has been attributed to the addition of a high molecular weight carbohydrate, such as a soluble dextrin.

The ability of dextrin, or more specifically maltodextrin, to stimulate exopolymer production by the indigenous flora, when simple sugars would not, is not well understood. In enrichments performed in the presence of hydrostatic pressure, it was discovered that simple sugars were more rapidly utilized than SD-1, thereby

resulting in a more rapid buildup of acids and gases (e.g., CO_2, acetate, hydrogen), which may have inhibited the production of polymer (results not shown). Another possibility is that dextrin may serve as a primer or template for the production of a modified or higher molecular weight insoluble polymer; enzymatic characterization of this insoluble polymer, however, suggested that a significant fraction may not be maltodextrin-like in nature (results not shown). Koldex 60, another dextrin, also stimulated notable exopolymer production, but its solubility in Burbank brine and its reproducibility in stimulating polymer were poorer than SD-1.

Ethyl acid phosphate, an organic phosphate ester, was chosen as the preferred phosphorus source based on its solubility, stability, and transport characteristics in rock cores. This is very important, since the ability to achieve plugging distal to the wellbore depends on the ability of the injected nutrients and bacteria to propagate in-depth. The lesser adsorption of the OPEs, versus the more commonly used inorganic orthophosphates and polyphosphates (e.g., potassium phosphate, ammonium phosphate, sodium pyrophosphate, or sodium trimetaphosphate), is due to their superior stability and solubility at higher temperatures, particularly in the presence of high concentrations of divalent cations. All OPEs had a PI greater than that of STMP, which is fairly stable in Burbank brine when compared to orthophosphates and other condensed phosphates (e.g., tripolyphosphate). Condensed phosphates, although more stable than orthophophates in the presence of divalent cations, hydrolyze readily to form orthophosphates as temperature increases.[16] Although the screening tests (see Table 3) suggested that EAP adsorbed more readily than several of the other OPEs tested, it was chosen as the phosphorus source because some retention of the phosphorus is desirable when applying a sequential nutrient injection process.[15]

The significant increases in MUB and total bacteria, observed in the reservoir brine following the near wellbore field tests, confirmed the results obtained in the laboratory enrichments and demonstrated that indigenous maltodextrin-utilizing bacteria could be stimulated in situ by the injection of these nutrients (see Fig. 2). It was not possible to directly measure exopolymer produced in situ; however, increasing numbers of MUB and the presence of insoluble polymer in some MPN tubes prepared from produced fluids is indicative of the potential for in-situ polymer production. A slight trend observed for increasing numbers of MUB in samples from wells 16-2 and 16-9 also suggests the possibility of in-situ maltodextrin-utilizing activity.

Although the NBU Field tests completed in the Fall of 1993 successfully demonstrated the ability of SD-1 and EAP to significantly reduce effective permeability and divert flow, the stability of the plug desired was not attained. Therefore, attempts were made following the pilot test to identify other nutrient and/or microbe combinations that might encourage better exopolymer production

than that of SD-1. The majority of initial screens for indigenous polymer-producers were performed on minimal media containing only a carbohydrate and phosphorus source, in order to minimize chemical costs. Following the field tests, it was felt that a more nutritionally complex nutrient media might encourage better production of exopolymer by indigenous bacteria. Complex media are rich in amino acids, proteins, vitamins, and other co-factors that are required by some bacteria to grow and produce polymer. Our results indicated that although good growth was observed, using complex media to stimulate polymer production in the injection brine was not successful (see Table 5).

In order to investigate the possibility that nonindigenous bacteria could be isolated with the ability to generate exopolymer under the conditions of the NBU reservoir, sources other than Burbank brine were screened (see Table 6). Samples obtained from the sediments of hypersaline ponds and from compost were the most effective. These environments likely contain large amounts of organic and other nutrients conducive to stimulating polymer production. Two isolates obtained from laboratory airborne sources most likely originated from spores produced by other cultures in the lab. Isolates 1–4 are halotolerant, but their ability to produce polymer is restricted to about 6% NaCl when grown at 45°C. All four isolates possess characteristics common to members of the genus *Bacillus*. Other investigators have also reported production of exopolymer by *Bacillus* sp. when grown at elevated temperatures and salinities.[17,18]

In conclusion, this paper demonstrates the potential of high molecular weight, water-soluble polysaccharides, such as corn starch dextrins or maltodextrins, to stimulate the biosynthesis of exopolymer in environmental samples, such as oil field reservoir brines. In contrast, attempts to use rich, complex nutrients or simple sugars as carbon sources did not stimulate exopolymer production by indigenous microbes. Several isolates, not indigenous to NBU brine, were eventually isolated from compost and from the sediment of a saline algal pond. These isolates could produce polymer when growing on a modified molasses media containing nitrate. Their ability to survive under Burbank reservoir conditions, however, is questionable. The ability of maltodextrin to stimulate indigenous MUB in Burbank brine under reservoir conditions was evidenced by the increases in MUB following nutrient treatment in the field, as well as by metabolic end-products in the brine. The importance of maltodextrin as a nutrient for exopolymer production, which is necessary for stable, in-depth, plugging, was demonstrated by experiments with laboratory cores.

Finally, it was found that organic phosphates esters were superior to inorganic phosphates and polyphosphates as sources of phosphorus for microbial growth and metabolism of indigenous microbes, due to their superior stability in hard brines at increasing temperatures, as well as their improved transport through reservoir rock.

Acknowledgments

The authors thank Phillips Petroleum Company for support of this project and the opportunity to publish this paper. A special thanks to LaVonne Melheim for reviewing this manuscript.

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Table 1 The Ability of Various Carbohydrates to Stimulate the Production of Exopolymer by Indigenous Microorganisms in NBU Brine

Carbohydrate	Type	Source	Polymer[a]
Star Dri 1	Dextrin	A. E. Staley	++
Koldex 60	Dextrin	A. E. Staley	++
Stadex 94	Dextrin	A. E. Staley	+
Maltrin M440	Dextrin	GPC	+
Lo Dex 5	Dextrin	American Maize	+
Clearsweet 95	Corn Syrup	Cargill	-
Clearsweet 99	Corn Syrup	Cargill	-
Star Dri 24r	Corn Syrup	A. E. Staley	-
Star Dri 35r	Corn Syrup	A. E. Staley	-
Maltrin M365	Corn Syrup	GPC	-
Molasses	Simple Sugar	Grandma's	-
Glucose	Simple Sugar	J. T. Baker	-
Sucrose	Simple Sugar	Sigma	-

[a] Results were obtained in duplicate or triplicate tests.

Table 2 Properties of the Nutrients Star-Dri 1 and Ethyl Acid Phosphate

Nutrient	Property
Star-Dri 1	Corn Starch Maltodextrin
	Dextrose Equivalent ~ 1.0%
	Average. M.W. ~ 300,000
	Solubility: at least 20% (25 C)
	Viscosity: 25 cp at 20% (25 C)
	Price: $0.55 / lb
Ethyl Acid Phosphate	Organic Phosphate Ester
	Mixture; ~ 99% Active
	Liquid in 55 Gal. Drums
	~ 22% As Phosphorus by Weight
	Price: $ 2.00 / lb.

Table 3 Adsorption Indices and Precipitation Indices of Various Phosphates

Type	Source	Adsorption Index	Precipitation Index
Sodium Trimetaphosphate	Monsanto	1.0	0.64
Triethylphosphate	Kodak	13	0.86
Methyl Acid Phosphate	Albright-Wilson	31	1.0
Dimethyl Acid Pyrophosphate	Albright-Wilson	1.1	0.88
Ethyl Acid Phosphate	Akzo	5.4	0.97
Butyl Acid Phosphate	Akzo	1.2	1.0
Monomethylphosphate	Sigma	65	0.90

Table 4 Effect of SD-1 and CS 95 on the RF and RRF along the Length of a Burbank Sandstone Core

Core-flood	Treatment (wt%)	Nutrient Injection PV[a]	Brine Injection PV[a]	RF[b] (RRF)			
				1	2	3	4
1	SD-1	350	65	130	12	11	10
	(0.4)			(118)	(11)	(11)	(10)
2	CS	396	40	100	<5	<5	<5
	(0.4)			(90)	(ND)	(ND)	(ND)
	CS:SD-1	57	ND	80	<5	<5	<5
	(0.2):(0.2)			(ND)	(ND)	(ND)	(ND)
	SD-1	188	1.2	130	55	<5	<5
	(0.4)			(80)	(55)	(ND)	(ND)

a PV= 9.55 cc (Coreflood 1) and PV= 9.85 cc (Coreflood 2)

b See Materials and Methods section for explanation.

Table 5 Effect of Complex Media on Stimulation of Growth and Polymer Production by Indigenous Microorganisms in NBU Brine

Carbon Sources	pH	AODC ($\times 10^8$)	Polymer
YM Broth*	4.7	5.4	-
Nutrient Broth (33%)	5.8	1.6	-
Brain Heart Infusion (33%)	5.6	3.1	-
Bacterium Medium*[a]	4.9	6.5	-
XSM Medium *[b]	4.6	4.4	-
Peptone Medium *[c]	5.6	4.3	-
Glycerol Beef Medium *[d]	6.3	5.3	-

* Prepared according to ATCC catalog with the following modifications: a) Glucose increased to 0.5%; b) liver extract omitted; c) average value of enrichments prepared with maltose, fructose, sucrose, and Star-Dri 1 as carbohydrate sources; fish extract omitted in all cases; d) sodium chloride omitted and 10 mM KNO_3 added.

Table 6 Description of Polymer-Producing Isolates Nonindigenous to NBU Brine

Isolate Number	Sample Description	Identification	Soluble Polymer (mg/l)	Insoluble Polymer (mg/l)
1	Sludge from *Dunaliella* pond	*Bacillus licheniformis*	300	-
2	Compost heap	*Bacillus licheniformis*	56	-
3	Airborne	Large rod	108	77
4	Airborne	Large rod	274	156

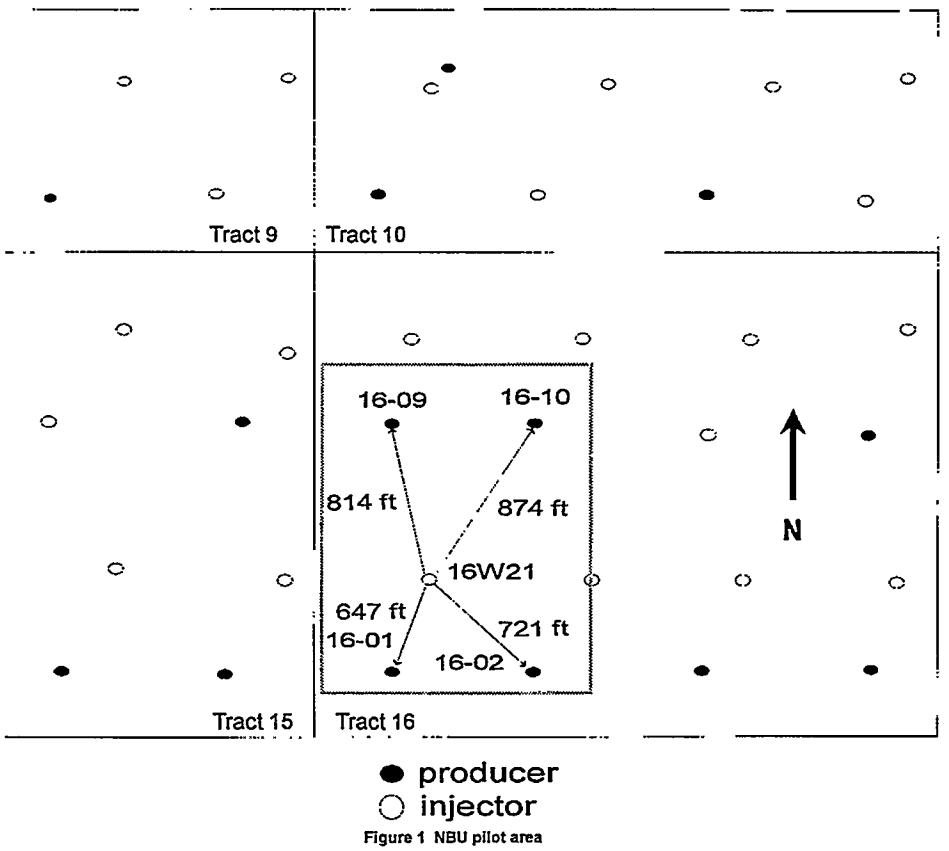

Figure 1 NBU pilot area

Figure 1 NBU Pilot Area

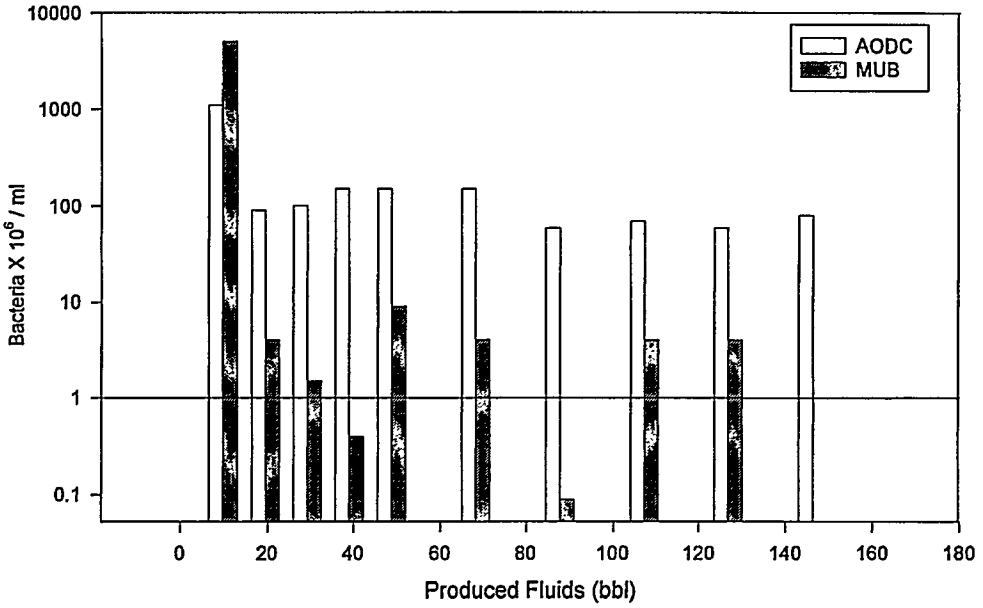

Figure 2 Effect of Coinjection of SD-1 and EAP on Concentration of Bacteria in Produced Fluids Backflowed from Well 12-9 following Near Wellbore Test

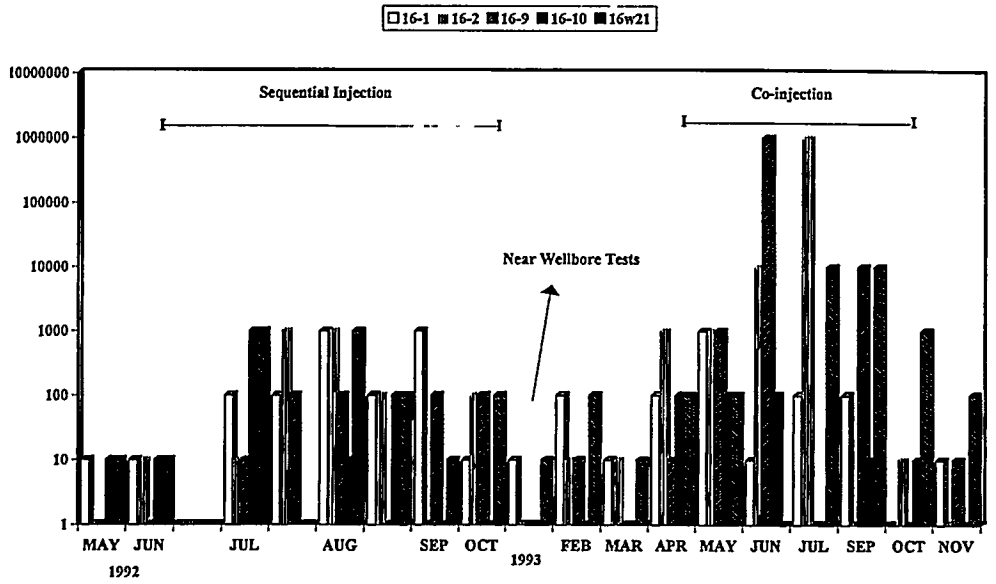

Figure 3 Bottle Counts of MUB in Produced or Injected Brine from Wells within the Pilot Area

A Case Study of the Intrinsic Bioremediation of Petroleum Hydrocarbons

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Abstract

Condensate liquids have been found to contaminate soil and groundwater at two gas production sites in the Denver Basin operated by Amoco Production Co. These sites have been closely monitored since July 1993 to determine whether intrinsic aerobic or anaerobic bioremediation of hydrocarbons occurs at a sufficient rate and to an adequate endpoint to support a no-intervention decision. Groundwater monitoring and analysis of soil cores suggest that intrinsic bioremediation is occurring at these sites by multiple pathways including aerobic oxidation, Fe^{3+} reduction, and sulfate reduction.

In laboratory experiments the addition of gas condensate hydrocarbons to saturated soil from the gas production site stimulated sulfate reduction under anaerobic and oxygen-limiting conditions, and nitrate and Fe^{3+} reduction under oxygen-limiting conditions, compared to biotic controls that lacked hydrocarbon and sterile controls. The sulfate reduction corresponded to a reduction in the amount of toluene relative to other hydrocarbons. These results confirmed that subsurface soils at the gas production site have the potential for intrinsic bioremediation of hydrocarbons.

Introduction

Amoco Production Co. presently operates over 800 natural gas wells within the Denver Basin, Colorado, which produce about 100 Mscf/D of gas with associated water and condensate liquids (< 3 bbl/D). Structural failures of concrete sumps, used to contain produced water, have resulted in hydrocarbon leaks into the environment at 86 sites which have adversely impacted groundwater and soils.

Within the context of Amoco's E&P operations in the Denver Basin, potential costs for active remediation of these sites are conservatively estimated at $10 million. Real costs are likely to exceed these estimates given the remote and inaccessible nature of many of these sites. Because of the large potential economic impact of these future environmental costs on E&P operations in this area, Amoco has sought an alternative to active remediation wherein costs might be reduced at an acceptable environmental risk. Natural or intrinsic bioremediation is one such option which, in principle, does not require costly active intervention. In this option it is recognized that indigenous microorganisms in the subsurface are capable of hydrocarbon degradation when critical environmental factors are not limiting (especially nutrients—nitrogen and phosphorous, temperature, moisture, pH, salinity and electron acceptor). Recently, researchers have convincingly demonstrated the natural attenuation of hydrocarbon plumes in groundwater through bioremediation under both aerobic and anaerobic conditions. Oxygen, nitrate, Fe^{3+} oxides, and sulfate have all been identified as potential terminal electron acceptors in the biochemical pathway for hydrocarbon degradation.[1,2]

Amoco Production Co. has initiated a study which seeks to determine whether intrinsic aerobic or anaerobic bioremediation of hydrocarbons occurs at the Denver Basin sites at a sufficient rate and to an adequate endpoint to support a no-intervention or passive remediation scheme. Tasks specific to this objective are: (1) long-term groundwater and soils monitoring (initiated July 1993) to document field hydrocarbon losses and bioactivity over time (quarterly sampling events for 2–3 years), (2) laboratory verification of hydrocarbon biodegradation by field microorganisms and identification of primary biodegradation mechanisms (initiated September 1993), (3) mathematical modeling to estimate biotic and abiotic losses for comparison with field observations, and (4) risk evaluation to determine potential environmental exposure pathways and anticipated doses.

In this paper we report the results of the initial site assessments and groundwater and soil monitoring results to date. The implications of these data to the natural attenuation of hydrocarbons at the sites are discussed. We also report the results of laboratory investigations which were conducted to determine whether the depletion of electron acceptors in the presence of high hydrocarbon concentrations at these

sites was biologically mediated and whether the presence of hydrocarbon stimulated the use of these electron acceptors. Four different electron acceptors were investigated: nitrate, sulfate, Fe^{3+}, and carbon dioxide (methanogenic) under anoxic and limited oxygen conditions. Saturated soil microcosms with excess hydrocarbon were used to simulate the field conditions as closely as possible.

Part I: Field Investigation

Site Characterization and Monitoring

In July 1993, two gas condensate contaminated sites near Ft. Lupton, Colorado, were chosen for in-depth site assessments. [One of these sites (KPU2) is described in Figure 1.] These sites are situated near the Platte River in agricultural areas. Preliminary evaluations had shown that both soils and shallow groundwater had been contaminated beyond the storage tank containment area. Given the highly permeable nature of the aquifer material (gravely sands, sands, and silty sands) and the dynamic fluctuations in water table elevations with seasonal irrigation, the potential for contaminant transport was deemed high. The groundwater velocity was estimated at 1.3 m/yr based on a gradient of 0.4 cm/m. Coupled with the proximity of surface water receptors, both sites were placed in a high-priority category.

Initial site assessment focused upon the delineation of the contaminant plume. Because of shallow groundwater (3–5 ft or 0.91–1.52 m), a soil gas survey of the vadose zone could be rapidly conducted. A minimum of 30 vapor probes were deployed per site initially along the anticipated direction of groundwater flow, and real time measurements of soil gas O_2, CO_2, and VOCs (Volatile Organic Carbon) were made.

Soil vapors were sampled with an AMS Soil Gas Vapor Probe (SGVP; Forestry Suppliers, Jackson, Mississippi). SGVP-dedicated sampling tips perforated with vapor inlet holes were driven 1.5 to 3 ft (0.46—0.92 m) into the subsurface with an electric roto hammer. The SGVP drive tubes were removed with a portable jack, leaving the vapor tip probe imbedded at the desired sampling depth. A Teflon® vapor tube, connected to the tip and extending to the surface, was used to sample soil gases near the tip.

The VOCs in soil gases were measured using a Gastech Trace-Techtor™ hydrocarbon analyzer with range settings of 100 ppm, 1,000 ppm, and 10,000 ppm. A dilution fitting permitted quantification of VOCs in soil gases to 20,000 ppm. The analyzer was calibrated against hexane calibration gas (4,350 ppm).

Soil gas concentrations of CO_2 and O_2 were measured using a Gastech model 32520 $\times$ CO_2 / O_2 analyzer. The CO_2 calibration was performed against atmospheric CO_2 concentration (0.05%) and a 2.5% standard. The O_2 was calibrated using an atmospheric standard (20.9%). Both analyzers had an internal vacuum pump for sampling soil gases.

The KPU2 site topsoil is a sandy loam, although a sandy/gravel road fill has been spread over a significant area of the site. Beneath the topsoil/road fill, the sediment type varies from a gravely sand to silt-rich sand horizons. Soil gas contours for the KPU2 site are shown in Figures 2, 3, and 4. Background soil gas VOC measurements were 0 to 30 ppm (VP3 and VP36, respectively). Soil gas VOC measurements suggested the presence of hydrocarbon-contaminated soil and/or a groundwater plume northeast of the contaminant source and a groundwater plume which had migrated to the north. Soil gas VOC levels exceeding 20,000 ppm were measured immediately east of the contaminant source (VP2) and at VP22 and VP28. Elevated VOC levels were measured approximately 110 ft (30.5 m) north of the contaminant source (VP26), suggesting the presence of a groundwater BTEX plume extending at least this distance from the source.

Soil gas O_2 and CO_2 data (see Figs. 3 and 4) were consistent with the VOC data. Background soil gas O_2 levels were 20.9% and 20.8% (VP3 and VP36, respectively). Background CO_2 levels were 0.5% and 0.6% (VP3 and VP36, respectively). In general, elevated VOC levels were associated with elevated CO_2 and depressed O_2 levels. For example, the soil gas VOC level measured at VP2 was >20,000 ppm while O_2 and CO_2 were 1% and 12.5%, respectively. Contours of soil gas O_2 and CO_2 data also suggested the presence of hydrocarbon-contaminated soils and/or a groundwater plume which had migrated in a northerly direction from the source.

Based on the soil gas survey data, permanent groundwater monitoring wells were installed to determine the extent of hydrocarbon losses and the degree of bioactivity over time. One inch (2.54 cm) OD vertically nested monitoring wells were installed in five-spot patterns (see Fig. 1) within a downgradient contaminated and upgradient uncontaminated area. This monitoring arrangement was adopted in an effort to define both areal and vertical variations of hydrocarbon and electron acceptor concentrations in groundwater. Vertical nesting consisted of a series of three wells screened over 18-in. (45.7-cm) intervals and placed 0, 5, and 10 ft (0, 1.52, and 3.05 m) below the water table at the time of installation. Additional 2-in (5.1-cm) monitoring wells were placed along the longitudinal axis of predominant groundwater flow in an effort to monitor plume migration and electron acceptor transport. These wells were arranged along a path extending from upgradient of the control area, through the source, and downgradient of the contaminated zone. The 2-in. (5.1-cm) wells were screened over a 10-ft (3.05-m) interval to allow for

seasonal groundwater fluctuations. Well completions were by standard practices for groundwater monitoring applications.

To adequately address hydraulic modeling requirements and thereby ultimately assess the role of abiotic mechanisms (e.g., dispersion, advection) in contaminant loss, both the contaminant and control study areas were contained within a larger hydraulic five-spot monitoring pattern. Hydraulic monitoring wells were completed identically to the 2-in. (5.1-cm) monitor wells. Pressure transducers were permanently installed at a fixed depth below the water table in each well. Average, maximum, and minimum water table fluctuations are recorded daily within a 0.5-in. (1.3-cm) resolution.

Soil cores were obtained from each site in November 1993 to document initial soil hydrocarbon and electron acceptor distributions. Four cores were taken from within each control and contaminated pattern. Coring locations were situated approximately halfway between the center and corner well clusters in each pattern quadrant. Continuous cores were obtained with a 2-in. (5.1-cm) split-spoon sampler from the surface to a total depth of 15 ft (4.6 m). Core samples were composited in 1.5-ft (45.7-cm) intervals and stored in a reduced oxygen atmosphere at 4°C until requisite analyses could be performed.

Analytical. Baseline groundwater samples were collected during the first week of November 1993. Fresh water samples were obtained by producing approximately 3 well volumes from each monitoring well prior to sampling. Dissolved oxygen (DO), pH, and temperature were determined immediately. Individual samples were collected and analyzed within 24 hours for inorganic constituents such as nitrate, sulfate, and Fe^{2+}. Samples for BTEX and TPH were collected in clean 40 cm^3 VOA vials, immediately extracted with Freon, and shipped to Amoco's Groundwater Management Section Laboratory, Tulsa, Oklahoma, for analysis. Nitrate, sulfate, and Fe^{2+} were determined as described below.

Soil solids were analyzed for moisture content, acid extractable Fe^{2+} and Fe^{3+}, porosity, bulk density, and saturated paste pH, nitrate, and sulfate. Moisture content was determined by drying a nominal 10 g sample of core material at field moisture content to constant mass in a Denver Instruments moisture balance (P/N 900207.1). To determine acid extractable Fe^{2+} and Fe^{3+}, a nominal 5 g of core material at field moisture content was weighed into a 60 ml capacity amber serum bottle. The bottle was then filled with a known volume of 0.77 N hydrochloric acid (57.0 to 58.5 ml) and capped and sealed without headspace. The bottle was then shaken by hand for 30 seconds prior to sonication for 60 minutes. After sonication, the bottle was allowed to stand for 72 hours prior to analysis. The extract was analyzed for Fe^{2+} and Fe^{3+} by ion chromatography using a Dionex AGP-1 ion chromatograph fitted with a Dionex CS5 IonPak separator. The eluant used was a

10% (v:v) methanol in water solution containing 50 mM acetic acid—50 mM sodium acetate and 6 mM 2,6-dicarboxylic acid (PDCA)—at a flow rate of 1 ml/min. After passing through the separator column, the eluant was mixed with a solution containing 0.3 mM 4-(2-pyridylazo) resorcinol, monosodium salt hydrate (PAR), 1 M acetic acid, and 3 M ammonium hydroxide in a post column reactor (PAR flow rate was 0.6 ml/min). The concentrations of Fe^{2+} and Fe^{3+} were then detected by measuring absorbance of their PAR complexes at 520 nm.

The saturated paste extract was prepared by placing a known volume and mass of core material at field moisture content in a 1-pint wide mouth jar and weighing in enough deionized water to achieve a fully saturated condition. The jar was then sealed and allowed to stand for 60 minutes. The water was then removed by vacuum filtration through a Whatman #4 filter paper. The pH of the filtered water was then determined. A 2 ml aliquot of this water was then filtered through a 0.45 µm syringe filter and stored at 4°C prior to analysis for sulfate and nitrate. The bulk density and porosity of the material were then determined by drying and weighing the remaining solids. The sulfate and nitrate content of the saturated paste extract was determined using a Dionex AGP-1 ion chromatograph fitted with a 4-mm Dionex AS5 IonPak separator. A gradient elution was performed using an NaOH gradient of 1 mM to 64 mM (changing linearly over a 25 minute period) which then switched to an isocratic 1 mM NaOH eluant for the remaining 20 minutes of the analytical program. Eluant flow was constant at 1 ml/min, and the eluant contained a constant concentration of methanol (10% v:v). Detection was by conductivity following chemical conductivity suppression in a Dionex membrane suppresser (suppresser reagent = 25 mM sulfuric acid at a flow rate of 10 ml/min).

Preliminary Field Results

Groundwater Analysis. Table 1 summarizes baseline groundwater data for the KPU2 site. (Data from the second site shows similar trends and are not reported here.) The data are organized by well depth for both control and plume volumes. As the data indicate, BTEX and TPH were confined primarily to the shallow well-depth within the contaminated area. At this depth, median electron acceptor concentrations were uniformly lower in the contaminated versus the control zone. $Iron^{2+}$, a product of the utilization of Fe^{3+} as an electron acceptor, is higher. At the intermediate well depth, hydrocarbon concentrations were considerably lower than similarly located shallow concentrations within the contaminated area. No BTEX or TPH were noted in the control samples. Trends in electron acceptor utilization at the intermediate depth within the plume were identical to those at shallow depths although less pronounced, presumably because of lower hydrocarbon concentrations. Deep well data indicated no appreciable hydrocarbon presence in either study area. Likewise, electron acceptor data showed no appreciable

differences. Finally, axial monitoring well data (not shown) indicated that highly soluble BTEX components had migrated a distance of only 165 ft (50.3 m) from the source over an estimated 20-year time period. Subsequent quarterly groundwater sampling events have produced similar results in terms of electron acceptor, Fe^{2+}, BTEX, and TPH concentrations. No clear decrease in BTEX or TPH concentrations has been observed to date due presumably to replenishment of dissolved hydrocarbons from a sink of adsorbed hydrocarbons and fluctuations in the water table.

The following observations are made on the basis of groundwater data acquired to date. The aerobic biodegradation potential of hydrocarbon appears limited due to uniformly low DO concentrations (1.4 mg/l or less) throughout the contaminant and control volumes. Nitrate, although present as a consequence of agricultural applications of fertilizer, also seems to have limited potential for hydrocarbon degradation. This again is attributed to low background concentrations (< 20 mg/l) within the subsurface. In contrast, the utilization of sulfate appears significant. Background (control) concentrations are on the order of 230 mg/l while concentrations within the shallow contaminated area are 0. Given the large initial concentration of sulfate and its favorable stoichiometric utilization for hydrocarbon degradation, it appears that sulfate reduction may be a major means of hydrocarbon remediation at these sites. Although evidence for iron reduction exists within the contaminated area, the utilization of Fe^{3+} cannot be quantified from groundwater samples due to its sparing solubility. In addition, the solubility of Fe^{2+} is very low in the presence of sulfide, which is the product of sulfate reduction.

In summary, the groundwater data suggest that significant hydrocarbon contamination is laterally and vertically confined to a small portion of the total aquifer. Given the age of the site (> 20 years), the limited extent of contamination, and supporting evidence for aerobic and anaerobic bioactivity, the data further suggest that intrinsic bioremediation may play a large role in attenuating hydrocarbon contaminants at these sites.

Soil Core Analysis. Soil core analyses for total iron, Fe^{2+}, BTEX, and TPH were performed on representative samples from each 1.5-ft (45.7-cm) interval. The average and median background BTEX concentrations within the KPU2 control volume were 0.036 and 0.02 mg/kg, respectively. Surface soil samples showed typically higher concentrations at about 0.1 mg/kg, possibly indicating exposure to airborne BTEX. TPH values from control core samples were uniformly nondetectable (< 1 mg/kg). Comparable values within the contaminated volume for average, median, and maximum BTEX concentration were 60.8, 0.27, and 817 mg/kg. The large difference between the average and median value reflects the vertical distribution of BTEX, which is narrowly confined to an approximate 3-ft (0.91-m) interval at the water table/air interface. The majority of samples outside of

this interval had BTEX concentrations well below 1 mg/kg. TPH values were similarly distributed with the average, median, and maximum of 379.6, nondetect, and 4,590 mg/kg. Finally, soil samples acquired from upgradient cores within the contaminated zone showed less BTEX and TPH than their downgradient counterparts. Since upgradient cores were situated nearest the original contaminant source, this evidence supports the contention that the cause of the existing hydrocarbon contamination had been effectively eliminated at these sites.

When control and contaminant distributions of Fe^{2+} and Fe^{3+} in soil samples were compared, it was noted that the ratio of Fe^{2+} to Fe^{3+} was shifted towards proportionately higher Fe^{2+} concentrations within the contaminated zone (see Fig. 5). Assuming both iron species were initially distributed uniformly across the site, it appears that Fe^{3+} was subsequently reduced to Fe^{2+} within the zone of significant hydrocarbon contamination. The reduction of iron in the presence of hydrocarbon is indicative of anaerobic biodegradation and further supports the hypothesis that intrinsic bioremediation of hydrocarbons is occurring at these sites by multiple pathways.

Finally, visual inspection of soil cores showed a significant accumulation of a black precipitate (acid volatile iron sulfide) associated solely with hydrocarbon contamination. The accumulation of iron sulfide (FeS) in the presence of hydrocarbon is consistent with anaerobic biodegradation of hydrocarbons by sulfate reduction.

Part II. Laboratory Microcosm Studies

Materials and Methods

Composition of Saturated Soil Microcosms. Microcosm studies used 25 g of native soil obtained from an uncontaminated region of the Denver basin field site. Native soil was dried for about 24 hours at 70°C to facilitate handling and then sieved through a standard 10-mesh sieve to remove larger stones and root ends. The inoculum was from the contaminated region and comprised about 10% (by wt) of the native soil used in the microcosms. The contaminated soil was a mixture of cores obtained from both saturated and vadose zones from a region downstream of the gas condensate spill including regions with free product and from the ground water plume area. Soil for use as inoculum was collected using precautions to maintain anaerobic conditions throughout the sampling procedure. A mineral and electron acceptor analysis of native soil and the soil inoculum are given in Table 2.

The soil was saturated with 6 ml of a mineral salts medium which was composed of 1.0 ml of trace metals solution, 2.0 ml of mineral solution, and 0.35 g of sodium bicarbonate per 100.0 ml of medium. The composition of trace metals solution and mineral stock solution are given by Tanner et al.[3] Electron acceptors were added to the mineral salts medium before saturating the soil. Amended nitrate and sulfate concentrations in the nitrate and sulfate-amended microcosms were 0.168 mM per microcosm and 0.144 mM per microcosm, respectively. An internal standard, sodium bromide (0.138 mM per microcosm), was used to account for any losses of anions to the soil. $Iron^{3+}$ was added as a separate phase in the form of an amorphous oxyhydroxide gel[4] to give 0.108 mM of amended Fe^{3+} per microcosm. The medium (mineral salts + electron acceptor) was anaerobically prepared using a Hungate gassing station[5,6] and transferred to an anaerobic chamber. The medium was then added to serum bottles containing the native with or without soil inoculum within the anaerobic chamber. The bottles were stoppered with Teflon-lined gray butyl rubber stoppers (Wheaton, Milleville, New Jersey) and the headspace exchanged from anaerobic chamber gas to 80% N_2 + 20% CO_2.[6] These will be referred to as the anoxic microcosms. A second identical set of microcosms was prepared outside the anaerobic chamber and the headspace exchanged to 21% O_2, 20% CO_2, with the balance being N_2. The total mM of oxygen in the gas phase was 0.258 mM per microcosm, with 0.0016 mM in the aqueous phase. Carbon dioxide was included in the headspace of all microcosms as a component of the buffering system (with bicarbonate in the aqueous phase) and as a potential electron acceptor in microcosms that did not receive exogenous electron acceptors. The pH of the aqueous phase under these conditions was 7.2.

Since the native soil and soil inoculum used in these experiments contained significant amounts of the potential electron acceptors, sulfate and Fe^{3+} (see Table 2), an additional series of experiments was done using 25 g of clean Ottawa sand in place of native soil. The background levels of electron acceptors in the Ottawa sand were much lower (see Table 2).

Three types of microcosms were prepared: biotic, sterile, and background. The biotic microcosms contained 25 g of native soil or Ottawa sand with 10 wt% soil inoculum saturated with the mineral salts medium with or without exogenous electron acceptor and with sufficient condensate to give a hydrocarbon concentration of 10,000 mg/kg of soil or sand on a dry weight basis. The sterile controls were prepared in an identical manner but were steam sterilized at 121°C for 60 min. The background controls consisted of native soil or Ottawa sand with the mineral salts medium with and without exogenous electron acceptor and did not contain any hydrocarbon or soil inoculum.

The gas condensate obtained from the Denver basin site was heated to remove part of the low boiling compounds to reduce the potential for volatile losses during the

experiment. The gas condensate was heated using a Soxtec Heating System 2 HT2 (Perstorp Analytical Inc., Silver Spring, Maryland) for a period of 8 hours at temperatures up to 110°C. The heat-treated condensate was filter sterilized using a 0.22-μm sterile, cartridge filter (Gelman Sciences) and then injected into each microcosm using a sterile syringe and needle. The microcosms were then shaken manually to mix the hydrocarbon with the soil and the aqueous phase, and transferred into anaerobic jars with a gas phase of 80% N_2 + 20% CO_2.

Groundwater temperatures at the Denver basin field site range from 6°C to 20°C due to seasonal variations. The microcosm experiments reported here were carried out at an incubation temperature of 10°C. Triplicate microcosms of each type were sacrificed at six different times over a period of 402 days. The contents were analyzed for electron acceptors, hydrocarbons, and possible products of hydrocarbon degradation.

Analytical. Microcosms were extracted with methylene chloride for hydrocarbon analysis, and then with 1N HCl to produce an aqueous phase for analysis of electron acceptors and possible products of biodegradation (low molecular weight organic acids). The extraction and sampling protocol is given in Figure 6. Hexacosane was used as an internal standard (300 mg/kg) during the methylene chloride extraction to account for any losses during the extraction procedure.

Total petroleum hydrocarbons (TPH) was obtained by gas chromatography; each chromatogram resulting from analysis of the methylene chloride extract was integrated using baseline root integration, and calibrated using an external standard made with heat-treated condensate to obtain the TPH (C6-C20). Data were normalized to account for recovery of the hexacosane internal standard.

Methylene chloride extract was analyzed for hydrocarbons on an HP 5880 gas chromatograph equipped with a flame ionization detector. A DB-1 capillary column was used with an injection temperature of 290°C and detector temperature of 345°C. The column was subjected to the following temperature program: prehold at 35°C for one min, gradient of 7°C/min from 35°C to 340°C, post-hold at 340°C for 10 min. Helium was used as a carrier gas with split injection at a split ratio of 1:100.

Nitrate, sulfate, bromide, and acetate were analyzed on a Dionex ion chromatograph (IC) model AGP-1 equipped with electrochemical conductivity detector. A 4 mm IonPak AS5 Dionex analytical column was used to carry out the separation. A gradient method was used with sodium hydroxide solution containing 5% methanol as the eluant. The sodium hydroxide concentration was ramped from 1 mM to 64 mM over a period of 22 min, at the end of which the eluant flowed isochratically at a concentration of 64 mM for a period of 3 min, followed by a gradient with sodium hydroxide concentration dropping from 64 mM to 1 mM over a period of 3

min. The total run time was 45 min with isochratic flow of 1 mM NaOH for the last 17 min. The eluant flow rate was 1 ml/min. A Dionex membrane suppresser was used with sulfuric acid as the reagent at a concentration of 25 mM and flow rate of 10 ml/min. Data was normalized to account for recovery of the bromide internal standard.

Fe^{2+} and Fe^{3+} were separated on a Dionex IonPak CS5 analytical column using a second IC instrument. The eluant was 50 mM acetic acid–50 mM sodium acetate solution with 10% methanol containing a chelating agent, 2,6-pyridine dicarboxylic acid (PDCA) at a concentration of 6 mM. The eluant flow rate was 1 ml/min. The metals were detected by measuring the absorbance of a complex formed with a post column reagent, 1 M acetic acid–3M ammonium hydroxide containing 0.3 mM 4-(2-pyridylazo) resorcinol, monosodium salt hydrate (PAR) flowing at 0.7 ml/min.

The low molecular weight organic acids were separated using a Dionex IonPak NS1 analytical column on a third IC instrument and quantified using a UV detector. A gradient method was used with 100% solution A going to 100% solution B over a period of 35 min. Solution A was 0.025 mM HCl in 24% CH_3CN–6% MeOH solution, and solution B was 0.05 mM HCl in 60% CH_3CN–24% MeOH solution. The eluant followed a gradient to 100% A over next 3 min. The eluant flow rate was 1.5 ml/min. A Dionex membrane suppresser was used with 2.5 mM KOH as the reagent at a flow rate of 2 ml/min. Separation of propionic acid to decanoic acid was achieved with this method in 35 min.

Results and Discussion

Apparent Loss of Hydrocarbon. Because of an excess of hydrocarbon relative to the amount of electron acceptor present (total amount of electron acceptor present in the microcosms was sufficient for complete oxidation of only about 1–2% of the hydrocarbon added to each microcosm), the hydrocarbon loss could not be used as a measure of biological activity. A 60–70% loss in TPH occurred in all microcosms during the first 40 days of incubation, including the sterile controls, under all electron acceptor conditions. The TPH concentrations remained relatively unchanged thereafter. This large total loss in hydrocarbon is not a result of biotic processes since similar losses were observed in sterile and nonsterile microcosms. A comparison of gas chromatograms from days 0 and 402 (data not shown) showed the presence of the light hydrocarbons in the day 402 chromatogram with relatively small losses compared to the high boiling compounds. This eliminates volatilization as a possible mechanism for TPH loss of the magnitude observed.

Adsorption of the hydrocarbon on the solid matrix is another possible explanation for the apparent disappearance of hydrocarbon. The methylene chloride extraction

procedure was unchanged over the period of the experiment; however, the extraction efficiency may have been reduced due to penetration of the hydrocarbon into the micropores of the soil/sand. To test this, the soil from a biotic microcosm that had been extracted with methylene chloride and acid was further extracted with methylene chloride using a Soxtec method for 8 hours. The amount of hydrocarbon recovered from the second extraction was only about 8.3% of the amount of TPH lost.

Anoxic Conditions. About 600–800 mg/(kg soil) (dry wt.) Fe^{3+} was present in the sulfate-amended, anoxic microcosms. Fe^{3+} reduction with Fe^{2+} production occurred simultaneously with sulfate reduction. At the end of 402 days, approximately 500 mg/kg Fe^{3+} was consumed and 800 mg/kg of Fe^{2+} was produced. The background soil microcosms without hydrocarbon were similar to the biotic microcosms with hydrocarbon, indicating that Fe^{3+} reduction was linked to oxidation of a background carbon source. Native soil contained about 1,000 mg and the soil inoculum contained about 900 mg of organic carbon per kg (dry wt) of soil (Leco Furnace test). No Fe^{3+} reduction was observed in any anoxic sand microcosms.

In the sulfate-amended, anoxic soil microcosms, a reduction in sulfate concentration occurred after 122 days, and sulfate was almost completely consumed by the end of 402 days (see Fig. 7). Sulfate depletion was observed only in biotic soil microcosms with hydrocarbon added and not in background microcosms that lacked hydrocarbon or sterile microcosms. This suggests that sulfate reduction may be linked to hydrocarbon oxidation. Moreover, about a 38% loss in the amount of toluene present was observed in the biotic soil microcosms compared to the sterile controls, as indicated by the decline in toluene:dodecane ratio (see Fig. 8). No noticeable change was observed in the ratios of other components of the heavy condensate. No sulfate depletion occurred in any of the sulfate-amended, anoxic sand microcosms.

Significant utilization of sulfate was also observed in the field. Groundwater samples collected from a zone upgradient of the contamination, representing background levels, indicated sulfate concentrations on the order of 230 mg/l, while those collected from the contaminated region near the top of the water table showed no sulfate. Also, visual inspection of soil cores showed a significant accumulation of a black precipitate (acid-volatile iron sulfide) associated solely with hydrocarbon contamination. Sulfate depletion was, therefore, linked to the presence of hydrocarbon. The microcosm experiments also indicate the potential for sulfate reduction as a possible mechanism for hydrocarbon remediation at the Denver basin field sites.

Hydrocarbon biodegradation linked to nitrate or iron reduction was not demonstrated in this study. Nitrate consumption was observed in biotic, nitrate-amended soil microcosms (see Fig. 9) but not in biotic, nitrate-amended sand microcosms (data not shown). Nitrate consumption was also observed in the background microcosms which contained no hydrocarbon, at a rate similar to that seen in biotic microcosms (see Fig. 9). These data indicate that the organic carbon in the soil rather than the hydrocarbon served as the electron donor. Native soil contained about 1,000 mg and the soil inoculum contained about 900 mg of organic carbon per kg (dry wt) of soil (Leco Furnace test).

Production of Fe^{2+} was observed in Fe^{3+}-amended microcosms with and without hydrocarbon under anoxic conditions, which suggests that Fe^{3+} reduction was linked to oxidation of background carbon in the soil rather than oxidation of added hydrocarbon. A corresponding decrease in Fe^{3+} was not observed. Therefore, a mass balance could not be closed on iron in the microcosm. No change in the Fe^{2+} to Fe^{3+} ratio was observed in the sterile anoxic soil microcosms or any of the anoxic sand microcosms.

In microcosms without an exogenous electron acceptor added, consumption of indigenous nitrate occurred first followed by Fe^{2+} production, regardless of whether hydrocarbon was present or not. Sulfate reduction also occurred simultaneously with the Fe^{2+} production in both biotic and background microcosms. The amount of sulfate reduced was, however, greater in biotic microcosms than in background soil microcosms. No Fe^{2+} production or sulfate reduction was observed in the anoxic sand microcosms. Again, sulfate reduction was linked to the presence of hydrocarbon.

Oxygen-Limited Conditions. Experimental results suggest that nitrate may have been used as an electron acceptor for hydrocarbon biodegradation under oxygen-limited conditions. About 1,200 mg/kg of nitrate was reduced in biotic, nitrate-amended soil microcosms with hydrocarbon, and little nitrate loss was observed in soil microcosms without hydrocarbon added or in sterile controls (see Fig. 10). About 500 mg/kg of nitrate was used in biotic, nitrate-amended sand microcosms. Since the amount of nonhydrocarbon organic matter was very low in sand microcosms, this supports the conclusion that nitrate reduction was linked to hydrocarbon use. No nitrate use was observed in sterile sand microcosms with hydrocarbon or in sand microcosms without hydrocarbon added.

The amount of oxygen present initially in oxygen-limited microcosms was sufficient for complete mineralization of only 1.2% of the initial TPH present. The presence of a limited amount of oxygen may have produced partially oxygenated intermediates which were more amenable to further oxidation with nitrate than the original hydrocarbon.

Sulfate reduction in the oxygen-limited sulfate-amended microcosms was similar to that observed in anoxic microcosms with strong evidence suggesting a linkage between sulfate reduction and bio-oxidation of condensate hydrocarbons. Sulfate reduction started only after a lag period of 222 days, which was longer than that observed in the corresponding anoxic experiment (lag of 122 days). About 700 mg/(kg aqueous phase) of sulfate was reduced in the sand microcosms and more than 2,000 mg/(kg aqueous phase) sulfate reduction was observed in the biotic, soil microcosms, both of which contained hydrocarbon. No sulfate reduction was observed in soil or sand microcosms that lacked hydrocarbon, or in sterile controls.

Fe^{2+} production was also observed after the 222-day lag period in the biotic microcosms amended with sulfate and hydrocarbon. The ratio of Fe^{2+}/Fe^{3+} increased from about 0.5 to 15.0 and from 1.0 to 10.0 in. soil and sand microcosms, respectively. Fe^{2+} production may have resulted from chemical reduction of Fe^{3+} by the sulfide produced by microbial sulfate reduction .[7]

In oxygen-limited microcosms with Fe^{3+} added as the electron acceptor, Fe^{2+} production and Fe^{3+} consumption were observed in the biotic—both soil and sand—microcosms with hydrocarbon added. The Fe^{2+}/Fe^{3+} ratio increased in 402 days from about 0.5 to 3.0 in the biotic soil microcosms and from 0.5 to 6.0 in 402 days in the biotic sand microcosms. No Fe^{2+} production or Fe^{3+} reduction occurred in the background soil or sand microcosms or sterile controls. This suggests that the presence of a limited amount of oxygen in the microcosms enhanced the utilization of Fe^{3+} as an electron acceptor, most probably by producing partly oxygenated intermediates from the hydrocarbons. Sulfate reduction was observed in the soil microcosms; however, the low amounts of sulfate in the sand microcosms precludes a similar conclusion in these microcosms.

Acetate accumulation was observed in the biotic, oxygen-limited sand microcosms with hydrocarbon added; however, no acetate production was seen in soil microcosms. Acetate concentration reached a peak of 400 mg/(kg aqueous phase) at 123 days and then declined to zero in 402 days.

In microcosms without an exogenous electron acceptor added, nitrate depletion followed by simultaneous Fe^{2+} production and sulfate reduction occurred in the oxygen-limited, biotic microcosms. No change was observed in the concentrations of electron acceptors in the background microcosms that lacked hydrocarbon or sterile controls. The Fe^{2+}/Fe^{3+} ratio increased in 402 days from 0.4 to 3.0 in the biotic soil microcosms amended with hydrocarbon and from 1.1 to 4.8 in the biotic sand microcosms amended with hydrocarbon.

Conclusions

Sulfate reduction was stimulated in the presence of gas condensate hydrocarbons under both anoxic and oxygen-limited conditions. A corresponding decrease in toluene was observed in the biotic, anoxic, and oxygen-limited soil microcosms concomitantly with sulfate reduction. Field observations show the depletion of sulfate from soil and ground water in contaminated areas. Nitrate and Fe^{3+} reduction occurred in anoxic microcosms; however, these could not be linked directly to hydrocarbon biodegradation due to the presence of an alternate carbon source in the soil. Nitrate was observed to inhibit microbial iron reduction; however, sulfate reduction occurred simultaneously with iron reduction.

Greater consumption of nitrate and Fe^{3+} was observed under oxygen-limited conditions in the presence of added hydrocarbon. This may be due to partial oxidation of hydrocarbons with the limited amount of oxygen present as the electron acceptor.

Acknowledgments

The authors wish to thank Dr. Joseph Suflita, Department of Botany and Microbiology, University of Oklahoma, for his comments and suggestions during the course of this work.

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Table 1 Baseline KPU2 Groundwater Analysis

Shallow	Average		Median		Minimum		Maximum	
	Control	Plume	Control	Plume	Control	Plume	Control	Plume
pH	6.92	6.95	7.27	6.81	6.00	6.74	7.38	7.43
Temperature, C	10.1	9.4	10.5	9.5	8.9	8.8	10.9	9.8
Sulfate, mg/L	246.3	0	233.3	0	200.0	0	294.4	0
Nitrate, mg/L	7.7	0.2	3.1	<0.4	1.3	<0.4	19.0	0.6
Fe^{2+}, mg/L	1.8	3.3	0.4	2.5	0.1	1.5	6.5	6.5
DO, mg/L	1.25	0.70	1.40	0.50	0.50	0.35	1.70	1.25
BTEX, mg/L	.003	14.2	ND	11.6	ND	8.9	.017	24.9
TPH, mg/L	ND	23.0	ND	20.5	ND	16.0	ND	35.0

Intermediate	Average		Median		Minimum		Maximum	
	Control	Plume	Control	Plume	Control	Plume	Control	Plume
pH	5.88	6.46	5.61	6.40	5.36	6.16	7.23	6.72
Temperature, C	10.4	11.2	10.9	11.0	9.2	10.5	11.2	12.0
Sulfate, mg/L	213.0	116.0	211.1	127.8	211.1	0.0	216.7	175.0
Nitrate, mg/L	20.2	1.9	21.7	0.8	16.8	<0.4	22.6	4.9
Fe^{2+}, mg/L	0.1	3.2	0.05	3.0	0.05	0.3	0.1	6.0
DO, mg/L	0.49	0.45	0.40	0.38	0.40	0.30	0.75	0.60
BTEX, mg/L	ND	1.673	ND	0.51	ND	0.11	ND	4.7
TPH, mg/L	ND	2.8	ND	2.0	ND	ND	ND	8.0

Deep	Average		Median		Minimum		Maximum	
	Control	Plume	Control	Plume	Control	Plume	Control	Plume
pH	6.25	6.71	5.83	6.62	5.15	6.54	7.47	7.00
Temperature, C	10.6	11.7	10.6	11.8	9.9	10.9	11.5	12.2
Sulfate, mg/L	206.0	215.0	206.3	218.8	188.9	187.5	216.7	240.5
Nitrate, mg/L	23.2	19.0	22.6	17.7	18.2	14.2	27.5	24.4
Fe^{2+}, mg/L	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DO, mg/L	0.87	0.53	0.75	0.50	0.45	0.30	1.35	0.95
BTEX, mg/L	ND	0.010	ND	ND	ND	ND	ND	0.052
TPH, mg/L	ND	ND	ND	ND	ND	ND	ND	ND

Table 2 Mineral and Electron Acceptor Analyses of the Solid Media, Saturated Soil Experiment

	Native Soil	Soil Inoculum	Ottawa Sand
Minerals			
Quartz	63%	63%	100%
Feldspar	23%	23%	0
Clays	10%	10%	0
Other	Dolomite, siderite, gypsum	Dolomite, siderite, gypsum	-
Leachable Electron Acceptors,* mg/kg (dry basis)			
Nitrate	11.8	0.0	0.0
Sulfate	85.2	49.2	0.0
Fe^{3+}	839.1	1024.4	39.5
Fe^{2+}	356.5	2362.2	20.8

**Leachable electron acceptors*: The solid media were extracted with an equal amount (by wt.) of 1 N HCl over a period of 48 hrs at room temperature without any shaking and the extract filtered and analyzed to obtain the *leachable electron acceptors.*

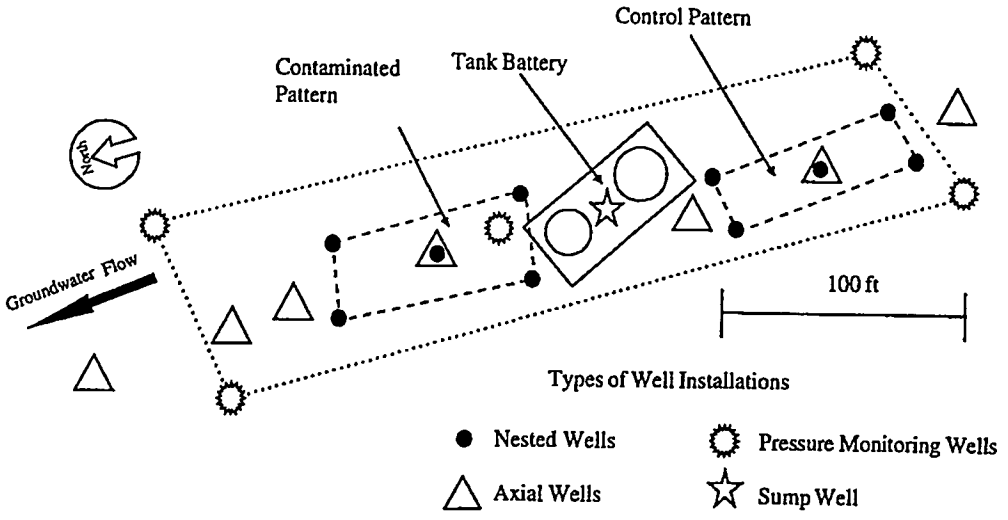

Figure 1 KPU-2 Site Map

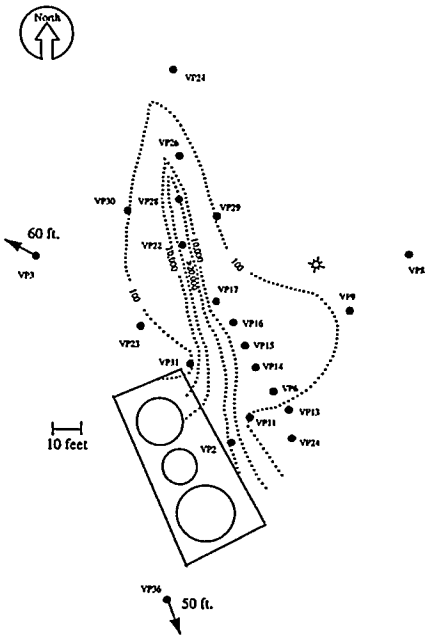

Figure 2 Results of the Soil Gas Survey for Volatile Organic Carbon (VOCs) at the KPU-2 Site. VOC Levels Are Given As PPM.

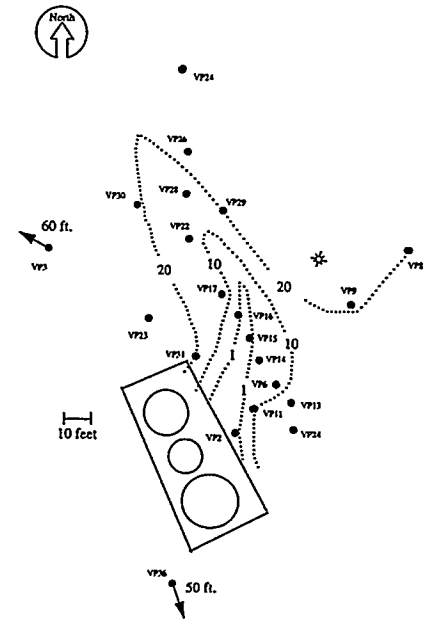

Figure 3 Results of the Soil Gas Survey for Oxygen at the KPU-2 site. Oxygen Levels Are Given As Volume Percent.

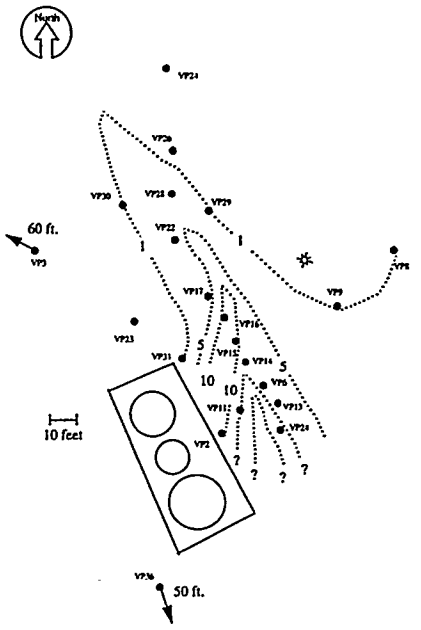

Figure 4 **Results of the Soil Gas Survey for Carbon Dioxide at the KPU-2 Site. Carbon Dioxide Levels Are Given As Volume Percent.**

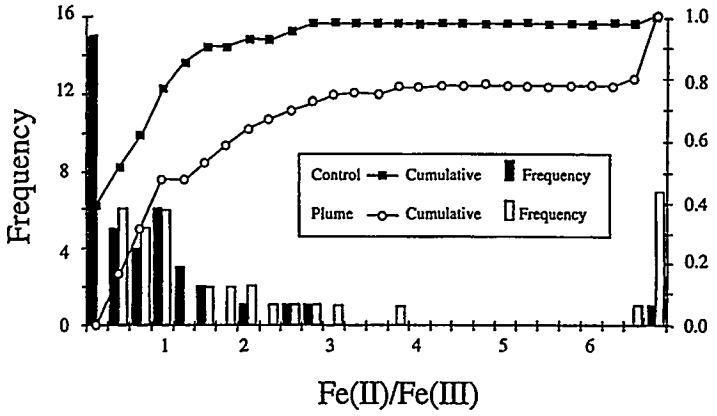

Figure 5 **Distributions of Fe^{2+} and Fe^{3+} in Soil Samples from the Plume and Control Areas of the KPU-2 site. The Right Axis Is the Cumulative Fraction of All Observations with Indicated Fe^{2+}/Fe^{3+} Ratios.**

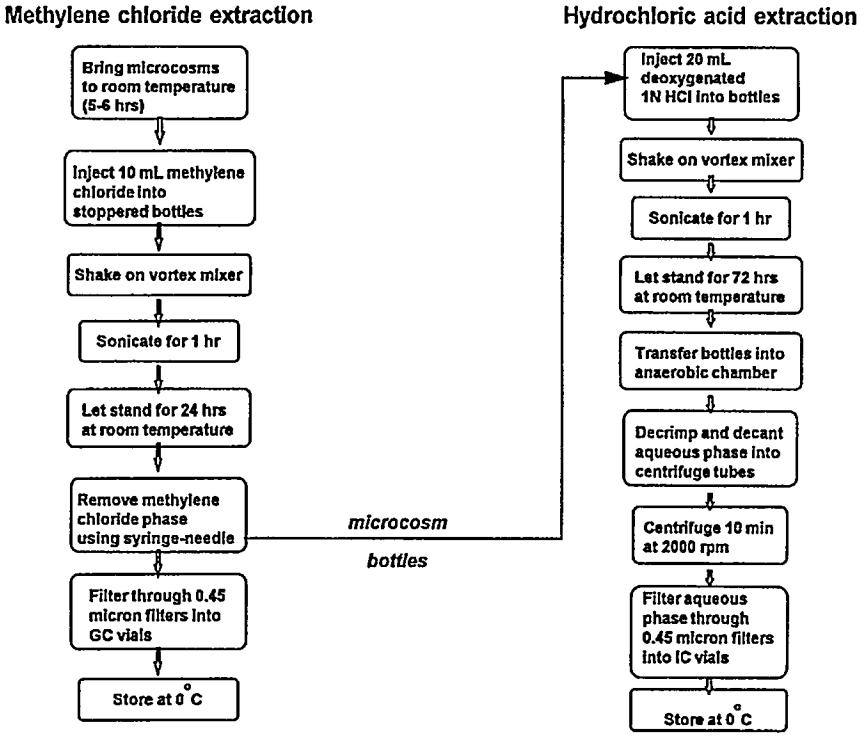

Figure 6 Experimental Protocol Used for the Analysis of Hydrocarbons and Electron Acceptors in Microcosms

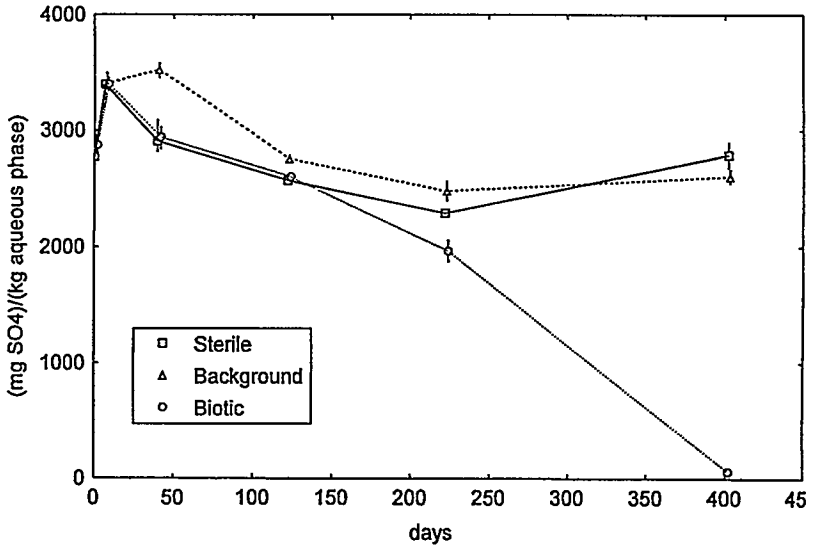

Figure 7 Sulfate Reduction in Anoxic Soil Microcosms with Exogenous Sulfate Added

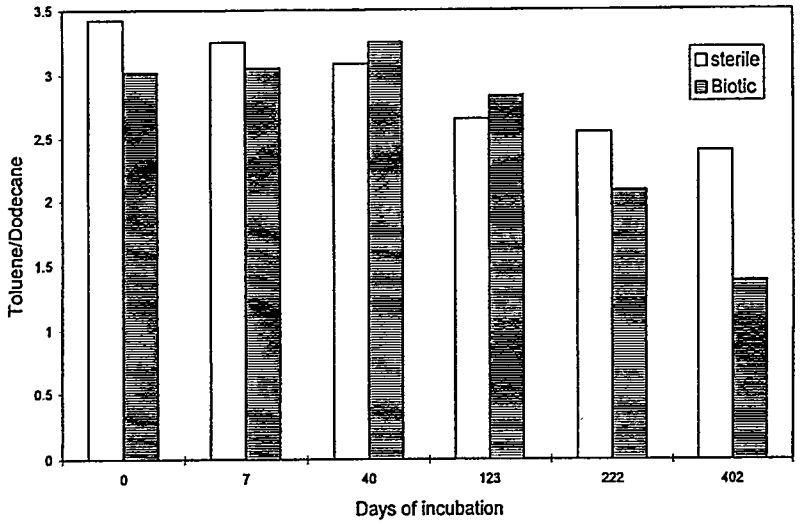

Figure 8 Ratio of Toluene to Dodecane in Anoxic Soil Microcosms with Exogenous Sulfate Added

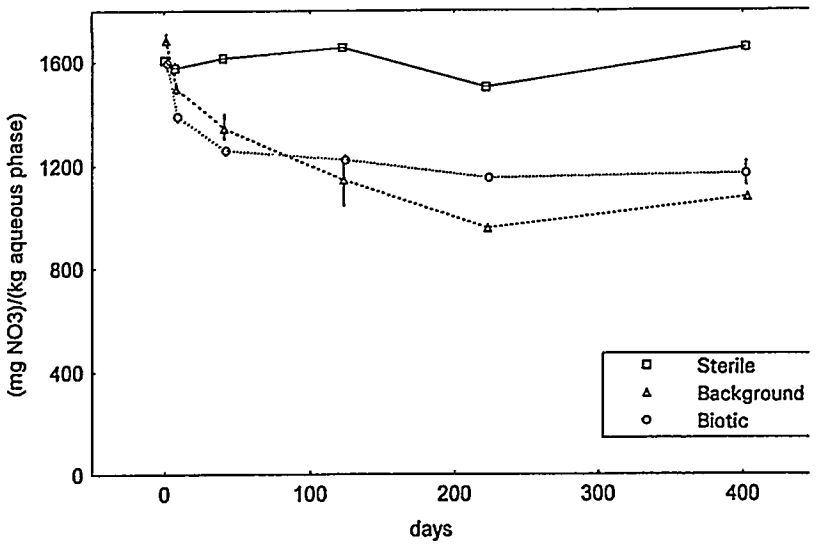

Figure 9 Nitrate Use in Anoxic Soil Microcosms with Exogenous Nitrate Added

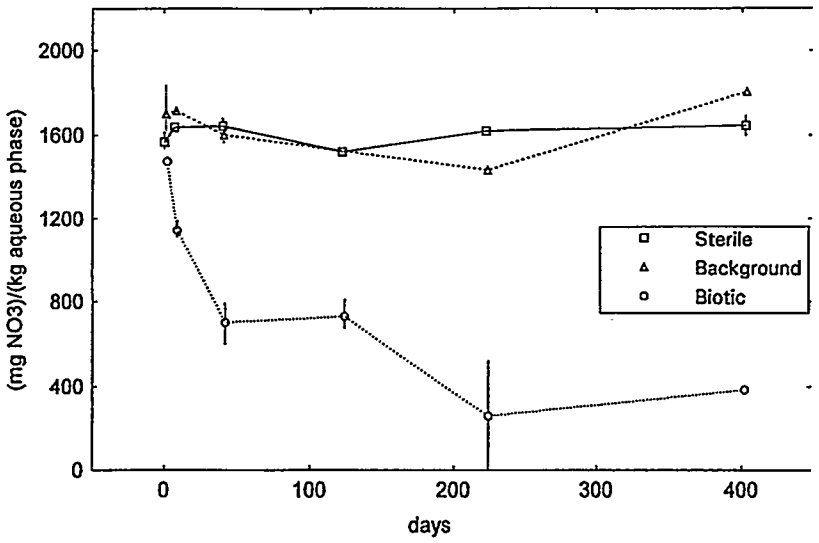

Figure 10 Nitrate Use in Oxygen-Limited Soil Microcosms with Exogenous Nitrate Added

FIELD TRIALS

A Commercial Microbial Enhanced Oil Recovery Process: Statistical Evaluation of a Multi-Project Database

J. T. Portwood
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Abstract

This paper discusses a database of information collected and organized during the past eight years from 2,000 producing oil wells in the United States, all of which have been treated with special applications techniques developed to improve the effectiveness of MEOR technology. The database, believed to be the first of its kind, has been generated for the purpose of statistically evaluating the effectiveness and economics of the MEOR process in a wide variety of oil reservoir environments, and is a tool that can be used to improve the predictability of treatment response. The information in the database has also been evaluated to determine which, if any, reservoir characteristics are dominant factors in determining the applicability of MEOR.

Introduction

Oil companies, both large and small, share a common problem—declining oil production and decreasing recoverable reserves. Producers need low cost, safe, effective technology to slow the rate of decline and add recoverable reserves. MEOR satisfies these requirements.

For years, factories have been manufacturing chemicals that have been used by producers in tertiary EOR projects to improve the mobility of oil and increase recoverable reserves. Specialized microorganisms also can manufacture large volumes of similar oil-mobilizing chemicals. Therefore, it can be said that the oil-release mechanisms associated with most MEOR processes are similar to those associated with traditional methods of tertiary EOR. Why then, shouldn't producers just continue using traditional technology? Because with the proper "know-how," microbes can be made to work cheaper, safer, smarter, and more effectively than traditional EOR processes. For example, conventional EOR chemicals can be referred to as "dead" materials, because whenever they are pumped into an oil reservoir, they depend on fluid flow to transport them to the desired locations. Often these chemicals "spend" prematurely, in the areas where they are least needed. Microbes, on the other hand, are alive and have motility which allows them to transport themselves through the aqueous phase of an oil reservoir, independent of fluid movement. Since the oil-mobilizing chemicals are produced only when the microbes are in the presence of oil, they spend in the areas where they are most needed.

Treatment strategies have been developed that afford MEOR microbes the highest probability that they will survive reservoir conditions and produce commercial amounts of oil-mobilizing chemicals. Data from the projects overwhelmingly substantiates that MEOR systems, when properly managed, can function effectively and economically in virtually any type of oil reservoir environment.

Background

Risk is inherent to the oil industry, but successful companies have learned how to manage it. Generally, the more data available, the lower the risk. Conversely, the less data available, the higher the risk. It has been difficult for producers to assign an accurate degree of risk to MEOR projects because commercial MEOR service companies have not made their statistical data available. Therefore, a database has been constructed of information collected from over 2,000 MEOR treated wells. The data have been organized to isolate ranges of individual reservoir characteristics like lithology, porosity, permeability, crude oil gravity, etc., so that they can be

compared to the corresponding average response in oil production observed after implementing MEOR. The database can be used as a tool to predict treatment response for any given reservoir type, and can also provide the oil producer with information needed to make informed, economic decisions when considering the feasibility of applying MEOR to an operation.

Mechanisms And Materials

Components of the MEOR Process

The projects in this report were all treated with special mixed cultures of naturally occurring aerobic and facultative microorganisms which, as a community, non-selectively metabolize a very wide range of hydrocarbons. In addition to microorganisms, inorganic growth stimulants and biocatalysts were used to enhance microbial activity in the reservoir environment. The inorganic growth stimulants consist of nitrogen, potassium, phosphorus, and trace elements and are used to supplement the microbes' natural diet of hydrocarbon. The biocatalysts increase the microbes tolerance to high salinity and maximize their ability to use available oxygen. In all cases, the MEOR materials were blended in water (usually lease brine or 2%–3% potassium chloride water) so that they could be pumped into the oil reservoir.

Oil Release Mechanism

The microorganisms used on the projects in this report utilize an oil-release mechanism which may be roughly summarized as follows. After the microbes are injected into the target reservoir, they transport themselves through the interstitial water and congregate adjacent to oil droplets trapped in pore spaces. The microbes then convert hydrocarbon molecules, located on the perimeter of the oil droplet, into organic solvent, surfactant, acid, and gas. These chemicals reduce oil viscosity, decrease interfacial surface tension between oil/rock and oil/water surfaces, and may also restore effective permeability to the near-wellbore by removing paraffin and scale blockage from perforations and pore throats. Finally, new microbial cells are produced and the process continues. More oil is produced because an incremental amount of previously immobile oil is mobilized. The process is applicable to waterfloods or single wells. The effect that each biochemical by-product has on the oil and the reservoir is summarized in Table 1.

Pre-MEOR Treatment Study

FIELD TRIALS

lowering the decline rate can extend the economic life of a well/reservoir and increase ultimate recovery.

Category 2: Figures 9 and 10 illustrate an initial increase in production rate after MEOR has begun, followed by a flatter decline pattern. This kind of response is more common to wells that are suspected to have paraffin and/or scale skin damage in the near-wellbore reservoir. It is theorized that the initial increase in production rate can be attributed to the microbes and/or their chemicals removing these suspected near-wellbore obstructions, and the flatter decline is an indication that additional oil is being mobilized and produced from deep within the reservoir.

Category 3: Figures 11 through 14 illustrate an initial increase in production rate after MEOR has begun, followed by a decline parallel to the historical decline trend. Like Figures 9 through 11, this kind of response is more common to wells that are suspected to have been cleaned, microbially, of paraffin and/or scale skin damage in the near-wellbore reservoir. The parallel decline suggests that MEOR has improved the relative permeability to oil in the near-wellbore area, and prevented the accumulation of new paraffin and/or scale deposits.

Category 4: Figures 15 through 20 illustrate how MEOR treatment can reverse the historical decline into a gradual incline in oil production, followed by a flat decline trend. This type of response is typical of many single-well MEOR projects conducted in pressure depleted reservoirs. This response is also common in microbial enhanced waterflood projects.

The MEOR Database

The MEOR database has been created using a Microsoft Excel Version 4.0 computer software program. Each project has been listed alphabetically by operator name, followed by project lease name, field name, location, recovery classification, and number of wells in the project. Pertinent reservoir data including lithology, porosity, permeability, depth, temperature, and pressure have been entered, as well as reservoir fluid properties such as oil gravity, brine salinity, and percent water cut. Incremental oil production resulting from MEOR has been entered for each project, expressed in barrels and as a percentage.

Results from Evaluation of Database

In order for a project to be considered in this statistical evaluation of the database, the following were required:

- A minimum of 12 months of consistent, historical pre-MEOR treatment oil production data
- A minimum of 12 months of post-MEOR treatment oil production data
- Documentation that incremental oil production includes only that oil produced as a result of MEOR, and not as a result of a workover, recompletion, increase in water injection rate or pressure, in-fill drilling, or any other outside variable

The information in the database was analyzed to determine how many of the projects responded to MEOR with a positive incremental increase in oil production of at least 5% or greater. According to the data, 81% of all projects undertaken have demonstrated arrested or decreased decline rate and production of incremental oil after initiating MEOR. The other 19% of the projects indicated that MEOR had no influence on oil production. No decrease in oil production has ever been observed as a result of this MEOR process. On average, MEOR causes an additional 36% more oil to be produced during the first 24 months of treatment, than would have otherwise been produced without this process. Normally, MEOR has a positive effect on oil production within 6 to 12 months of the first treatment.

Technological Evaluation

The database has been used to determine if any single reservoir characteristic is a dominant factor in determining the applicability of MEOR. Bar graphs have been constructed to illustrate the average incremental increase in oil production observed in various lithologies, porosities, permeabilities, oil gravities, formation temperatures, and water cuts.

Figure 21 illustrates that approximately 73% of all MEOR projects have been conducted in sandstone reservoirs and that 27% have been conducted in carbonate reservoirs.

Figure 22 suggests that reservoir lithology neither enhances or impedes the effectiveness of MEOR and should not be considered a limiting factor in its use.

Figure 23 indicates that as reservoir porosity increases, the percent incremental increase in oil production resulting from MEOR decreases. However, it should be noted that even in the highest porosity range, the average percent incremental increase in oil production has been nearly 20% and should, therefore, not be considered a limiting factor.

Figure 24 illustrates that as reservoir permeability increases, the percent incremental increase in oil production which can be expected from MEOR also increases. This has been generally true with the exception of reservoirs exhibiting average permeability in excess of 501 md. Since there have been only four recent MEOR projects conducted in reservoirs with permeability in excess of 501 md, it is possible that the lower average presented in this range is due to a lack of data. As more projects are conducted in this range and as more data is collected from current projects, it is anticipated that the average percent incremental oil increase will rise.

Figure 25 suggests that as oil gravity decreases, the percent incremental increase in oil production which can be expected from MEOR increases. Although no MEOR projects have been conducted in reservoirs containing sub 20° API gravity oil, the trend indicates that the best results from MEOR can be expected in this range.

Figure 26 indicates that reservoir temperature has not been a limiting factor with respect to MEOR. It appears that hydrocarbon-utilizing microorganisms can survive temperatures present in most oil reservoirs.

Finally, Figure 27 illustrates that the percent of total fluid produced in the form of water neither enhances nor impedes the effectiveness of MEOR and should not be considered a limiting factor in its use.

The data indicates that MEOR technology has been effective in a broad range of reservoir environments. It does not appear there is any single reservoir characteristic that is a dominant factor in determining the applicability of MEOR.

Economical Evaluation

An economic section of the database calculates the net value of the cumulative incremental oil produced from each project as a result of MEOR. The net value of the additional oil produced from MEOR has been calculated using the following equation:

$$\text{Net Value} = \text{bbl oil} \times \text{oil price} \times \text{net revenue interest} \times \text{production tax}$$

The producers Return on Investment (ROI) is calculated by comparing the net value of the incremental oil produced from each project to the cumulative cost of the MEOR process for the same period of time. ROI is calculated using the following equation:

ROI = cumulative net value of incremental oil ÷ cumulative MEOR cost

MEOR is affordable. According to the data, the average ROI to the producer has been 5:1 during the first 24 months of MEOR, and the average time to project payback has been six months. The average cost of MEOR has been less than $2.00 per incremental barrel of oil produced during the first 24 months of treatment and can be calculated using the following equation:

Cost per bbl oil = cumulative MEOR cost ÷ cumulative incremental bbl oil

Additional Benefits

There are other benefits and applications that have been realized from the MEOR process. Historically, production problems associated with paraffin, scale, corrosion, and emulsion have been reduced with conventional surfactant-based chemicals. Since hydrocarbon-utilizing microorganisms produce surfactant from metabolizing oil, it seemed reasonable to assume that these same problems would be reduced as a result of MEOR treatment. This assumption has been validated on numerous occasions, and MEOR is used by many producers as an alternative to traditional chemical treating techniques.

Saltwater disposal (SWD) and water injection wells (WIW) are especially prone to experience operational problems. Reduction of injectivity caused by oxygen, sulfate-reducing bacteria (SRB), iron sulfide, iron oxide, calcium carbonate, and oil carryover are common. However, improved injectivity and reduced operational problems at WIWs and SWDs have been observed during many of the MEOR projects that have been conducted.

The MEOR microbes, which are added to the water holding tanks, metabolize oil that is carried over with water from the separation equipment (see Fig. 28). Subsequently, surfactants are produced at the expense of the oil, so less oil is pumped down the SWD or WIW. The resultant surfactants are effective at solubilizing and removing precipitated iron sulfide scale and can also inhibit calcium carbonate scale formation. In addition, the surfactants make the water slicker, reducing friction and pressure during well injection/disposal.

The MEOR process inhibits iron sulfide formation in SWDs and WIWs because the hydrocarbon-utilizing microbes compete with SRB for the available nutrients. This competition for nutrients reduces the amount available to SRB and suppresses the production of hydrogen sulfide. Since many of the microbes used in the MEOR process are aerobic, they will scavenge free or dissolved oxygen present in many saltwater disposal and waterflood systems, and reduce the amount of oxygen available to corrosion.

The microbes and their surfactants, which are pumped down the well with the injected/disposed water, will eventually remove existing oily sludge and precipitated iron sulfide obstructions from the near-wellbore area, ultimately causing pressure to be reduced. To date, more than 100 SWDs and WIWs have been treated with the MEOR process. Routinely, injection pressure is reduced 15% to 50% with no negative effect on injection rate. Reduced injection pressure means less wear and tear on equipment and lower electricity cost. Figures 29 through 30 are water disposal/injection rate vs pressure graphs from actual projects and are presented to illustrate the response that can be anticipated after treatment of SWDs and WIWs.

MEOR Tax Incentives

MEOR has been qualified for Enhanced Oil Recovery/Improved Oil Recovery tax incentives currently offered by many states in the United States, and producers have successfully used MEOR to access these tax incentives. A few of the states currently offering these incentives are Alabama, Kansas, Louisiana, Mississippi, Montana, New Mexico, Oklahoma, Texas, Utah, and Wyoming.

Conclusions

1. The MEOR process has been commercially field tested in more than 2,000 producing oil wells in the U.S.

2. Statistical analysis of economic and technological data gathered from the broad use of this commercial technology provides producers with a tool to more accurately forecast the risked, economic potential of MEOR in any given reservoir.

3. The MEOR process contacts and mobilizes residual reservoir crude oil that would otherwise remain immobile and unrecoverable.

4. The MEOR process is safe for the oil field and the environment. It poses no threat to plants, animals, or humans.

5. The MEOR process has been observed to significantly reduce common operations problems associated with paraffin, emulsion, scale, and corrosion in producing wells; and improves injectivity in water disposal/injection wells by reducing problems associated with oxygen, sulfate-reducing bacteria (SRB), iron sulfide, iron oxide, calcium carbonate, and oil "carryover."

6. The MEOR process has been qualified for Enhanced Oil Recovery/Improved Oil Recovery tax incentives currently offered by many states in the United States and has been used successfully by producers to access these tax incentives.

7. MEOR treatment of single producing wells still in their primary phase of production and microbially enhanced waterflooding are economically and technologically feasible.

8. MEOR is easily applied, usually requiring little or no modification of existing production/injection equipment to accommodate it.

9. The status of MEOR can be easily monitored.

10. The MEOR process has been effective at incrementally improving oil production, and 81% of all projects undertaken have demonstrated a positive incremental increase in oil production after initiating this process.

11. On average, the MEOR process causes 36% more oil to be produced during the first 24 months of treatment than would have otherwise been produced without this process.

12. No decrease in oil production has ever been observed as a result of this MEOR process.

13. The MEOR process can function effectively in most oil reservoir environments.

14. The MEOR process produces quick results. The producers' average return on investment from MEOR has been 5:1 within the first 24 months of MEOR, and the average time to project payback has been six months.

15. The MEOR technology is affordable. The average cost of MEOR has been less than $2.00 per incremental barrel of oil produced during the first 24 months of treatment.

Acknowledgments

The author wishes to thank Alpha Environmental Midcontinent, Inc. for their permission to publish this paper, and Alpha Midsouth for their data contribution and permission to publish that data.

Table 1

By-Product	Effect
Solvent	•Dissolves in oil to reduce viscosity •Improves effective permeability by dissolving and removing heavy, long-chain hydrocarbons from pore throats
Surfactant	•Reduces interfacial tension between oil and rock/water surfaces
Acid	•Improves effective permeability by dissolving carbonate precipitates from pore throats •Localized etching of quartz and carbonate surfaces improves porosity and permeability. •Carbon dioxide produced from chemical reaction between acid and carbonate reduces oil viscosity and causes oil droplet to swell.
Gas	•Dissolve in oil to reduce viscosity •Encourages physical displacement of oil droplet by causing it to swell
New Microbial Cells	•Physically displace oil by growing between oil and rock/water surface

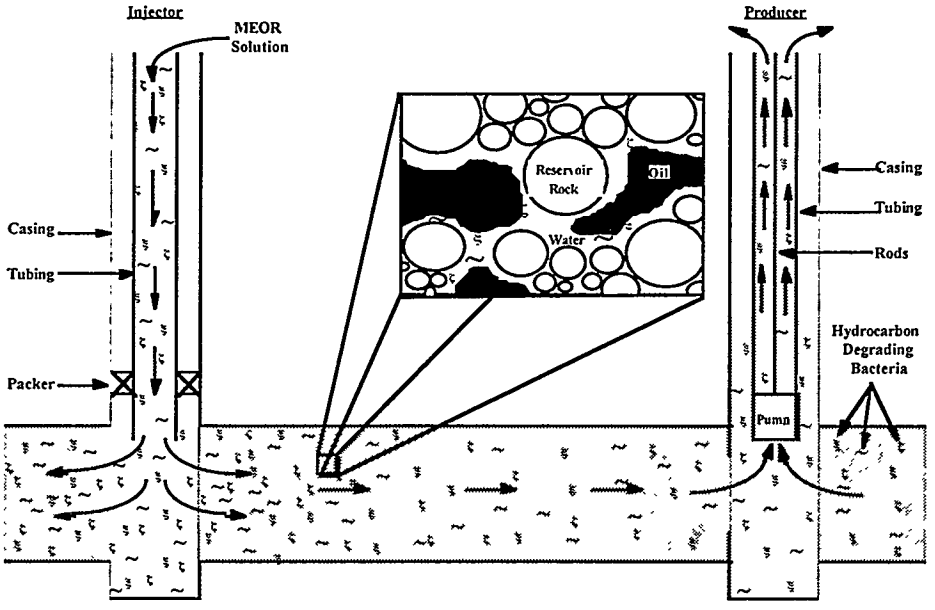

Figure 1 Schematic Diagram of Waterflood MEOR Treatment

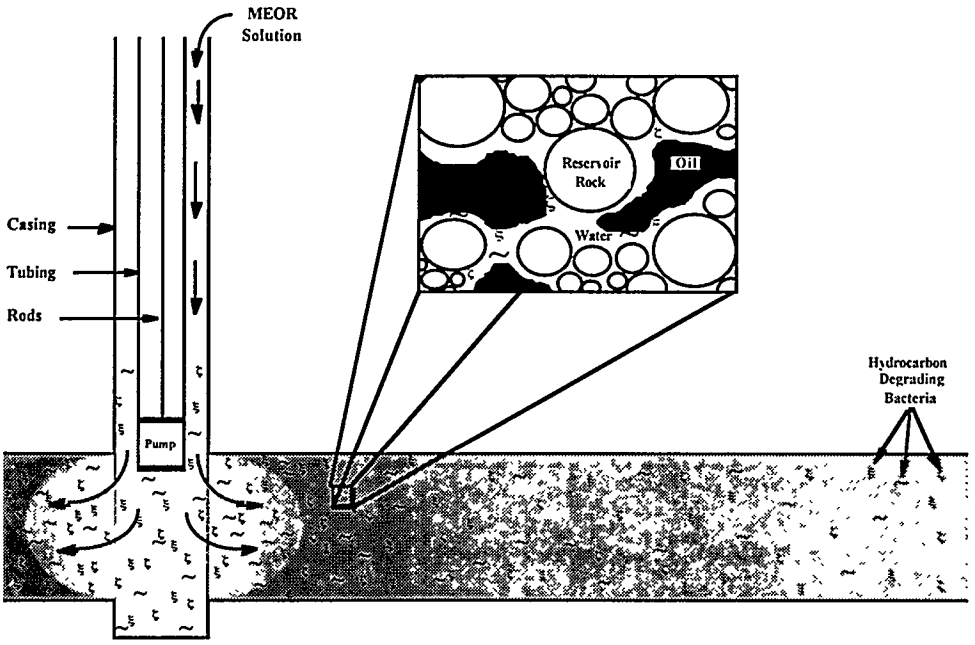

Figure 2 Schematic Diagram of Single-Well MEOR Treatment

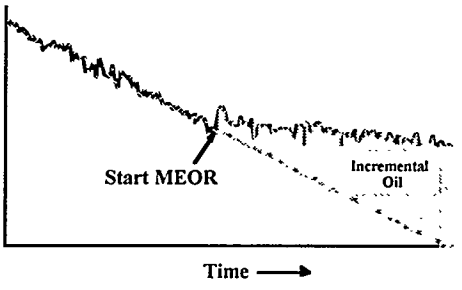

Figure 3 Example of a Production Graph Defining Incremental Oil Production

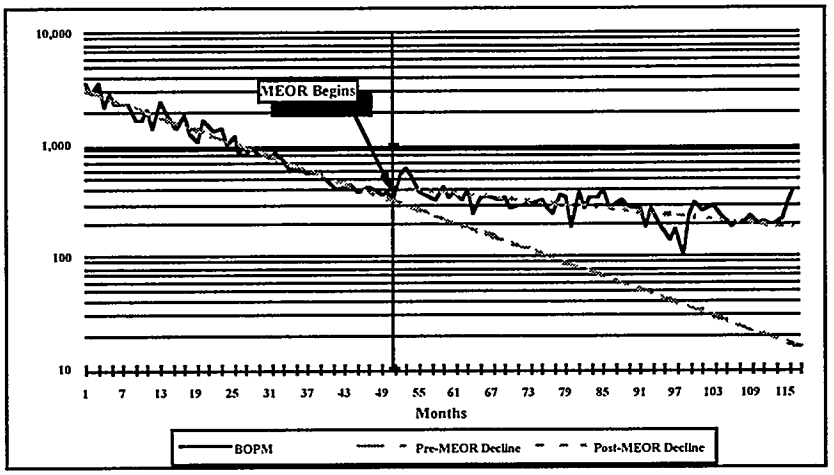

Figure 4 MEOR Waterflood—Wayne County, Illinois, Aux Vases Sandstone Formation Cumulative Incremental Oil = +11,730 bbl

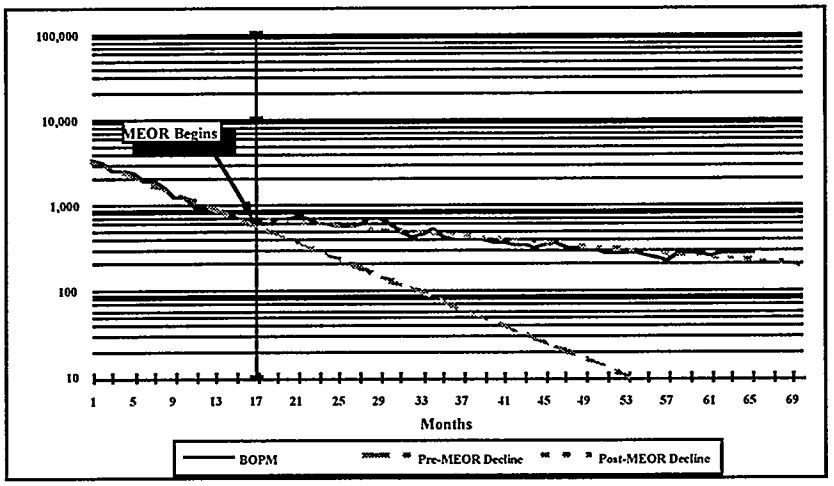

Figure 5 Single-Well MEOR—Grady County, Oklahoma, Medrano Sandstone Formation Cumulative Incremental Oil = +14,869 bbl

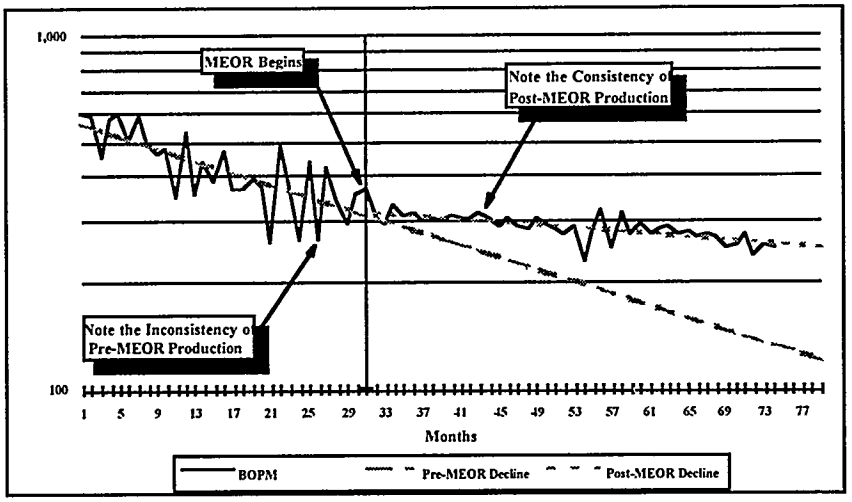

Figure 6 Single Well MEOR—Ochiltree County, Texas, Cleveland Sandstone Formation Cumulative Incremental Oil = +3,377 bbl

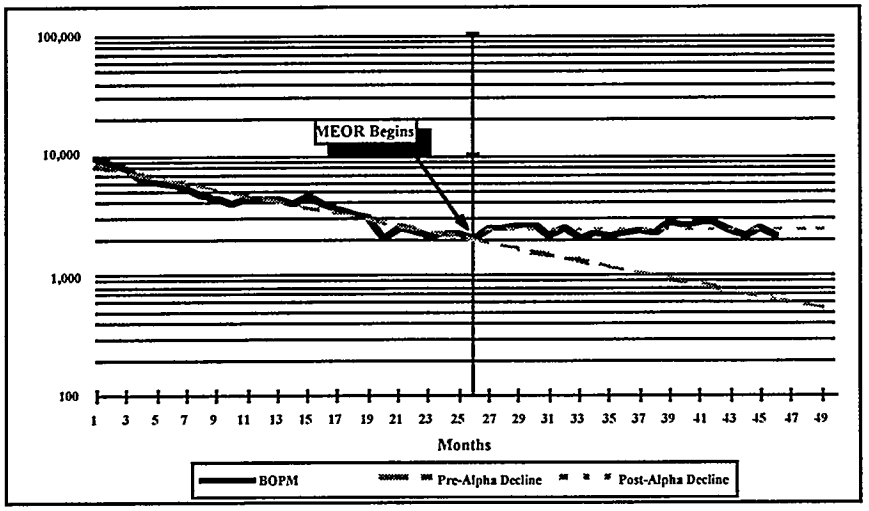

Figure 7 Single Well MEOR—Cheyenne County, Colorado, Morrow Sandstone Formation Cumulative Incremental Oil = +25,916 bbl

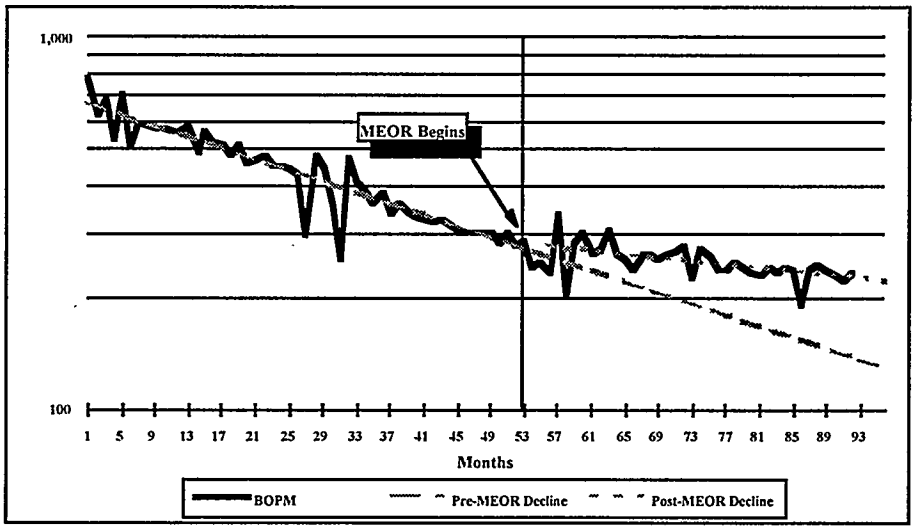

Figure 8 Single Well MEOR—Caddo County, Oklahoma, Marchand Sandstone Formation Cumulative Incremental Oil = +2,140 bbl

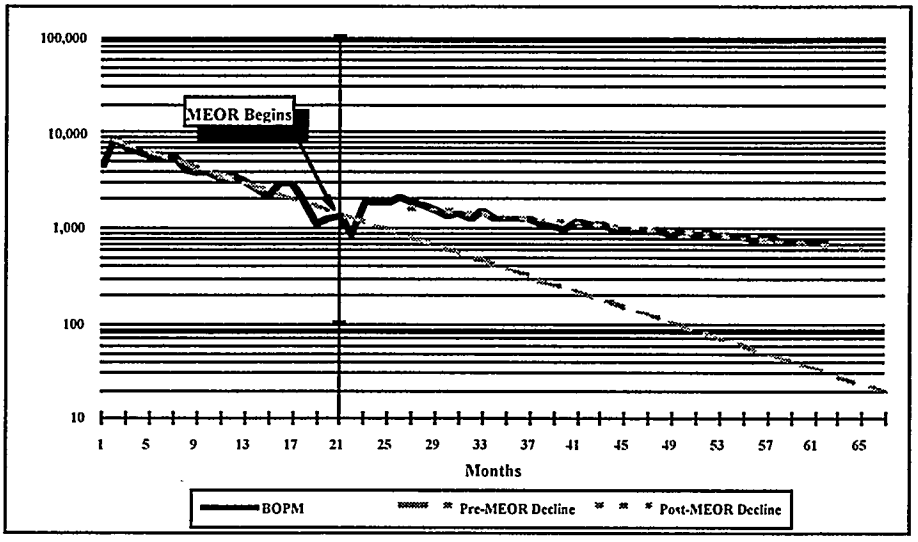

Figure 9 Single Well MEOR—Grady County, Oklahoma, Wade and Medrano Sandstone Formations Cumulative Incremental Oil = +33,069 bbl

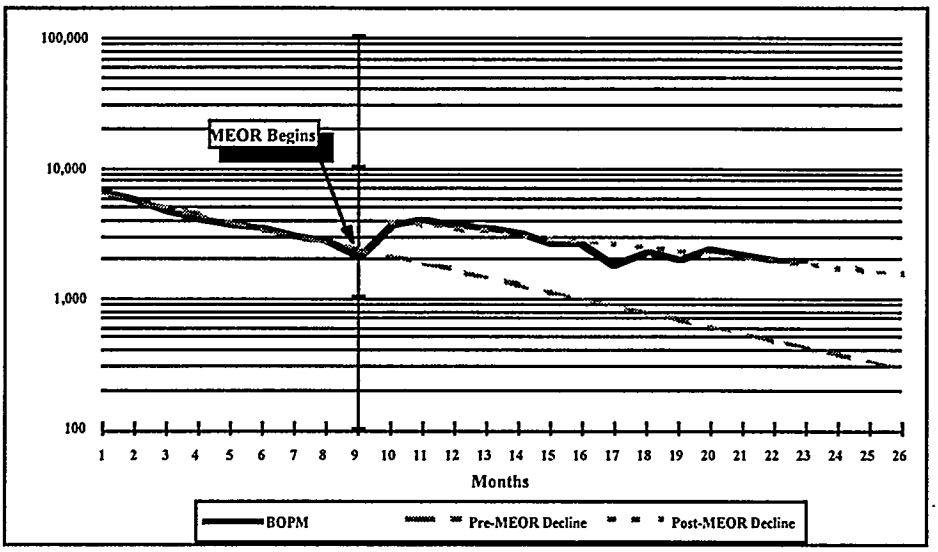

Figure 10 Single Well MEOR—Grant County, Kansas, Chester Limestone Formation Cumulative Incremental Oil = +23,136 bbl

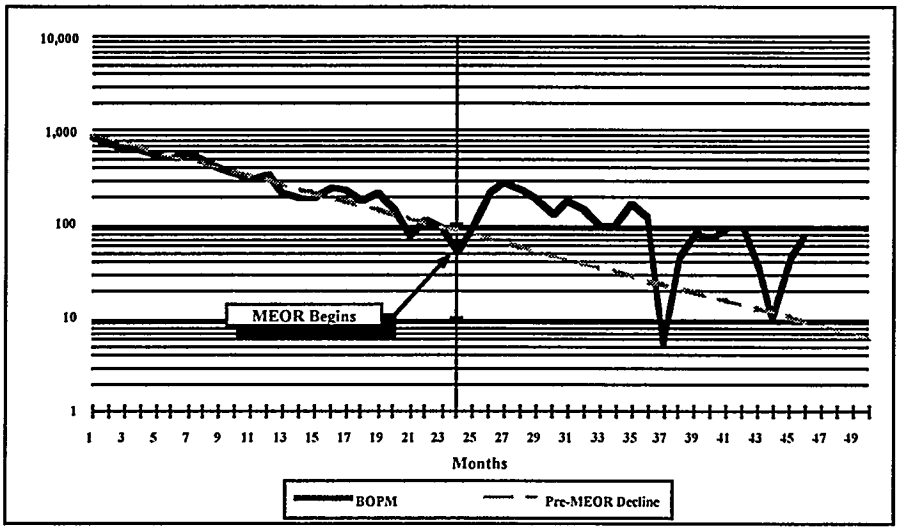

Figure 11 Single Well MEOR—Baca County, Colorado, Lansing Limestone Formation Cumulative Incremental Oil = +1,814 bbl

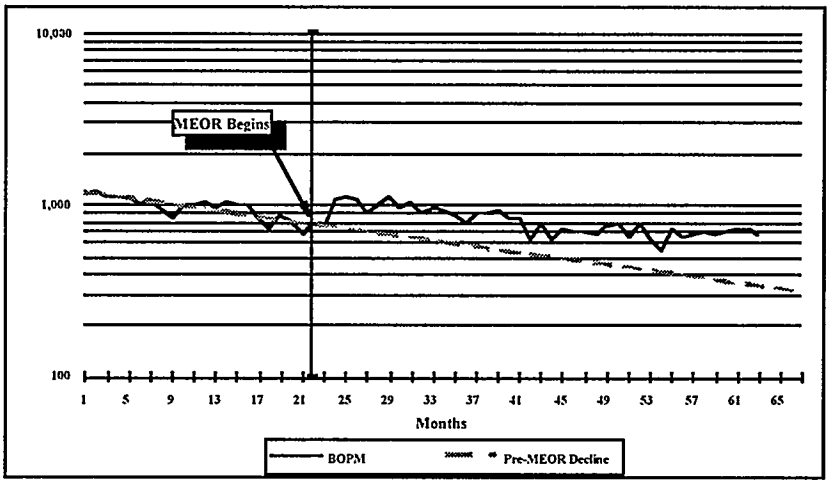

Figure 12 Single Well MEOR—Woodward County, Oklahoma, Oswego Sandstone Formation Cumulative Incremental Oil = +11,476 bbl

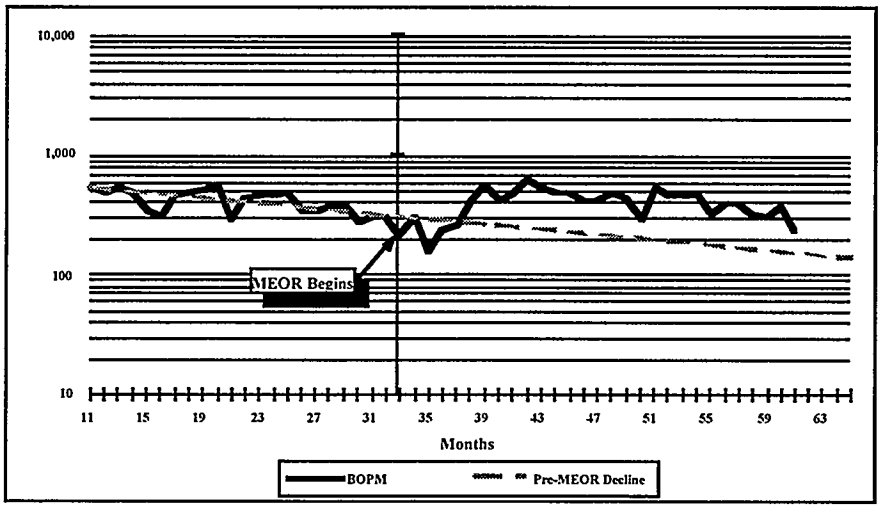

Figure 13 Single Well MEOR—Haskell County, Kansas, Lansing Limestone Formation Cumulative Incremental Oil = +5,363 bbl

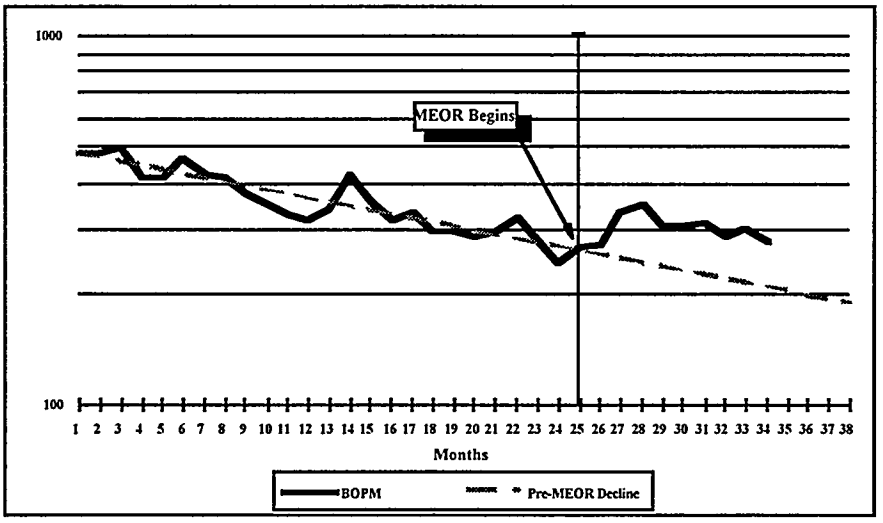

Figure 14 Single Well MEOR—Crook County, Wyoming, Muddy Sandstone Formation Cumulative Incremental Oil = +673 bbl

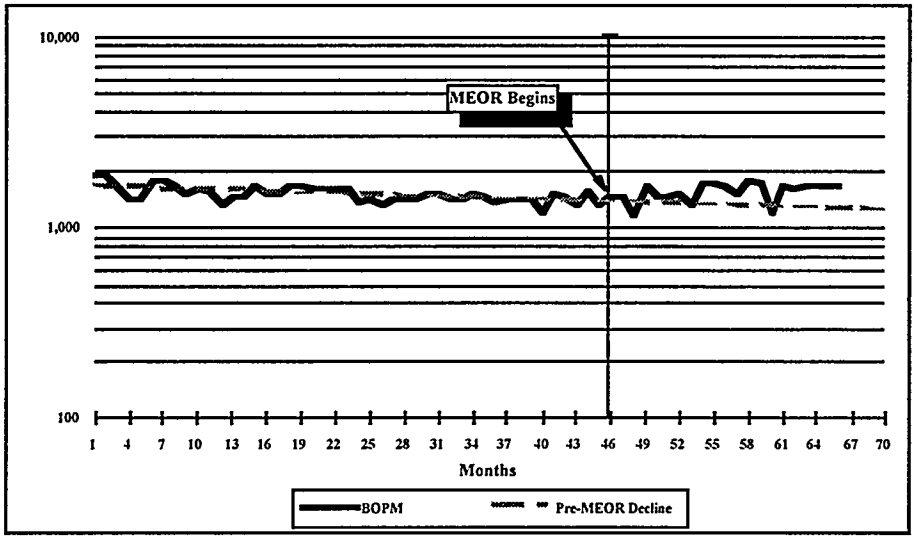

Figure 15 MEOR Waterflood—Union County, Arkansas, Blossom Sandstone Formation Cumulative Incremental Oil = +5,076 bbl

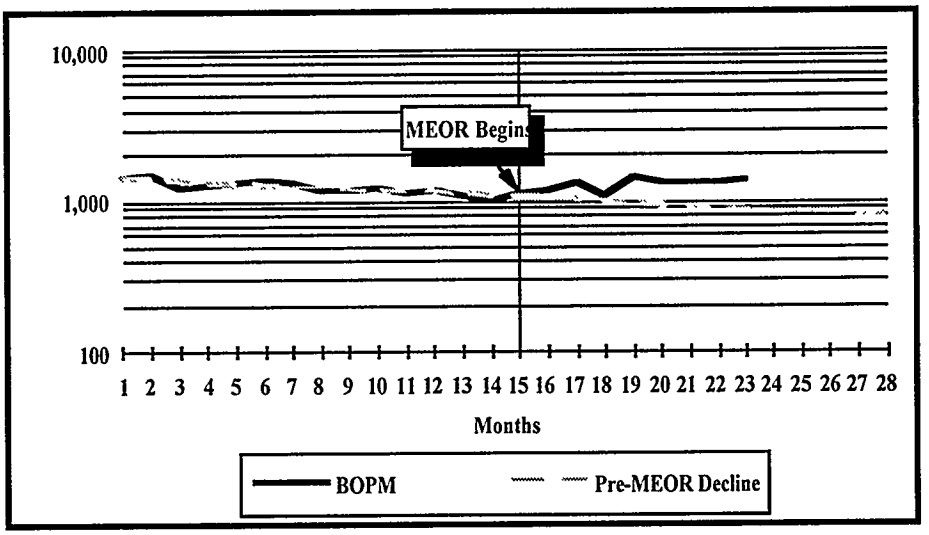

Figure 16 MEOR Waterflood—Carter County, Oklahoma, Tatums Sandstone Formation Cumulative Incremental Oil = +2,615 bbl

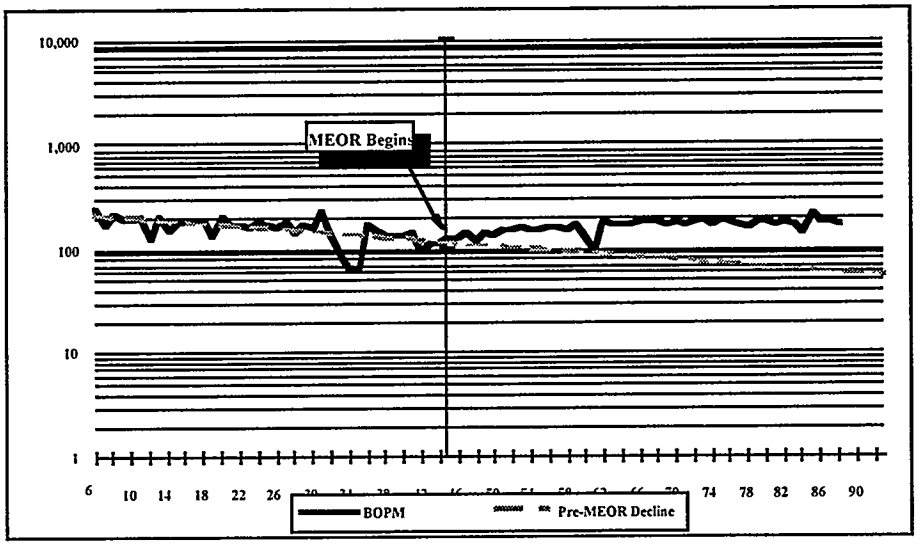

Figure 17 Single Well MEOR—Cleveland County, Oklahoma, Unconformity Sandstone Formation Cumulative Incremental Oil = +3,611 bbl

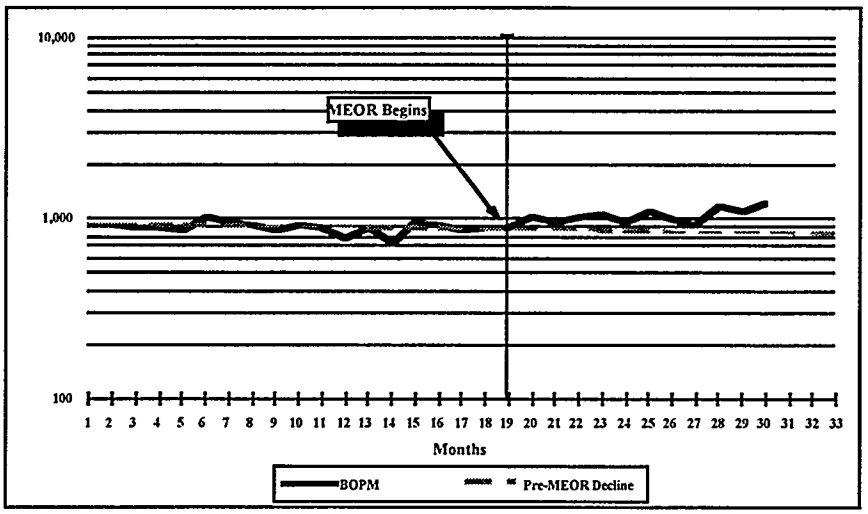

Figure 18 MEOR on 7 Single Well—Gray County, Texas, Granite Wash Formation Cumulative Incremental Oil = +2,220 bbl

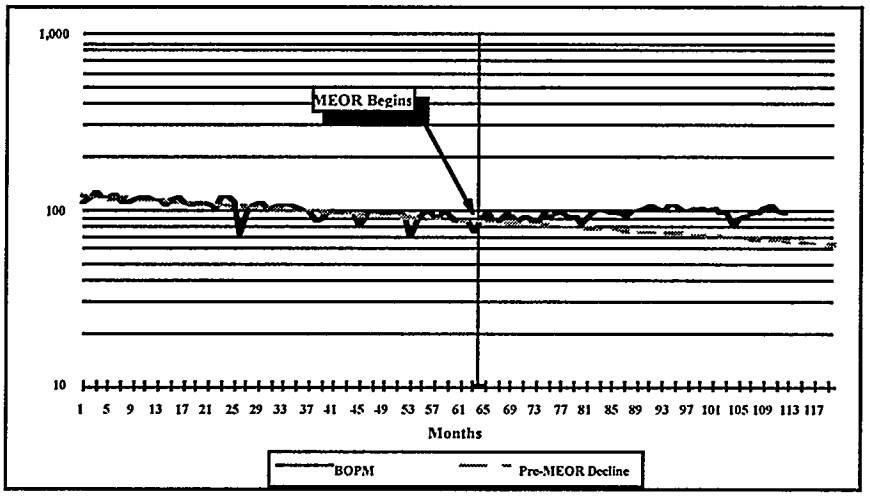

Figure 19 Single Well MEOR—Carter County, Oklahoma, Deese Sandstone Formation Cumulative Incremental Oil = +990 bbl

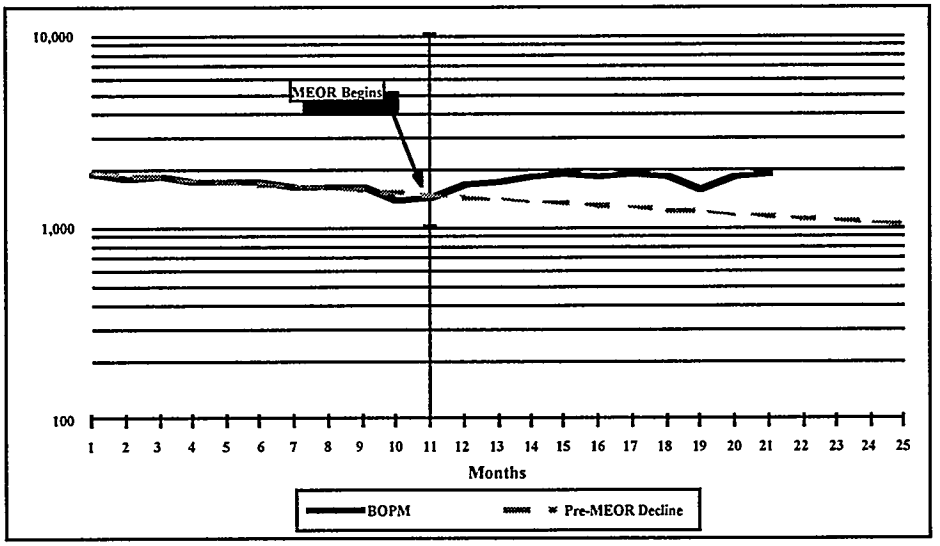

Figure 20 MEOR Waterflood—Johnson County, Wyoming, Curtis Sandstone Formation Cumulative Incremental Oil = +5,344 bbl

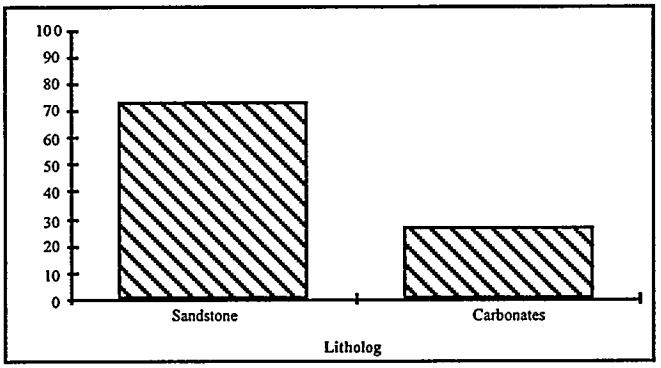

Figure 21 MEOR Projects in Sandstone (73%) vs. Carbonate (27%) Reservoirs

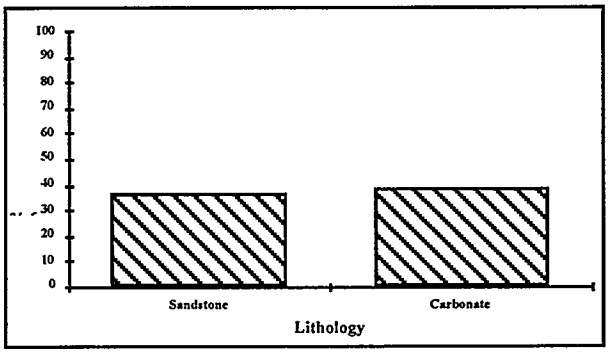

Figure 22 Reservoir Lithology Neither Enhances nor Impedes MEOR Use

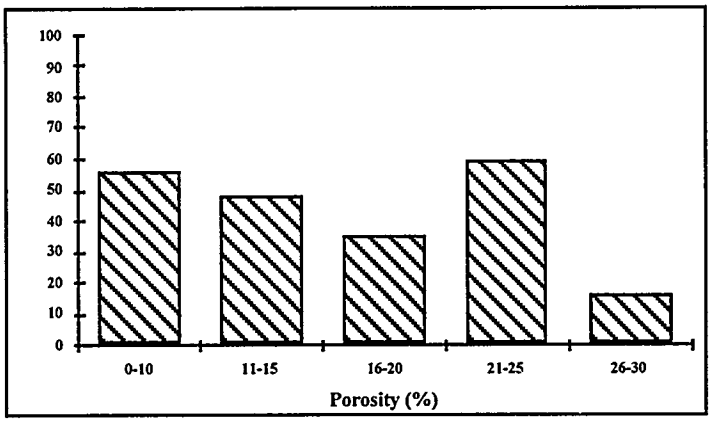

Figure 23 MEOR and Reservoir Porosity

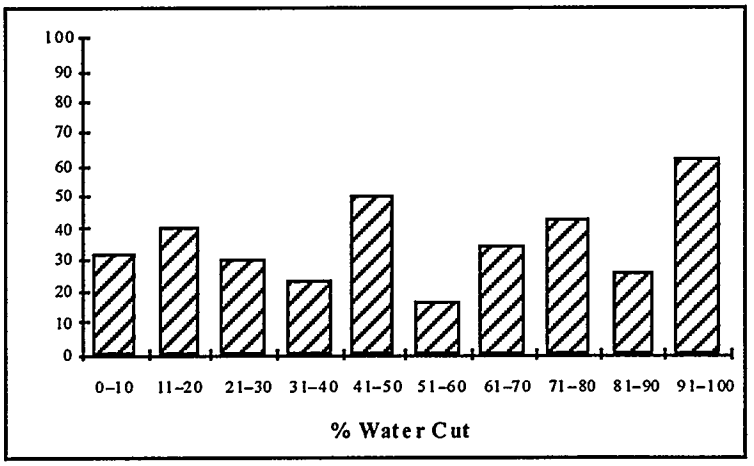

Figure 24 MEOR and Reservoir Permeability

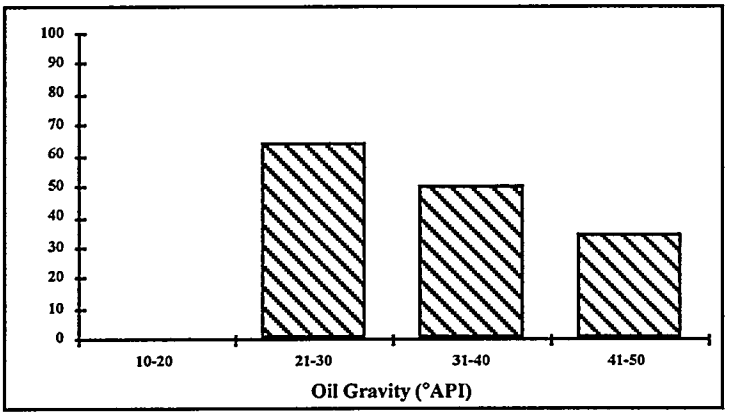

Figure 25 *No Projects in 10–20° API Gravity Oil

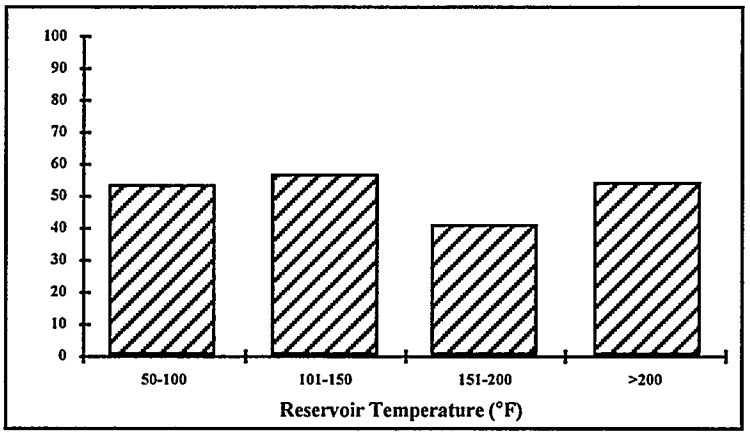

Figure 26 Reservoir Temperature Not a Limiting Factor in MEOR Use

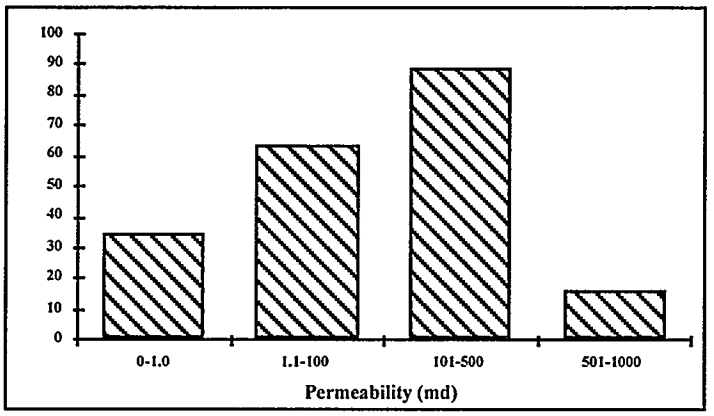

Figure 27 Percent of Total Fluid Produced in the Form of Water Neither Enhances nor Impedes MEOR Use

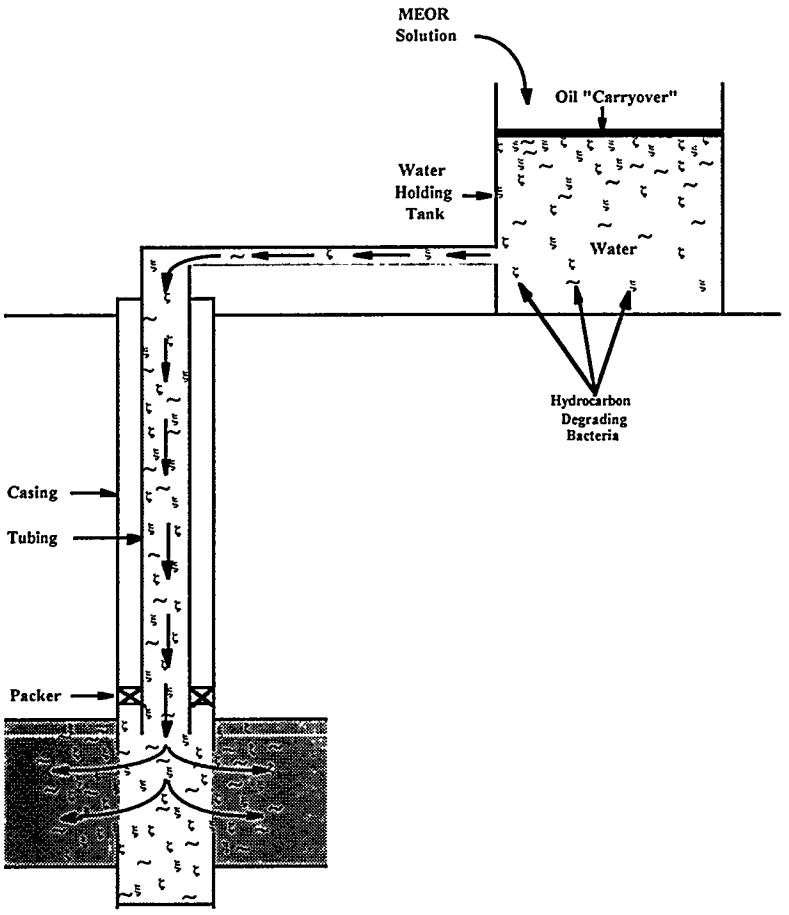

Figure 28 MEOR Microbes Added to Water Holding Tanks Metabolize Oil That Is Carried Over with Water from Separation Equipment

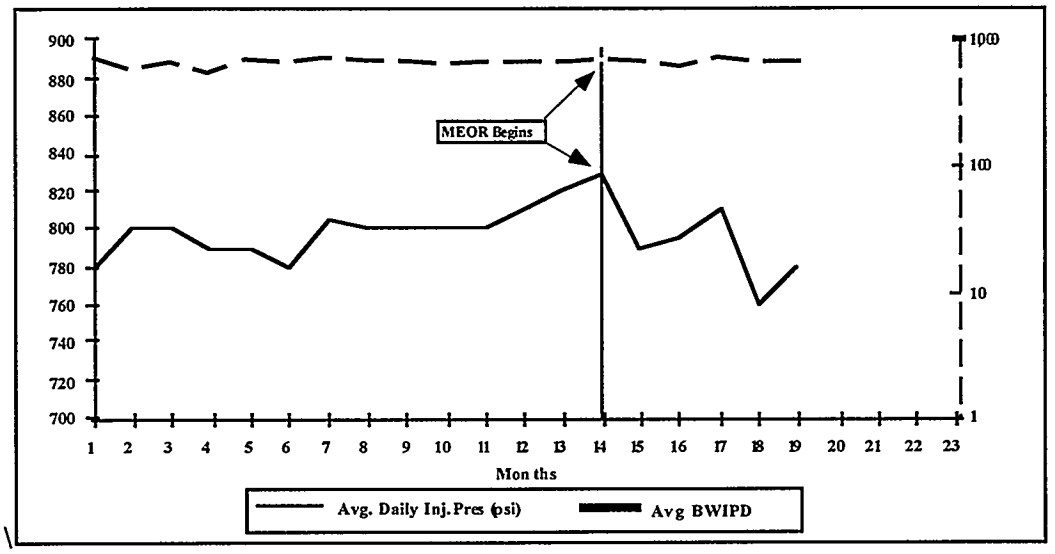

Figure 29 Water Injection Well Pressure Reduction—Johnson County, Wyoming, Curtis Sandstone Formation

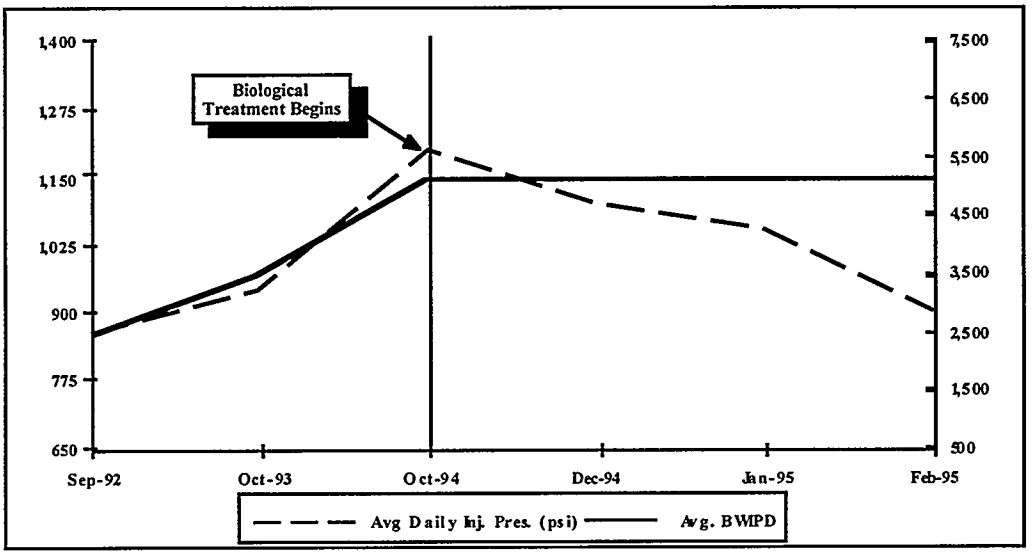

Figure 30 Water Disposal Well Pressure Reduction—Upshur County, Texas, Tokio and Blossom Sandstone Formations

Mississippi Exploration Field Trials Using Microbial, Radiometrics, Free Soil Gas, and Other Techniques

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Mississippi Office of Geology
Lewis R. Brown
Mississippi State University
Stanley C. Thieling
Mississippi Office of Geology

Abstract

The Mississippi Office of Geology has conducted field trials using the surface exploration techniques of geomicrobial, radiometrics, and free soil gas. The objective of these trials is to determine if Mississippi oil and gas fields have surface hydrocarbon expression resulting from vertical microseepage migration. Six fields have been surveyed ranging in depth from 3,330 ft to 18,500 ft. The fields differ in trapping styles and hydrocarbon type. The results so far indicate that these fields do have a surface expression and that geomicrobial analysis as well as radiometrics and free soil gas can detect hydrocarbon microseepage from pressurized reservoirs. All three exploration techniques located the reservoirs independent of depth, hydrocarbon type, or trapping style.

The Theory of Vertical Migration

Since this is one of the few papers given at this conference which deals with the microbial application to oil and gas exploration, a brief explanation of the theory of vertical migration is in order. The fundamental principle involved is that most oil and gas fields are leaking hydrocarbons through their seal and these escaping molecules rise to the surface of the earth. The mechanism responsible for the vertical migration is still debated by believers; skeptics say it doesn't happen at all. There are so many variables involved that the skeptics are right in part—in some areas, using certain techniques, it doesn't happen. But, thankfully, there are areas where microseepage is occurring, and there are techniques which can detect the microseepage or its effects. If one bears in mind that the rule of thumb for the pore throat size of unfractured shales below 5,000 ft is 50 Å (angstroms) and a methane molecule is 1.80 Å, ethane 3.33 Å, propane 4.86 Å, butane 6.39 Å, one can begin to accept the physical possibility of this process. If fracturing, even microfracturing, is included, the possibility for microseepage seems even more plausible. Once hydrocarbons escape the reservoir, they face what can be a very thick and complex geological section between the reservoir and the surface. Again, due to the extreme variations of this overlying geological section, some areas may never see those escaping molecules reach the surface while other geological settings seem to allow a remarkable degree of vertical migration.

Another factor that affects our ability to detect reservoirs at depth is the pressure in the reservoir. There are numerous cases documented which show that the surface expression weakens as the reservoir pressure decreases. Don't be surprised if you get little or no surface expression over depleted and/or abandoned fields.

Although modern geochemistry has made great advances, it cannot claim discovery of the vertical migration phenomenon or the techniques to detect surface microseepage. Those honors are due Russians and Germans working in the 1920s and 1930s. During that time Russians had developed and successfully used radiometrics and microbial analysis for locating oil and gas reservoirs. Germans were successful with free soil gas and even were able to predict whether the field would produce gas or oil based on molecular ratios of the free soil gases.

Study Objectives

The initial objective of this study was to determine whether oil and gas fields in Mississippi have detectable levels of hydrocarbons associated with vertical migration over the reservoirs. If that proves true, then reservoirs of differing

depths, products, and trapping styles would be surveyed to see if any of these factors affect surface expression.

The techniques used by the Mississippi Office of Geology are (1) geomicrobial as analyzed by Lewis Brown of Mississippi State University, (2) radiometrics, and (3) free soil gas. Dr. Brown's technique analyzes the microbial utilization of ethane, propane, n-butane, and isobutane.

Throughout the field trials the Mississippi Office of Geology has attempted to keep the test blind from the contractors. In the case of the microbial technique, the samples were collected by the Mississippi Office of Geology staff and shipped to Dr. Brown for analysis. Each single sample station was actually a combination of four samples located about 25 ft apart. Dr. Brown had no idea of where the samples were collected. When running radiometrics and free soil gas, Mississippi Office of Geology staff were present during all field operations.

Thus far, the Mississippi Office of Geology has run microbial analysis on six fields, free soil gas on four fields, and radiometrics on 14 fields. Due to the limited amount of sampling and analysis available to the Mississippi Office of Geology, the trials were conducted using single traverse lines in most cases.

Field Trial Results

Holly Grove Field (see Fig. 1) is located in Amite County, Mississippi. The field produces gas from the Frio Formation at a depth of 4,160 ft. The microbial analysis was able to locate the field. From Figure 1 one sees the microbial analysis showed a positive response over the known part of the field and extended the positive readings to the southeast. The radiometrics and free soil gas confirm the microbial indications. In this trend the use of microbial and radiometrics could be a very cost-effective method for identifying areas to shoot seismic for bright spot indications. Holly Grove Field is an excellent example where vertical migration is active and detectable at the surface. Because this area can be shown to have hydrocarbons at the surface, it is one of the areas that the Mississippi Office of Geology and NASA/Lockheed are studying together using hyperspectral analysis.

Berwick Field is also located in Amite County, Mississippi. It produces oil from the Tuscaloosa Formation at the depth of 11,800 ft. As seen in Figure 2, the microbial analysis identified the field with nearly equal shows updip and downdip of the field. In this stratigraphic play such a microbial signature would greatly aid in the exploration effort. Both radiometrics and free soil gas located the reservoir and both showed a slight updip shift. With all three methods giving positive results in this

area the field was included in the NASA/Lockheed-Mississippi Office of Geology hyperspectral study.

Freedom Field in Wilkinson County, Mississippi, produces Tuscaloosa oil from about 11,300 ft (see Fig. 3). This is another example of a stratigraphic trap where surface techniques point out the area of interest to the explorationist. Here the microbial method covers most of the field plus some updip area. The free soil gas shows a similar updip shift. Radiometrics picked up part of the field and missed part.

Johns Field in Rankin County, Mississippi, produces gas with 30% H_2S from the Smackover Formation at a depth of 18,500 ft. As shown in Figure 4 the field is a fault closure trap. Note how well the microbial analysis defined the fault and reservoir crest. The microbial shows to the north may be interpreted as a continuation of a single continuous show, or it may be interpreted as two possible areas of interest. Either way the microbial technique was identifying a gas reservoir at 18,500 ft. Radiometrics and free soil gas also were able to locate the field.

Peachtree Field is located in Clarke County, Mississippi. The field produces oil from the Cotton Valley Formation at a depth of 9,000 ft. The interpretation of the microbial survey shown in Figure 5 would certainly lead the explorationist to this area. If one were to review the raw data from the microbe survey, it would reveal good shows on the south portion of the survey line. These shows line up with the structural shift at the top of the Lower Cretaceous according to the operator's 3D seismic survey. The two existing wells indicate that the Lower Cretaceous has production potential in this area. It may be that Dr. Brown's interpretation actually covers the Cotton Valley production and the undrilled Lower Cretaceous sands south of the existing wells. Radiometrics also showed the location of this field.

Our last microbial survey was conducted over a recent Independence Field Frio gas well located in Wilkinson County, Mississippi. This well produces from a depth of 3,330 ft. As indicated on Figure 6, the microbial survey successfully located the reservoir and matches the seismic bright spot location.

Using this field as an example, let us consider the impact the microbial exploration technique could have in the Frio trend. If initial speculative surveys were run, with sample stations located every 330 ft and collected by company personnel, a mile of survey would cost about $650. If the resulting areas of interest were then sampled with an additional 20 samples, one could conceivably end up with a rough reservoir outline of the prospect with only $1,440 invested. In a play like the Frio of southwest Mississippi, where operators have used up most of the leads that can be developed from previously shot seismic, the cost-effective use of microbial exploration to focus expensive seismic acquisition seems promising. For operators

in the area such use would be a road less traveled by, but it could make all the difference in their world.

Conclusion

Based on the results so far, the Mississippi Office of Geology believes it has good evidence that some oil and gas fields in Mississippi have a surface hydrocarbon expression, and this surface expression can be detected using microbial surveys. Six fields which produce from depths of 3,330 ft to 18,500 ft, some oil producing and others gas, some being structural traps and others stratigraphic traps, were successfully located using microbial analysis of soil samples.

If these results are truly indicative of the capabilities of surface exploration techniques, one can easily imagine that using them in combination with other exploration technologies will result in lowering the finding cost in the future.

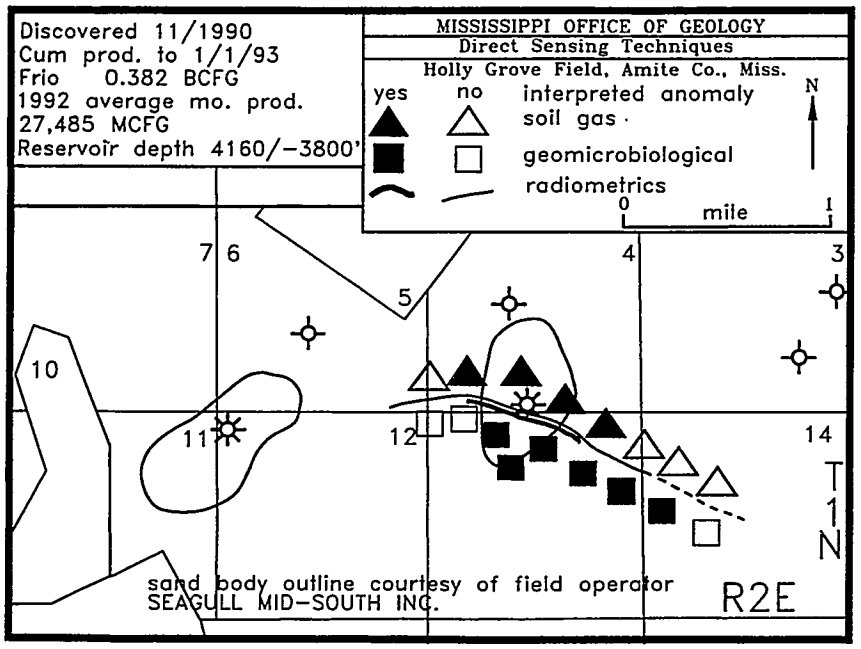

Figure 1 Summary Map of Holly Grove Field Comparing the Contractor Interpreted Results of Geomicrobial, Radiometric, and Soil Gas Surveys

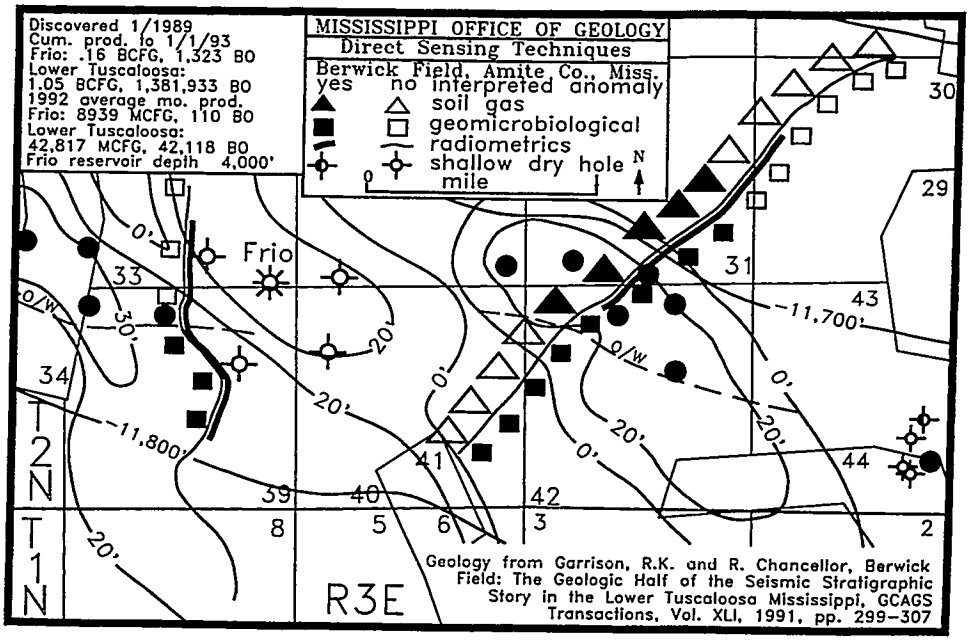

Figure 2 Summary Map of Berwick Field Comparing the Contractor Interpreted Results of Geomicrobial, Radiometric, and Soil Gas Surveys

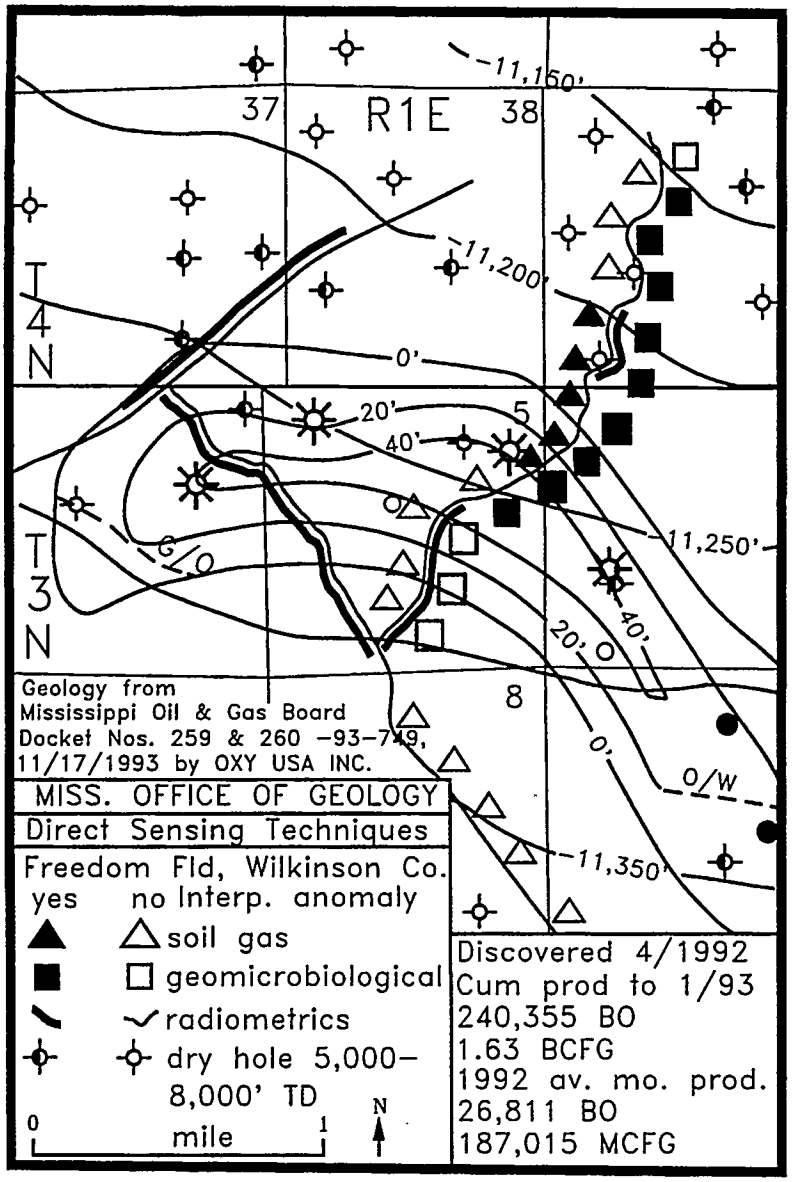

Figure 3 **Summary Map of Freedom Field Comparing the Contractor Interpreted Results of Geomicrobial, Radiometric, and Soil Gas Surveys**

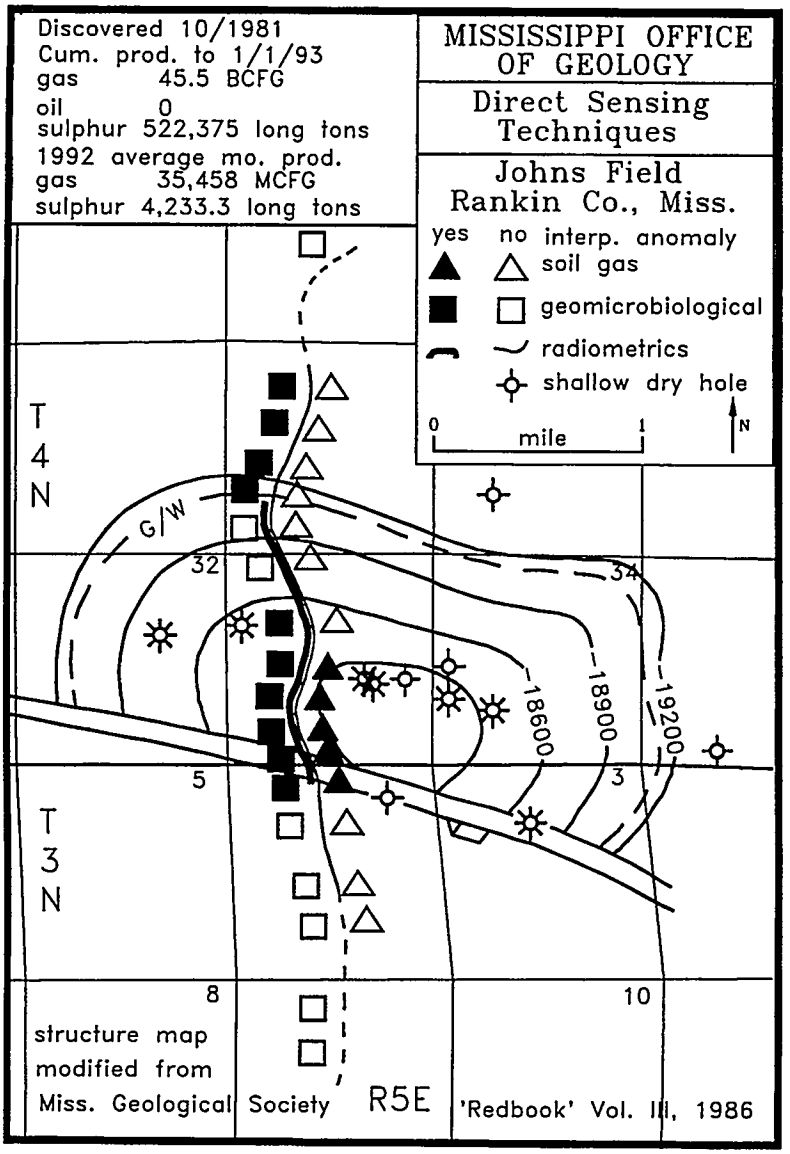

Figure 4 Summary Map of Johns Field Comparing the Contractor Interpreted Results of Geomicrobial, Radiometric, and Soil Gas Surveys

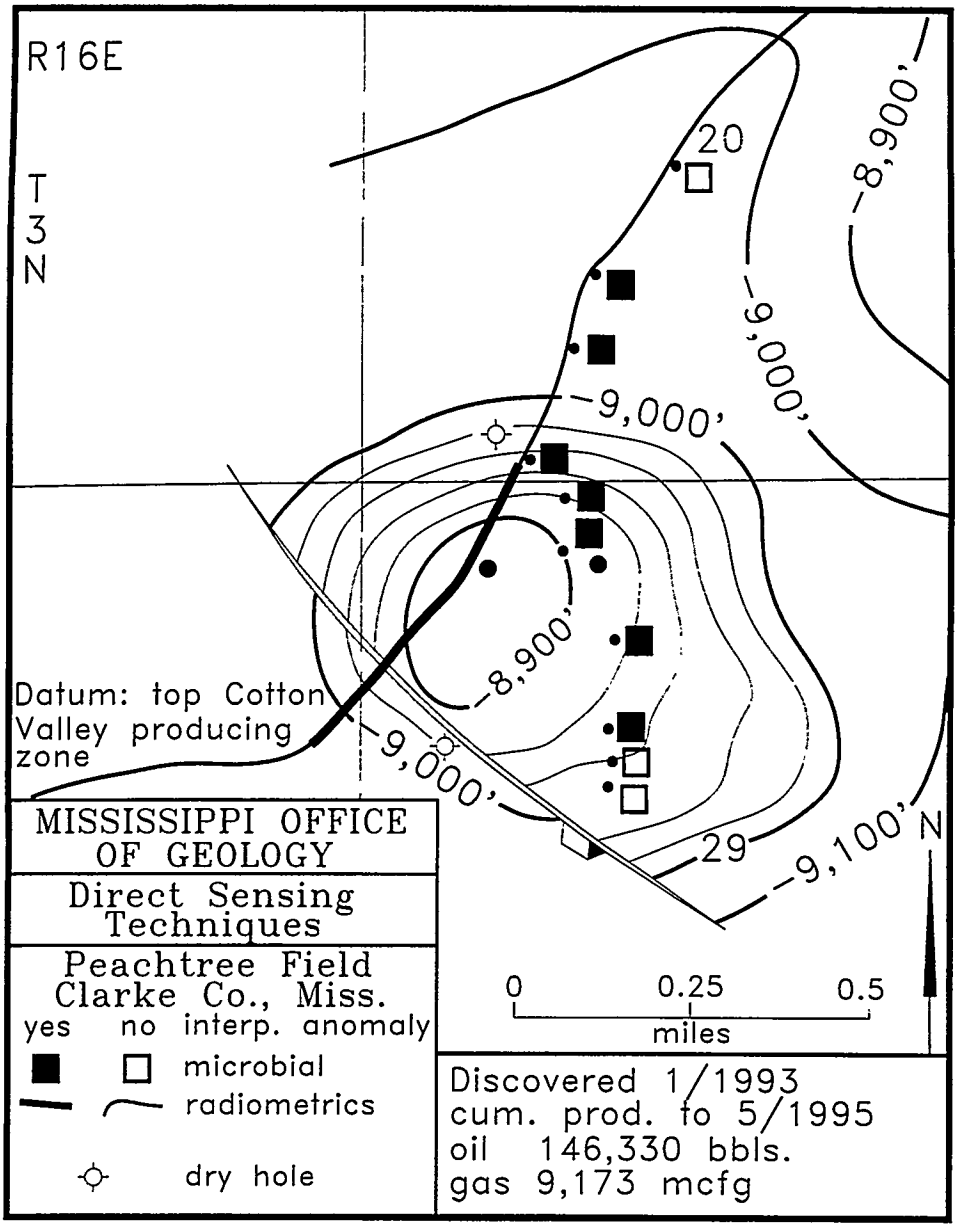

Figure 5 Summary Map of Peachtree Field Comparing the Contractor Interpreted Results of Geomicrobial, Radiometric, and Soil Gas Surveys

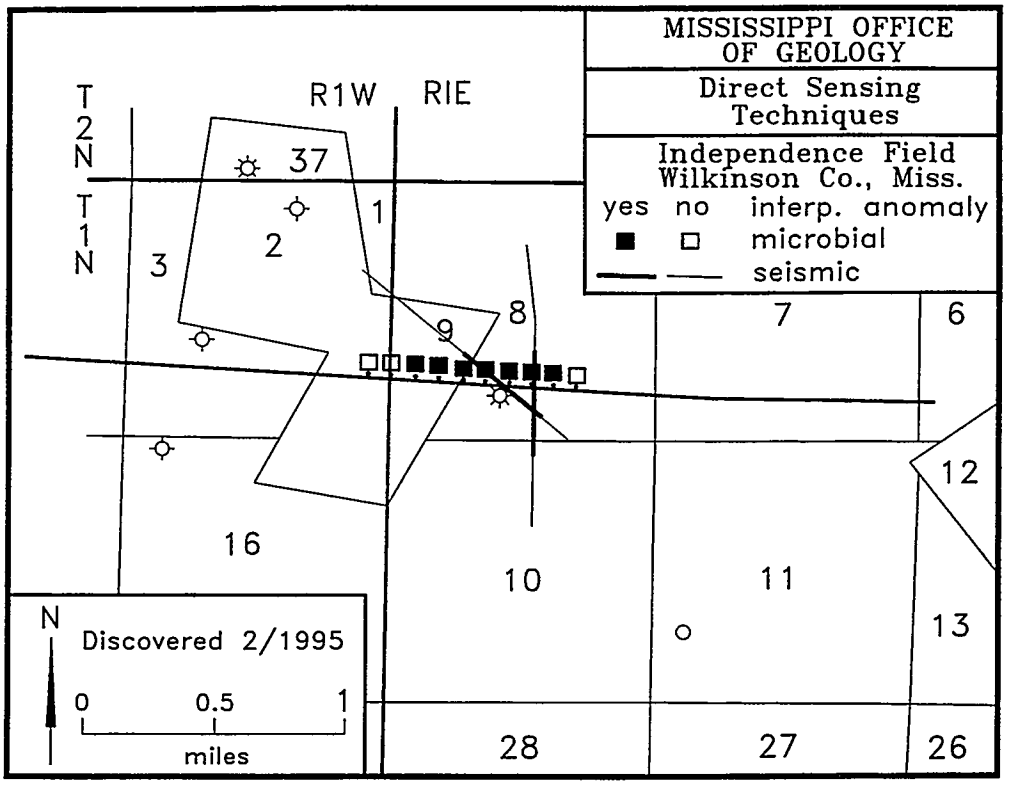

Figure 6 **Summary Map of Independence Field Comparing the Contractor Interpreted Results of Geomicrobial, Radiometric, and Soil Gas Surveys**

Oil Field Experiments of Microbial Improved Oil Recovery in Vyngapour, West Siberia, Russia

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Department of Oil and Gas Production, "Zapolyarneft"

Abstract

Experiments on microbial improved oil recovery (MIOR) have been performed in the Vyngapour oil field in West Siberia for two years. Now, the product of some producing wells of the Vyngapour oil field is 98–99% water cut. The operation of such wells approaches an economic limit. The nutritious composition containing local industry wastes and sources of nitrogen, phosphorus and potassium was pumped into an injection well on the pilot area. This method is called "nutritional flooding." The mechanism of nutritional flooding is based on intensification of biosynthesis of oil-displacing metabolites by indigenous bacteria and bacteria from food industry wastes in the stratum. 272.5 m^3 of nutritious composition was introduced into the reservoir during the summer of 1993, and 450 m^3 of nutritious composition—in 1994. The positive effect of the injections in 1993 showed up in 2–2.5 months and reached its maximum in 7 months after the injections were stopped. By July 1, 1994, 2,268.6 tons of oil was produced over the base variant, and the simultaneous water extraction reduced by 33,902 m^3 as compared with the base variant. The injections in 1994 were carried out on the same pilot area.

Introduction

Now nontraditional methods of improved oil recovery are being developed all over the world, since the traditional methods allow the recovery of this most significant geological source of energy at an average 45%. Nontraditional methods of oil recovery enable one to produce an additional 5%–10% of residual sources. These methods include microbiological ones, or MIOR.

Some microbial methods are based on injecting the sludges or wastes of biochemical or food industries into the formation to intensify the growth and biosynthesis of oil-displacing metabolites by microorganisms in situ. These sludges and wastes contain nutrient substances: sugars, proteins, vitamins, trace elements, and often some specific microorganisms. These technologies are developed in Russia, Tatarstan, Bashkortostan, Azerbaijan.[1–3]

Pilot tests of microbial improved oil recovery were performed in the Vyngapour oil field (Tyumen Region). The oil field is characterized by heterogeneous fractured low-permeable reservoir of 0.03–0.07 mkm^2 (which significantly complicates the conditions of its development), with high temperature (up to 94°C) and low mineralization of the formation waters. More than 90% of the reserves were timed to Jurassic and Lower Cretaceous deposits. The depth of bedding of the major productive stratum BV8 is 2,730–2,810 m. The stratum is presented by sandstones and aleurolites. The oil-saturated depth averages 5.3 m. The surface oils are sweet crude and low-resinousness (with a density of 822 kg/m^3). The Vyngapour oil field has been exploited since 1982, and is mainly worked out with the use of contour flooding.

Now the products of a part of producing wells on the Vyngapour oil field is 98–99% water cut. The operation of such wells approaches an economic limit; thus, the development and testing of MIOR is justified and up-to-date.

The aim of the pilot-field test (PFT) was to estimate the efficiency of the improved oil recovery biotechnology developed for the Vyngapour oil field (the nutritional flooding), which is based on intensification of biosynthesis of oil-displacing metabolites by indigenous bacteria and lactobacteria from food industry wastes in the stratum.

Materials and Methods

Field Data. PFT of the MIOR was carried out on the pilot area (cluster No 30) of Vyngapour oil field, Department "Zapolyarneft," the territory of Yamalo-Nenetsky

autonomous area and, partially, Nizhne-Vartovsky region of Khanty-Mansiysky autonomous area. The area of cluster 30 is 276 ha; it contains 4 injection and 13 producing wells. The average density of the well-net is 12.5 ha/well. The pilot area is 32.5 ha. It contains 9 wells (see Fig. 1). Three injection (726, 695, and 1859) and 3 producing (725, 754, 755) wells were operated in 1993 and 1994. The distances between injection well 726 and producing wells 725, 754, and 755 were 600, 1150, and 700 m, respectively.

Accumulated oil production in the pilot area from the beginning of development till the end of May 1993 was 653,486 tons. The coefficient of oil extraction was 0.351. By the end of May, the total daily oil output for the pilot area was 10 tons, the monthly one—206 tons; that of the water per month—6,632 m^3; that of the gas per month—37 m^3; the level of water-cut oil production—98–99%. This was a background for the beginning of the MIOR test (see Fig. 2). Well 726 was injected with the nutritious composition containing nitrogen and phosphorus sources and local food industry waste. Wells 695 and 1859 worked under common conditions.

Biochemical composition of the waste appeared to be very rich: sugars, free protein, bound protein, amino acids, pH = 4.5–5.0. Microbial investigation of the local food industry waste showed that the common quantity of lactobacteria makes 10^8–10^9 cell/ml. Besides, the nutritious composition contained nitrogen and phosphorus salts in concentrations of 0.5–1.5 and 1.0–1.5 g/l, respectively, in 1993. In 1994 KH_2PO_4 of the nutritious composition was replaced by KCl added in the amount of 0.001%. The salt $(NH_4)_2HPO_4$ was used in a concentration of 0.4% for buffering of the nutritious composition. Sometimes we had to use dry milk in a concentration of 0.04% and industrial inoculum of lactobacteria in amounts of 3 liters per 10 m^3 for the nutritious composition instead of the waste. In total, 60 kg of dry milk were used for the preparation of modified nutritious composition.

The work of producing wells was under regular microbiological and chemical control. The production of oil, petroleum gas, and simultaneous water and the formation pressure were registered in Department "Zapolyarneft." The efficiency of microbial improvement of oil recovery was analyzed by the STAT program developed at the All Russian Oil and Gas Research Institute with the account for special features of the Vyngapour oil field.

Results and Discussion

Pilot Field Test. The nutritious composition in the amount of 5, 10, or 20 m^3 was introduced into the bottomhole zone of injection well, and then the flooding was stopped for 24 hours. The operation was repeated to model the feed-batch

process in the natural bioreactor. Some microbial metabolites with oil-displacing properties (volatile fatty acids, alcohols, acetone, CO_2) were squeezed through the stratum by injected water from the injection well to the producing wells.

In 1993 two cycles of the nutritious composition injection into the formation were completed during the PFT. At the first cycle (2/06/93–10/07/93) 158.5 m^3 of nutritious composition was pumped into injection well 726. After that the well was included into the system of usual flooding for 40 days. During the second PFT cycle (20/08/93 –15/09/93) the same injection well was pumped with 114 m^3 of nutritious composition with the subsequent inclusion into the flooding system. Thus, the total of 272.5 m^3 of nutritious composition was pumped into one injection well of the pilot area.

Dynamics of oil production on the pilot area of the Vyngapour oil field before, during, and after microbial treatment are presented in Figures 2 and 3. Presented data show that the wells were not equally efficient after the injection of nutritious composition. Oil production per month from well 725 increased twice, from well 754—for 30% and from well 755—for 2%.

The enhanced oil recovery by July 1, 1994, after the PFT was 2,268.6 tons over the base variant (see Fig. 4). Water extraction reduced for 33,902 m^3 as compared with the base variant. The level of water-cut oil before PFT-93 was 98–99%, after PFT-93 nutritious flooding, the average of water-cut oil was 94.5%.

The positive effect of the MIOR in 1993 on pilot area of the oil field Vyngapour was manifested up to July 1, 1994, and an extra 3,231.7 tons of oil was expected to be produced by the end of 1994. However, there was a decrease of oil production during July–October 1994. This was probably caused by the fact that the injections of the nutritious composition were started in the end of August instead of the middle of May 1994, as had been planned previously.

The delay of PFT-94 was due to economical difficulties in obtaining the necessary components for the nutritious composition. The injection of the nutritious composition on this pilot area of the oil field Vyngapour was performed continuously from August 24 to October 3, 1994. The 450 tons of nutritious composition was injected into the formation during this period.

Unfortunately, we had to change some components of the nutritious composition, as shown above. Moreover, wells 725 and 754 were out of action by the end of 1994, and well 755 was stopped in September 1994. These wells have not yet been restored. So, it is impossible to evaluate the efficiency of MIOR on this pilot area in 1995. The difficult economic situation in Russia severely complicates the

performance of MIOR tests and all the more their wide application in spite of the positive results obtained.

Conclusion

The nutritional flooding resulted in decrease of water-cut oil on the pilot area on the average 3%, improvement of oil recovery for 2,268.6 tons, reduction of simultaneous water extraction for 33,902 m^3 in the 7 months following PFT-93.

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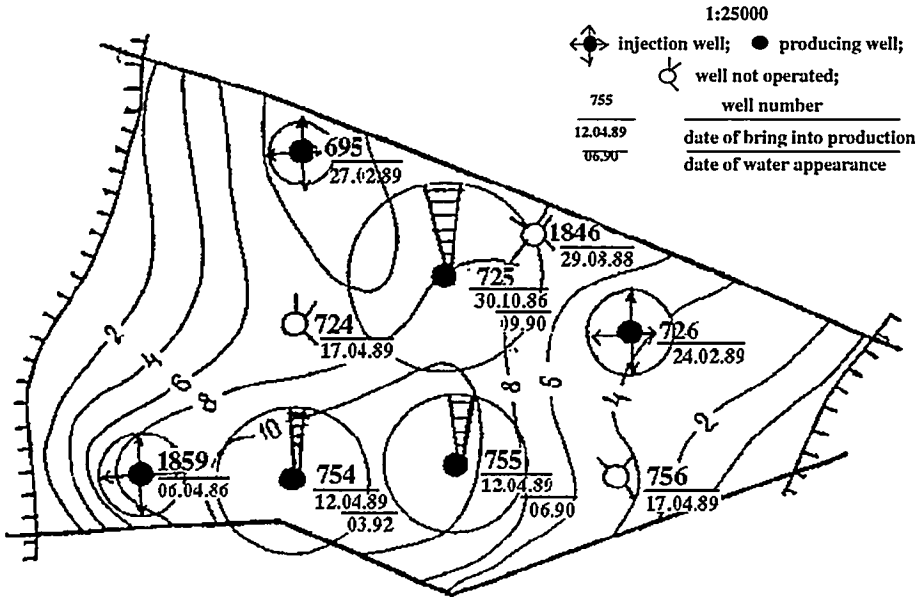

Figure 1 Map of Pilot Area of the Vingapour Oil Field for Microbial Treatment

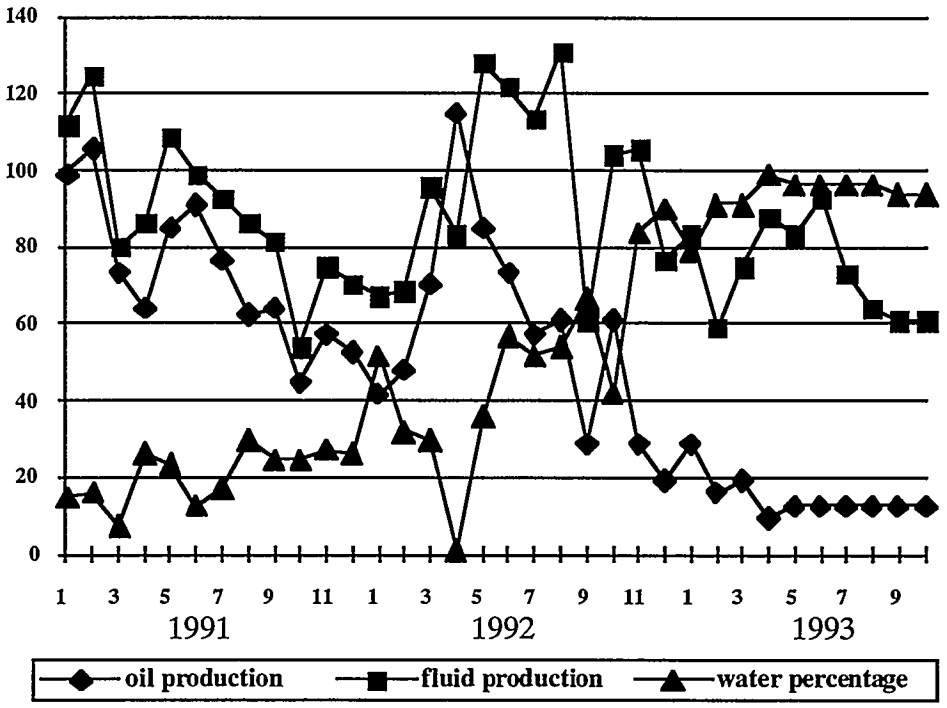

Figure 2 Dynamics of Oil and Fluid Production and Water Cut Oil on Pilot Area of the Vyngapour Oil Field (Cluster 30)

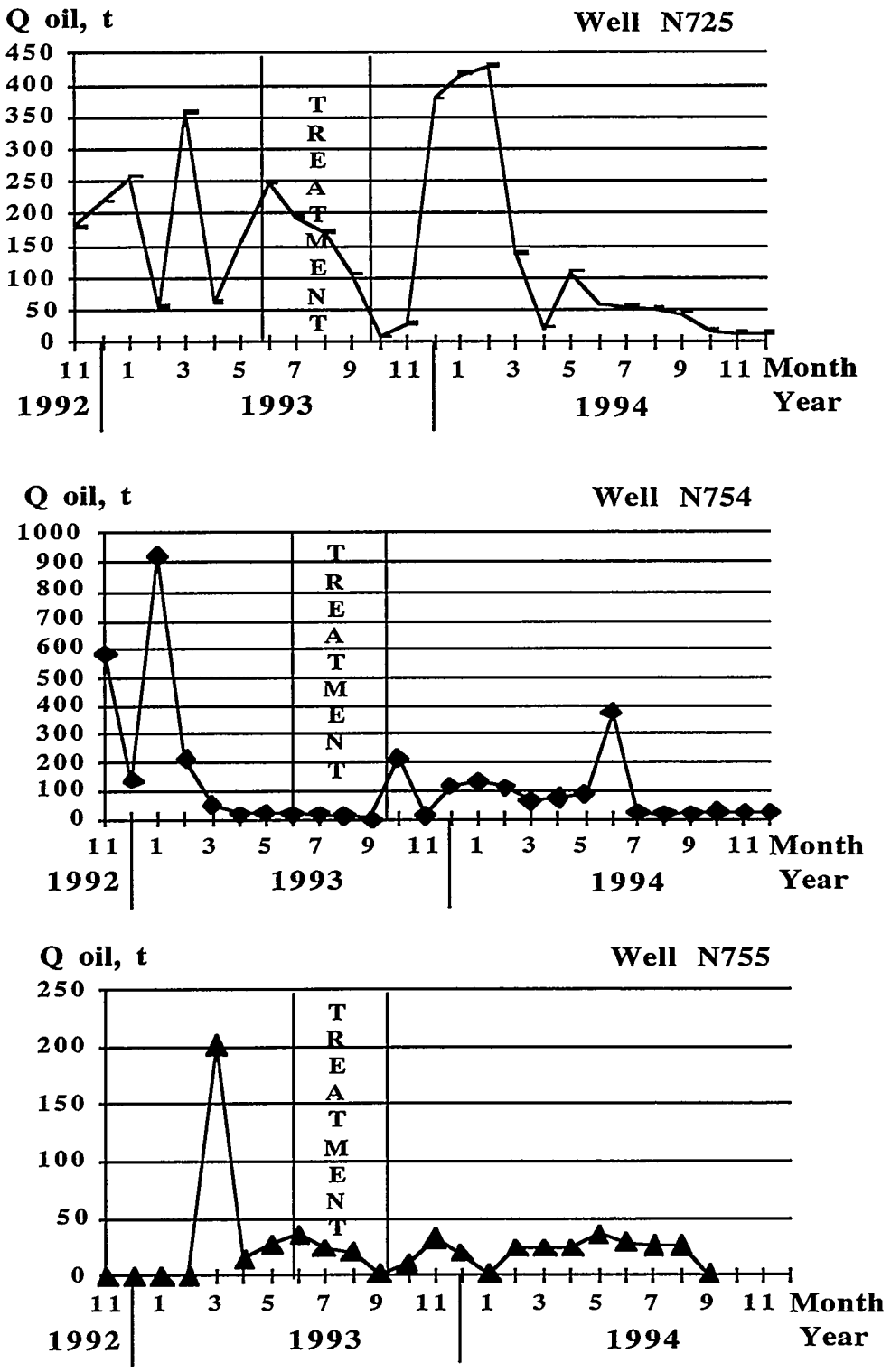

Figure 3 Dynamics of Oil Production on Pilot Area on the Vyngapour Oil Field after Microbial Treatment

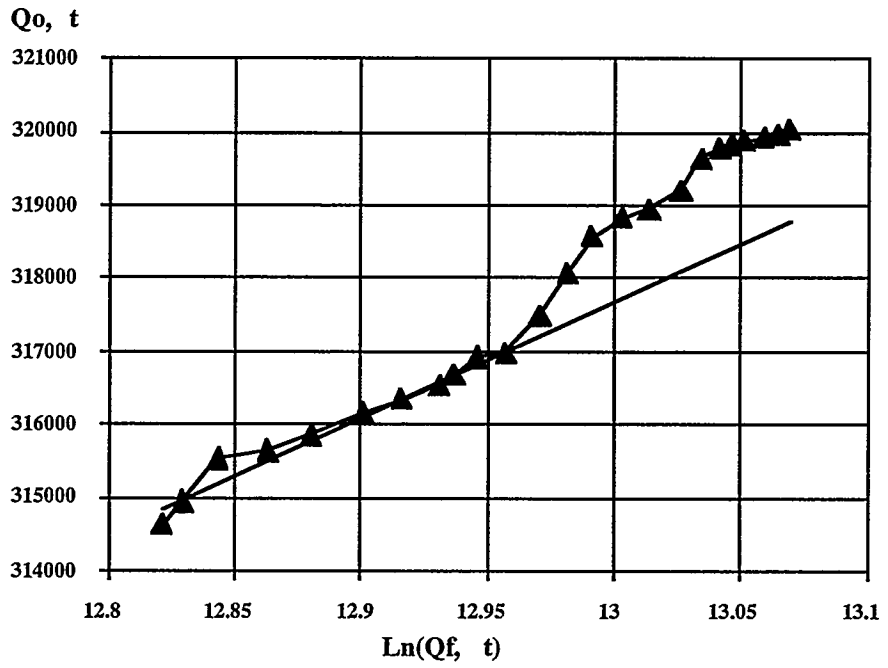

Figure 4 **Oil Production on Pilot Area of the Vyngapour Oil Field after Microbial Treatment. Qo—Oil Production, Tons; Qf—Fluid Production, Tons. Baseline (without Treatment); Production (after Treatment).**

Enhancement of the Sweep Efficiency of Waterflooding Operations by the In-Situ Microbial Population of Petroleum Reservoirs

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Abstract

Live cores were obtained from five reservoirs using special precautions to prevent contamination by exogenous microorganisms and minimize exposure to oxygen. The depths from which the cores were obtained ranged from 2,705 ft to 6,568 ft. Core plugs were cut radially from live cores, encased in heat-shrink plastic tubes, placed in core holders, and fitted with inlets and outlets. Nutrient additions stimulated the in-situ microbial population to increase, dissolve stratal material, produce gases, and release oil. Reduction in flow through the core plugs was observed in some cases, while in other cases flow was increased, probably due to the dissolution of carbonates in the formation. A field demonstration of the ability of the in-situ microbial population to increase oil recovery by blocking the more permeable zones of the reservoir is currently underway. This demonstration is being conducted in the North Blowhorn Creek Unit situated in Lamar County, Alabama. Live cores were obtained from a newly drilled well in the field and tested as described above. The field project involves four test patterns each including one injector, four to five producers, and a comparable control injector with its four to five producers. Nutrient injection in the field began November 1994.

Introduction

In addition to producing by-products that can assist in oil recovery, microorganisms also can restrict the wider microchannels in the formation, thereby increasing the sweep efficiency of waterflooding operations. While some investigators rely on microorganisms that are injected into the formations, others make use of the in-situ microbial populations. This microflora in the oil reservoirs undoubtedly exists in numerous loci, each with its own micro-environment. As pointed out by ZoBell,[1,2] the microflora in the reservoir is in dynamic equilibrium with its environment, and reconstructing such a situation in order to conduct laboratory experiments would be virtually impossible. Microbes taken from cores will grow in microbiological media in the laboratory, but the question to be answered prior to field trials is will these same organisms grow when offered small amounts of supplemental nutrients in their native environment. To help answer this question, coreflood experiments were conducted using live core plugs so that formation materials, microorganisms, oil, and water were in as near a natural state as possible. Based upon the results obtained, a field trial has been initiated in the North Blowhorn Creek Unit in Alabama's Black Warrior Basin.

This manuscript is a report on some of the laboratory work conducted on the ability of in-situ microorganisms to grow in petroleum reservoirs and a description of the field tests of a MEOR method.

Method and Material

Acquisition of Live Cores

Arrangements were made by the Petroleum Engineering Department at Mississippi State University with several oil companies to acquire cores directly from the core barrels immediately as they came from the wells (live cores). The cores were obtained from previously unswept areas of oil-bearing formations. The cores were received as they were pulled from the core barrels. The cores were broken into 1-ft sections, wiped with 70% ethanol, and immediately placed in BBL®Gaspak® System containers under anaerobic conditions. This procedure was completed within minutes, thus exposure to air was minimal. It also should be pointed out that the pressure in the core tends to force fluid and/or gases outward, thereby reducing further the possibility of exposing the internal section of the core to air and preventing contamination. The anaerobic containers were packed in ice, transported to the laboratory, and placed in a refrigerator at 4°C until needed. In the case of the cores from the North Blowhorn Creek Unit, the anaerobic containers were

transported to the laboratory within two hours and placed in a refrigerator at 4°C until needed.

Preparation of Core Plugs for Coreflood Experiments

Cores were removed from the GasPak® container systems under a nitrogen atmosphere, and two adjacent core plugs were cut radially from each core—one to serve as the test core plug and one as the control core plug. The plugs were 3–4 in. long and 1.5 in. in diameter. While still under a nitrogen atmosphere, each plug was inserted immediately into a special heat-shrink plastic tube. The plastic wrap shrank as it was heated and wrapped tightly around the core plug. An entry and an exit port were placed on opposite ends of the core plug. These stainless steel ports contained grooves for the reduction of end effects and for more homogenous distribution of flowing fluids. The entire assembly then was inserted into a thick rubber sleeve (Viton neoprene sleeve, 1.5 in. in diameter, with a 0.25-in. wall). The ends of the entry and exit ports were fitted with rubber tubing and clamped shut. Both ends were completely sealed with high-strength epoxy glue. The glue was allowed to harden for 24 hrs before the core plugs were used. Figure 1 is a diagrammatic sketch of the assembled core plug. Figure 2 is a photograph of the core plug.

Treatment of the Core Plugs

Initially, simulated production water contained in a 13.5-gal carboy was allowed to flow through the core plug. The carboy was situated approximately 25 in. above the core plug, and this hydrostatic head constituted the total pressure applied to the influent. The water was allowed to flow through the core plug until no oil was visible in the effluent (usually within 24 hrs). After a 24-hr waiting period, experimentation commenced. Control core plugs received simulated production water only while the test core plugs received simulated production water containing added nutrients—nitrate as sodium nitrate (0.05% w/v); orthophosphate as disodium hydrogen phosphate (0.01 or 0.5% w/v); and either ethanol (15 M) or glucose (0.5% w/v).

Simulated production water used for the waterflooding operation was prepared with the following inorganic salts per eight liters of distilled water.[3]

NaCl	778.00 g
Na_2SO_4	130.00 g
$MgCl_2 \cdot 6H_20$	352.00 g
$CaCl_2 \cdot 2H_20$	36.00 g
KCl	11.00 g
Na_2HCO_3	3.20 g
KBr	1.60 g
$SrCl_2 \cdot 6H_2O$	0.67 g
H_3BO_3	0.41 g
$Na_2SiO_3 \cdot 9H_2O$	0.08 g
NaF	0.05 g
NH_4NO_3	0.03 g
$FePO_4 \cdot 4H_2O$	0.02 g

The pH was adjusted to 7.0 using 10% (v/v) HCl

Analyses of Effluent from Core Plugs

Fluid volume, pH, oil content, and microbial content were measured and recorded periodically for all core plugs. Plate counts were conducted on selected samples using Plate Count Agar (Difco) prepared using simulated production water. Plates were incubated for 72 hrs under aerobic conditions or two weeks for anaerobic conditions.

Preparation for Field Trial

The simulated production water employed for the coreflood experiments using core plugs from the North Blowhorn Creek Oil Unit, Lamar County, Alabama, was based on chemical analyses of production water from the field and consisted of the following inorganic salts per 50 liters of distilled water.

$CaCl_2$	10.90 g
$MgCl_2$	2.71 g
$BaCl_2$	4.56 g
Na_2SO_4	1.84 g
$NaHCO_3$	34.86 g
NaCl	147.90 g

The pH was adjusted to 7.0 using 10% (v/v) HCl

Results

Experiment One

The core plugs used in this experiment were prepared from a core obtained from a depth of 4,725 ft from the Mabee Oil Field situated in Andrews County, Texas. The control core plug received only simulated production water, while the test core plug received simulated production water containing sodium nitrate or disodium hydrogen phosphate sequentially. During the first four weeks of treatment:

The Control Core Plug Effluent:

- Remained clear
- Had no signs of oil
- Contained approximately 10,000 microbes/ml

The Test Core Plug Effluent:

- Was turbid much of the time and contained fine particulate matter
- Contained oil
- Contained approximately 1,000,000 microbes/ml
- Became plugged after 16 days, and flow had to be restarted by increasing pressure on the influent

Glucose in simulated production water was added to the feeding regime, and the experiment continued for another 27 days. The test core plug stopped flowing again after another 15 days, and the flow restarted as before. Microbial counts and other observations continued as described above. Flow rates through the cores are shown in Figure 3.

Upon completion of the experiment, the core plugs were evaluated petrophysically with the following results.

The Control Core Plug:

- Had a much darker color due to residual oil still in pores

- No microchannels were visible in the core
- Had a porosity of 12.8%

The Test Core Plug:

- Showed a dissolution of carbonate and some microchannels were visible
- Was bright in color with only a few dark spots due to oil
- Had quite visible newly formed channels on the outside of the samples
- Chunks of calcite were dissolved causing relatively large cavities along with wide channels
- Wherever there were traces of calcite in general carbonaceous material, the calcite had dissolved, leaving cavities and channels with a porosity of 17%.

Changes in permeability were minimal due to very tight channels. Appearance of the core plugs after the tests is shown in Figure 4.

Experiment Two

The core for this experiment was obtained from a depth of 4,050 ft from the Johnson Oil Field situated in Ector County, Texas. The test core plug received a supplemental nitrogen and phosphorus source, while the control core plug only received simulated production water. Microorganisms were present in the effluent from the control core plug, but there were no visible signs of oil or particulate matter in the effluent. Contrariwise, not only did the effluent from the test core plug contain microorganisms, gas was produced, and both oil and particulate matter were present. Also, on one occasion, the test core plug became plugged, but flow was restarted by application of a slightly increased pressure on the influent.

Since the core plugs contained dolomite and there was evidence of microbially produced acids, it was conjectured that acid production could be enhanced by supplying the microflora with a metabolic precursor of acid, namely ethanol. The results of this addition were striking in the test core (see Fig. 5). Within several days, the flow rate increased significantly and large amounts of particulate matter were present in the effluent. While ethanol also was added to the control core plug, no observable changes occurred, indicating that it was not the ethanol *per se* causing the results observed for the test core plug. It must be remembered that the control core plug did not receive supplemental nitrogen or phosphorous.

Upon completion of the experiment the core plugs were subjected to petrophysical analyses with the following results. These core plugs were highly heterogenous and consisted of a very tight dolomite. Water would not flow through the core plugs due to the extremely low permeability and low inlet fluid pressure (hydrostatic head only). Consequently, fluids found their way around the core plug adjacent to plastic wrap. Therefore, microbial activity occurred only on the surface of the test core plug with the development of visible cavities and microchannels. No visible changes were evident on the control core plug surface. The test core plug appeared whitish due to removal of oil while the control core plug was brownish in color from the oil present in it. The test core plug porosity was 7.4% while the control core plug porosity was 5.9%. No data on permeability was collected due to a lack of measurable flow. Figure 6 shows the appearance of the core plugs after the experiment was completed.

Experiment Three

The protocol for this experiment was the same as for Experiment Two, except for the following:

- The core plugs were prepared from a core obtained from a depth of 4,300 ft from the Emsu oil field situated in Lea Co., TX.
- During treatment the microbial content of the effluent from the control core plug was low (10,000/ml), there was no particulate matter or oil in the effluent
- The flow rate through the core plug was very low

After treatment of the test core plug with a supplemental nitrogen and phosphorus source, there were more microorganisms (1,000,000/ml) in the effluent but only a slight trace of oil. After one month of treatment, both the control and test core plugs were given ethanol and within several days there was a dramatic increase in the flow rate through the test core plug, and particulate matter and oil were present in the effluent (see Fig. 7).

Petrophysical examination of the core plugs after completion of the experiment showed that both the test and the control core plug samples were of a very tight dolomite type. It seemed that whatever channels were available for fluid flow were plugged by formation clay due to waterflooding, some loose fines, and the effects of core cutting. Permeability and porosity of the two core plugs were the same (0.9–0.95 md) and porosity was approximately 11%.

Experiment Four

The core plugs for this experiment were prepared from a core that was predominately dolomite and obtained from a depth of 2,705 ft from the McElroy Oil Field situated in Crane County, Texas. Once again flow through the core plugs was low, but after treatment with a supplemental nitrogen and phosphorus source the effluent from the test core plug had an increased microbial content, some gas, particulate matter, and oil. The addition of ethanol to the influent of the test core plug increased the flow through the core significantly, while flow through the control core plug remain unchanged (see Fig. 8).

Petrophysical evaluation of the core plugs after completion of the experiment showed that both core plugs had lost a lot of fines and had become somewhat more porous, but differences in porosity were minimal (16.8% for the control and 15% for the test). On the other hand, the permeability of the test core plug increased three-fold (from 3.3 md to 9.3 md) while the permeability of the control core plug did not change.

Experiment Five

The core plugs for this experiment were prepared from a core that was predominately sandstone from a depth of 6,568 ft from the Cellers Ranch Oil Field situated in Johnson County, Wyoming. The control core effluent contained approximately 10,000 microorganisms/ml, there was no evidence of particulate matter or oil, and the pH only dropped to 6.5 even after ethanol was added to the injection water. After treatment of the test core plug with a supplemental nitrogen and phosphorus source for only a few days, the effluent showed a 100-fold increase in microbial content, and contained particulate matter and oil. With the addition of ethanol to the test core plug, flow through the core plug increased dramatically, and the pH dropped to 5.0 (see Fig. 9).

Petrophysical examination of the cores showed the following:

Control Core Plug. No oil was evident on the heat shrink plastic wrap. The wrap was quite clean and whitish (see Figs. 10 and 11). On the other hand the core plug was dark, and most of the oil seemed to be in place.

Test Core Plug. Sizable amounts of the oil blackened the heat shrink plastic wrap (see Figs. 10 and 11). The core plug was relatively clean and whitish.

Fine particulate matter was visible on the heat shrink wrap.

The porosity of the test core plug was 33% higher than that of the control (17.7% vs. 13.3%). The permeability of the test core plug was higher than the control core plug (54 md vs 8.6 md).

Preparation for Field Trail

The MEOR method to be field tested involves stimulating the in-situ indigenous microbial population to expand in the reservoir thereby increasing the effectiveness of the waterflooding operation in the North Blowhorn Creek Oil Field situated in Lamar County, Alabama. Prior to commencing field trials, coreflood experiments, as described above, needed to be conducted to ensure that the indigenous microflora in the formation would respond in an appropriate fashion to the feeding regime. Therefore, two wells were drilled into the producing formation in an area believed to be poorly swept by the existing waterflooding operation. Analysis of the cores from one of the wells proved that viable microorganisms were present and since sulfate-reducing bacteria (SRB) were not present, the area in which the wells were drilled probably had not been impacted by the waterflooding since SRB were prevalent in fluids from other wells in the field.

Coreflood experiments were conducted as described above with the following results. Two sets of core plugs were prepared from two different sections of cores from this reservoir. As would be expected, the flow rates of simulated production water through the core plugs varied from core plug to core plug and ranged from 5.5 ml (control core plug 1) to 12.5 ml (control core plug 2) per day (see Figs. 12 and 13) for the control cores to 10.5 ml (test core plug 1) to 148 ml (test core plug 2) per day (see Figs. 14 and 15) for the test core plugs. Surprisingly, the pH values for the effluent from both control and test cores were slightly alkaline and remained steady in the range of 7.9 to 8.4. Similarly, the microbial content of the effluent remained essentially constant at about 10 organisms/ml for both the control and the test core plugs.

Both control core plugs showed a steady increase in flow rate with time while both test core plugs decreased in flow rate with time. In fact, test core 2 ceased to flow twice and had to be restarted by gently increasing the pressure on the influent. Test core plug 1 ceased to flow and had to be restarted on six occasions. Oil was detected only one time in each of the control core plugs (day 1 in control 1 and day 10 in control 2). Oil was detected in the effluent from test core plug 2 only on day 2 but was found in the effluent from test core plug 1 on days 9, 10, and 12.

The above coreflood experiment was repeated using two new core plugs. Once again the pH of the effluent from both core plugs ranged from 7.4 to 8.4, and no increase in microbial content was observed. Oil in the effluent of the control core

plug occurred only once (day 29), while in the test core plug it was found six times (days 11, 13, 14, 16, 19, and 25) as shown in Figures 16 and 17. The flow rate in the control core plug increased slightly while the flow rate in the test core plug decreased appreciably. Thus, the results of this second experiment confirmed the results of the first experiment in that the addition of potassium nitrate and disodium hydrogen phosphate to the injection water caused a decrease in the flow rate of the injection water through the core plug plus an increase in oil in the effluent.

Therefore, it was concluded that the addition of nitrate and orthophosphate to the injection water employed in the injection wells in the North Blowhorn Creek Unit should result in a decrease in the diameter of the channels in the reservoir formation caused by the growth of the in-situ microflora. This decrease in flow through the main channels in the reservoir should divert injection water to the less permeable areas of the formation, thereby increasing the sweep efficiency of the waterflooding operation.

Description of the Field Trial

The North Blowhorn Creek Unit is a 52-well field that produces from the Carter oil sand at an average depth of about 2,200 ft. The field is spread over 9 sections in Townships 13 and 14 South, Range 14 West, Lamar County, Alabama, covering 1,580 productive acres. The field is drilled on 40-acre spacing and includes 20 injection wells and 32 producers. It is a fluvial-dominated deltaic reservoir. Surface production and injection facilities are centrally located, and each well is connected via a flowline, manifold, and truckline gathering system. All producing wells are on rod pump. A net pay isopach map of the North Blowhorn Creek Unit is shown in Figure 18.

For the field demonstration, four injector wells were selected for nutrient additions and the surrounding producer wells are being monitored. Results obtained in these tests will be compared to historical data for the wells and the data obtained from four additional injectors and their surrounding producers which are serving as controls (see Fig. 1).

That control wells be included in the experimental design was considered essential to the scientific integrity of the project. As a consequence, control wells will be included for the duration of the 37-month trial. That some producing wells may be influenced by more than one of the injectors will be taken into account when interpreting the data. Performance of the production wells is being monitored. Additionally, chemical, microbiological, and petrophysical characteristics of the injection water and produced fluids are being monitored.

Nutrient additions to the injector for test pattern one began in November 1994.

Conclusions

It has been shown that the in-situ microbial population in live cores obtained from five different petroleum reservoirs will respond to the addition of supplemental nutrients. Depending upon the feeding regime, nutrient additions to core plugs resulted in one or more of the following: increased microbial populations, decreased pH, increased oil recovery, reduction of flow through the cores due to microbial growth or increased flow through the cores due to acid production, increased permeability, increased porosity, and gas production.

Core plugs prepared from cores obtained from the North Blowhorn Creek Oil Unit responded to treatment with inorganic nitrate and orthophosphate by a decrease in flow rate of simulated production water through the core plugs. A field trial to test the ability of in-situ microorganisms to grow, thereby restricting the wider microchannels of the formation and increasing the sweep efficiency of the waterflooding operation began in November 1994.

Acknowledgments

The authors would like to express gratitude to the following oil companies for their assistance in obtaining the cores—Texaco, Oxy USA, Inc., and Chevron. The financial assistance of the U.S. Department of Energy under Contract No. AC22-90BC 14665 and DE-FC22-94BC14962 is gratefully acknowledged.

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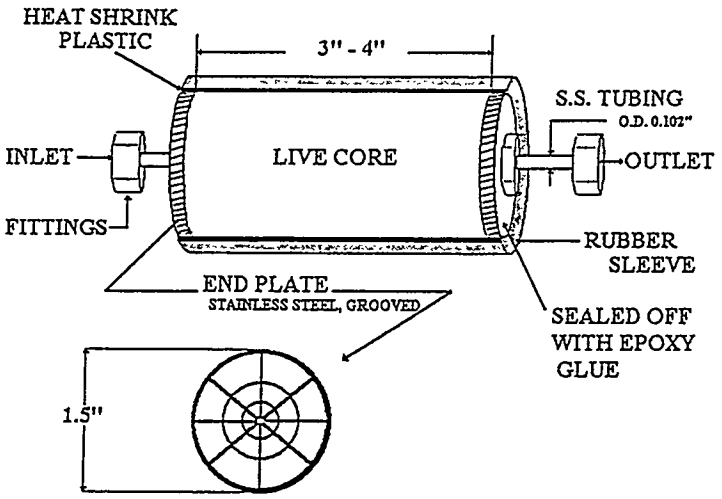

Figure 1 Live Core Assembly

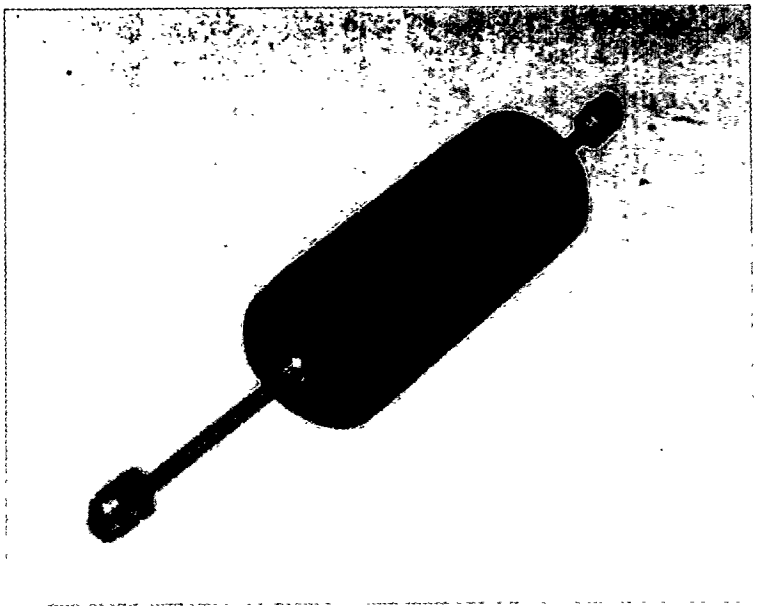

Figure 2 Photograph of Completely Assembled Core

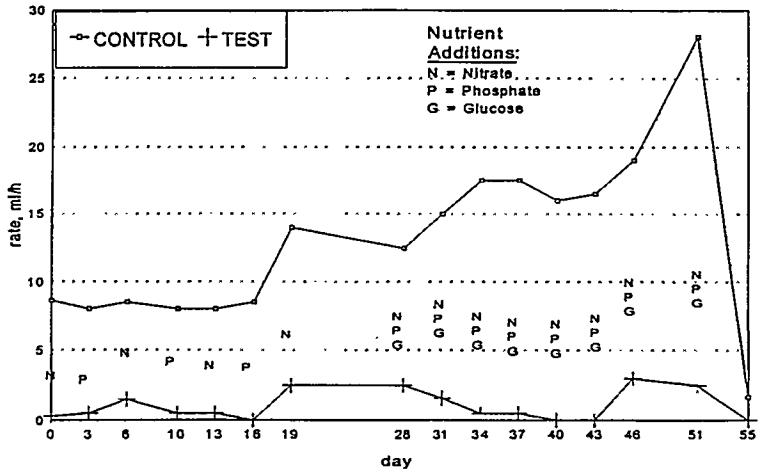

Figure 3 Treatment Schedule and Observations for Coreflooding Experiment Using Core Plugs from the Mabee Oil Field

Figure 4 Photograph of the Control (Left) and Test Core Plugs (Right) from the Mabee Oil Field after Treatment

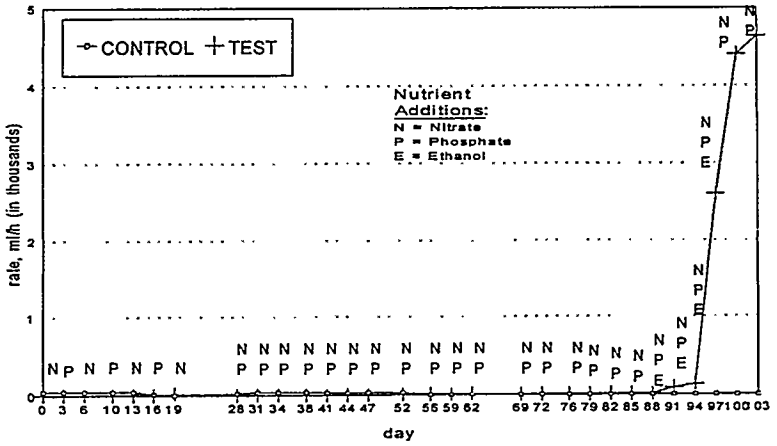

Figure 5 Treatment Schedule and Observations for Coreflooding Experiment Using Core Plugs from the Johnson Oil Field

Figure 6 Photograph of the Control (Left) and Test Core Plugs (Right) from the Johnson Oil Field after Treatment

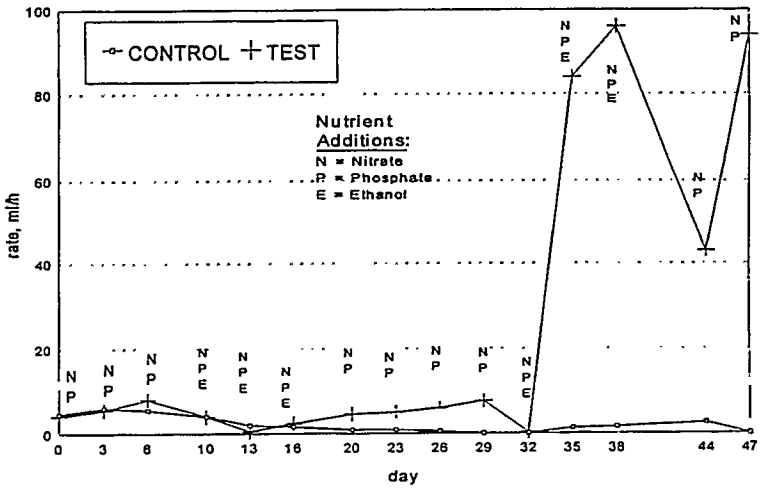

Figure 7 Treatment Schedule and Observations for Coreflooding Experiment Using Core Plugs from the Emsu Oil Field

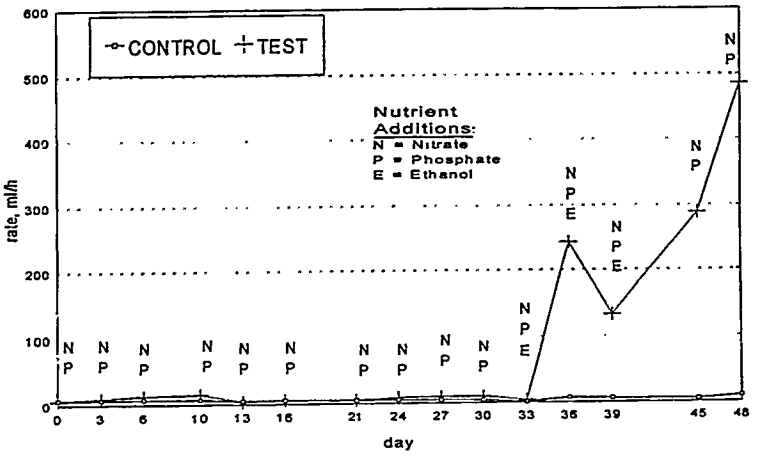

Figure 8 Treatment Schedule and Observations for Coreflooding Experiment Using Core Plugs from the McElroy Oil Field

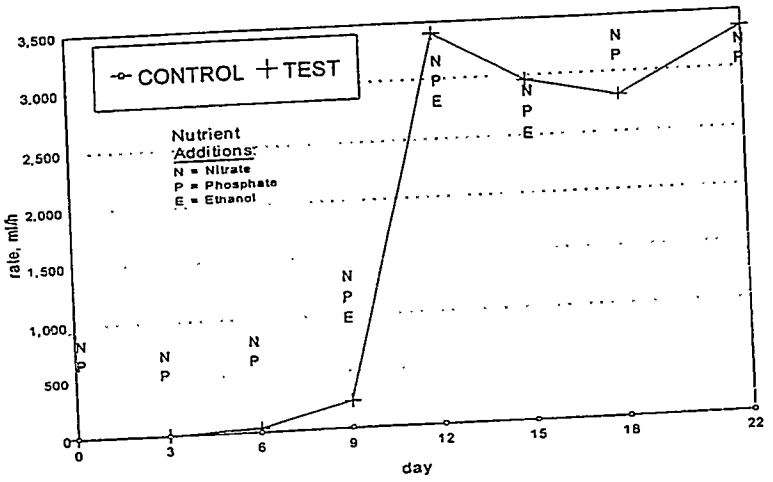

Figure 9 Treatment Schedule and Observations for Coreflooding Experiment Using Core Plugs from Cellers Ranch Oil Field

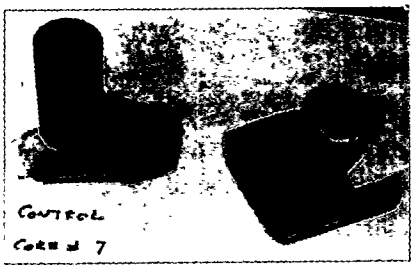

Figure 10 Photograph of the Control and Test Core Plugs from the Cellers Ranch Oil Field after Treatment (Note the Oil on the Wrap of the Control Core)

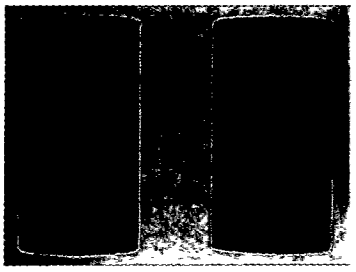

Figure 11 Photograph of the Control (Left) and Test Core Plugs (Right) from the Cellers Ranch Oil Field after Treatment

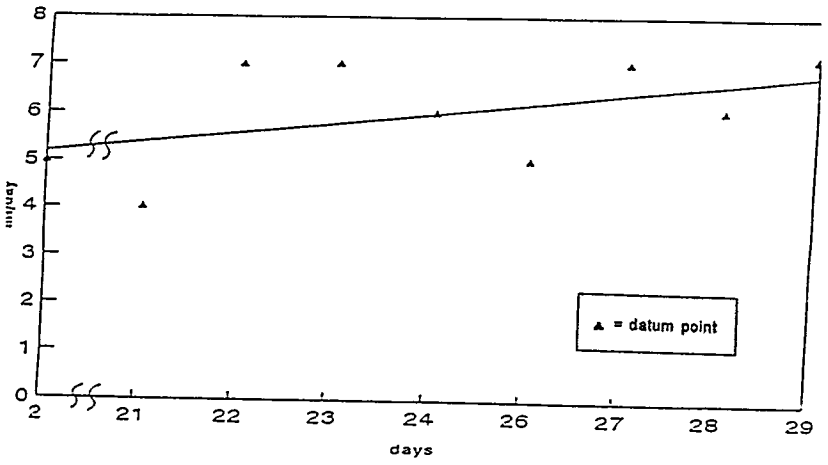

Figure 12 Flow Rate of Simulated Production Water through Control Core Plug 1 in Experiment 1

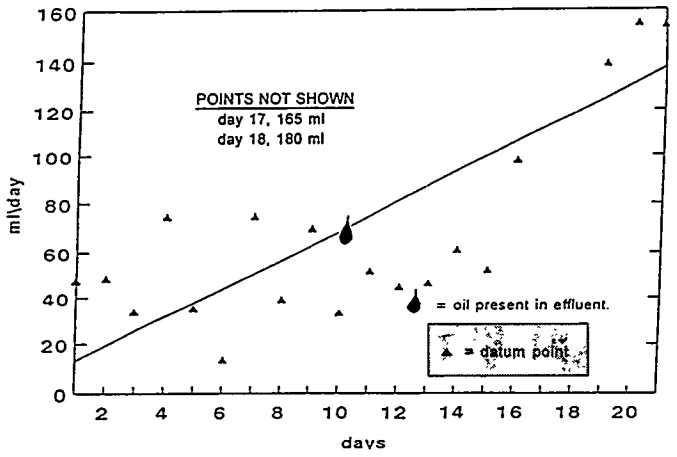

Figure 13 Flow Rate of Simulated Production Water through Control Core Plug 2 in Experiment 1

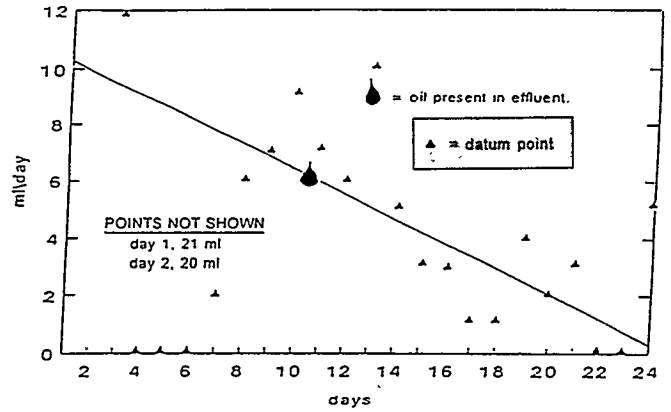

Figure 14 Flow Rate of Simulated Production Water Containing Potassium Nitrate and Disodium Hydrogen Phosphate through Test Core Plug 1 in Experiment 1

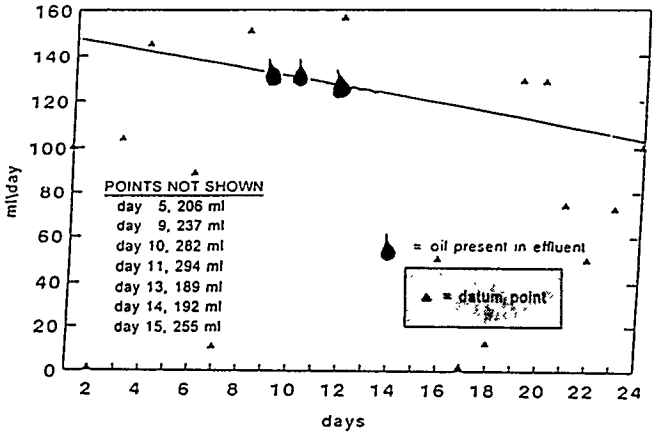

Figure 15 Flow Rate of Simulated Production Water Containing Potassium Nitrate and Disodium Hydrogen Phosphate through Test Core Plug 2 in Experiment 1

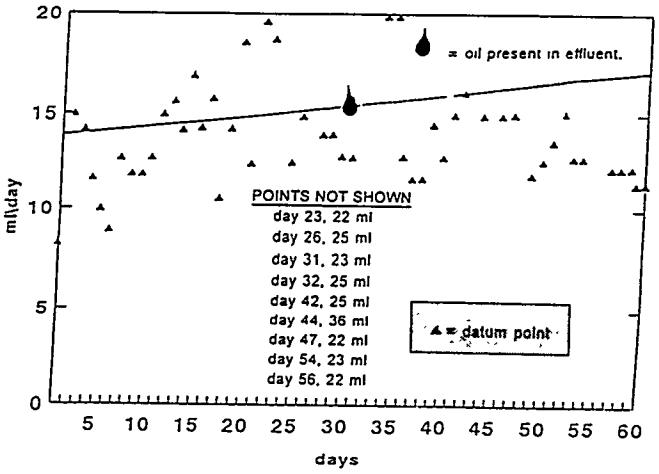

Figure 16 Flow Rate of Simulated Production Water through Control Core Plug in Experiment 2

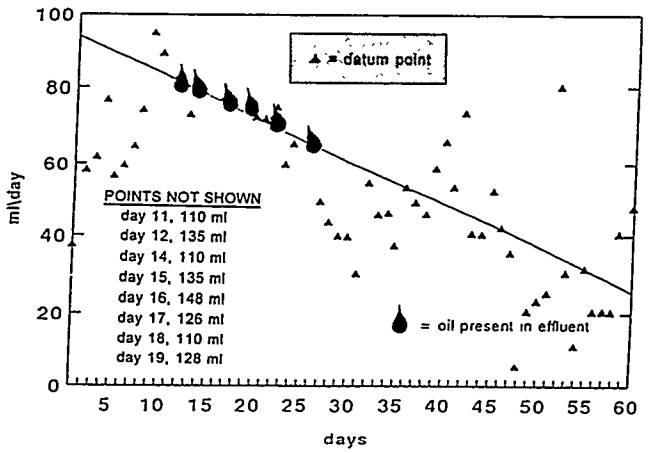

Figure 17 Flow Rate of Simulated Production Water Containing Potassium Nitrate and Disodium Hydrogen Phosphate through Test Core Plug in Experiment 2

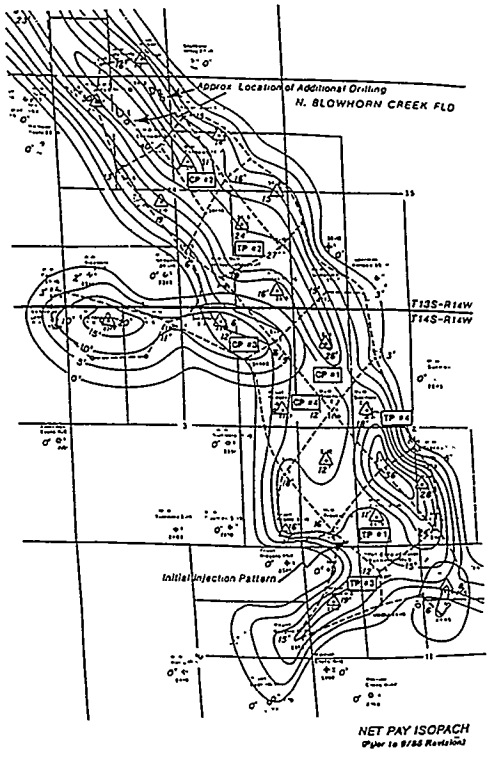

Figure 18 Isopach Map of the North Blowhorn Creek Oil Field

Application of Bio-Huff-'n'-Puff Technology at Jilin Oil Field

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Abstract

An enriched culture 48, capable of adapting to the reservoir conditions and fermenting molasses to produce gas and acid, was used as an inoculum for bio-huff-'n'-puff tests at Fuyu oil area of Jilin oil field. The production well was injected with water containing 4–6% (v/v) molasses and inoculum, and then shut in. After 15–21 days, the well was placed back in operation.

A total of 44 wells were treated, of which only two wells showed no effects. The daily oil production of treated wells increased by 33.3–733.3%. Up to the end of 1994, the oil production was increased by 204 tons per well on average. Results obtained from various types of production wells were discussed.

Introduction

Since the first patent on MEOR was published in 1946,[1] considerable amounts of laboratory and field researches in this area have been performed and have made much headway.[2–5] Several kinds of MEOR processes have been developed, of which the single-well biostimulation process is a simplest, cheapest, and easiest to control. This process might be first established by Kuznetsov for a well at Sernovodsk oil field in the fifties.[6] Since then, more production wells were treated by use of this process in many countries, but in most cases only the results obtained from 1–3 treated wells were published.

Fuyu reservoir, located in the south of Songliao Basin, is a fractured and bedded sandstone reservoir with depth of 280–480 m. Fuyu reservoir went into production in 1970. Its effective pay thickness is 8.1 m with a low permeability of 0.210 μm^2 on average and a porosity of 24%. The original average reservoir pressure was 3.95 MPa and its saturation pressure 3.06 MPa. The reservoir water belongs to the type of $NaHCO_3$ with a mineralization of 5,994 mg/l. Its average temperature is 30.3°C, and oil saturation is 73%. The oil surface and subsurface viscosities are 46.3 MPa/s and 40 MPa/s in their given order. The reservoir has an oil density of 0.8468 g/cm^3 and a low carbonate content (1.4%). Its oil condensation point is 16°–26°C and water cut reached 87% already. These conditions, with the low content of carbonate excepted, mainly are suitable for MEOR technology. Based on the present development level, it will be expected that the oil recovery is as low as 20.6%. Due to its high water cut and low recovery rate (0.54%), Fuyu reservoir needs applyication of new EOR technologies. The successful field trials of single-well stimulation process at Daqing oil field[7] gave us great confidence in the practical application of this technology. Consequently, this technology was extended to Fuyu reservoir.

This paper presents the results from 44 wells treated by single-well bio-huff-'n'-puff process during 1992–4.

Material and Methods

Bacterial Inoculum and Nutrient

On the basis of the properties of enriched culture 48 and its oil recovery efficiency obtained from laboratory model experiments, this culture was used as an inoculum for bio-huff-'n'-puff trials. Detailed information on this culture is described by Wang et al. in this volume.[8]

A solution of 4%–6% (v/v) molasses as a nutrient was injected into oil-bearing formation.

Procedure of MEOR Treatment

Figure 1 shows the procedure for microbial single-well stimulation process.

Sampling

Water and gas samples were directly collected from sampling valves into sterilized flasks and gas collectors, respectively.

Assays

The enumeration of anaerobic bacteria was carried out by the MPN (most probable number) method with five tubes at each dilution. Molasses (4%, v/v) broth was used as medium. Cultures were incubated at 30°C.

Analyses of gases and organic acids were performed through gas chromatography using a GC-7AG gas chromatograph (Shimadzu) or SC-3A gas chromatograph (Sichuan). Surface viscosity of oil, HCO_3–content and others were determined by conventional methods.

Results and Discussion

During 1992–4, 44 wells of Fuyu reservoir were subjected to single-well bio-huff-'n'-puff technology. A production well was injected with water containing 4–6% of molasses and 4–5% of inoculum broth. Its total volume was equal to 2–3 m of pore space around the wellbore. Total viable cell counts in the injected fluid were X × 103-X × 104/ml (see Table 1). The outcomes of microbiological treatment were as follows.

Responses after MEOR Treatment

Total Viable Counts of Anaerobes and Persistance of Injected Bacteria. The success of MEOR technology depends on the predominance and persistance of injected bacteria in the reservoir ecosystem. If a bacteria group is a predominant one in an environment, then isolates from this bacterial group are

easily obtained. For this reason, we randomly estimated the viable counts of gas- and acid-producing anaerobes in coproduced water and re-isolated the injected culture 48 from water sample of the treatment well. The results indicated that the gas- and acid-producing anaerobes were absent or a few cells/ml in the coproduced water before treatment. After treatment, numbers of this bacterial group in coproduced water increased to X × 102-X × 106 cells/ml (see Table 1). In addition, the injected culture 48 was successfully obtained from the produced fluids, too. The injected culture 48 in the horizons around treated wellbores resulted in some changes as follows.

Metabolite Contents. The analysis of CO_2 in the accompanying gas and of HCO_3^- in the coproduced water indicated that their amounts had increased significantly. The average amount of CO_2 was increased from 2.64% before treatment to 25.25% after treatment (see Table 2), meanwhile, wellhead pressure increased by 0.3–0.5 MPa. The HCO_3^- production has increased from 1,767 mg/l to 5,219 mg/l (see Table 3) on average in the 7 wells.

The determination of organic acids was carried out in stratum water of well 16-18. The well was subjected to MEOR treatment on June 9, 1992, and placed back in operation on August. Organic acids in its accompanying water were not found before treatment, but revealed after treatment. Their total amounts in five samples collected at different times during three months (August–October) fluctuated between 3,180 and 4,718.6 mg/l. At that time, the pH of the reservoir water also dropped from 6.8–7.5 to about 6.0. These results provided strong evidence of the bacterial fermentation process in treated oil strata.

Water Cut. The average water cut (65.8%) in the producing fluid has decreased as compared with the average values (78.5%) before treatment. Greater decrease of water content occurred in the well with high water content (see Table 8).

Oil Viscosity. The viscosities of the crude oil from 7 wells were determined before and after the microbial treatment. As shown in Table 4, their surface viscosities dropped by 5–14 cP through the microbial treatment.

Fluid and Oil Production. According to the statistical data of 44 wells, fluid and oil production had increased after the microbial treatment. Table 5 shows the average fluid and oil production in ton/D. Before the microbial treatment, the average fluid and oil productions of per well were 2.97 and 0.64 t/D. After that, we observed an increase of fluid and oil productions to an average of 5.35 and 1.83 t/D. MEOR technology obviously had a positive effect on fluid and oil production.

Applicability of the Single-Well Bio-Huff-'n'-Puff Process to Various Types of Wells

Thick Oil Wells. As shown in Table 6 and Figure 2, the MEOR efficiency of culture 48 in five thick oil wells is noticeable. The average wellhead pressure increased by 0.6 MPa. Oil viscosity decreased from 45.8 to 37.5 MPa. The CO_2 content increased from 2.44% to 20.4%. Oil production increased by 0.95 t/D after biotreatment. As CO_2 dissolved in crude oil, oil vicosity was decreased and oil mobility was increased. The biogas generated from molasses fermentation, therefore, led to the increase of oil production.

Scaling Well. Fuyu reservoir was subjected to waterflood with river water for a long time. Its producton and flooding wells were strong scaled by $CaCO_3$. After the injection of enriched culture 48 into seven scaling wells, oil production per well increased from 0.5 t/D to 1.9 t/D on average (see Table 7 and Figure 3). It might be attributed principally to the microbially produced organic acids. They resulted in the dissolution of carbonates and the improvement of permeability, as well as oil mobility, in the formation.

Wells with Different Water Cuts and Permeabilities. Based on water cut, 44 treated wells may be classified as two groups—7 wells with low (<50%) water cut (Group I) and 27 wells with high (>50%) water cut (Group II). Data shown in Table 8 and Figure 4 indicated that the treatment process had favorable effects of increasing oil and decreasing water. The oil production was increased on average by 213 t/well under a steady water cut for 241 days to Group I and by 262.6 t/well under a decreasing water cut of 11% for 278 days to Group II.

Fuyu reservoir is a heterogeous reservoir with low permeability. The treated wells include 10 wells with permeability less than 200 md, 11 wells with permeability of 200–300 md, and 9 wells with permeability more than 300 md. In any case, microbiological treatments substantially increased fluid, increased oil, and decreased water cut. The greatest effects are observed in wells with permeability more than 300 md. Its fluid and oil production increased by 2.69 t/D and 1.47 t/D per well respectively, but its water cut decreased by 15.5% (see Table 8).

We can suppose that when the injected culture first entered into the high permeability zone, its growth and metabolism led to plugging high-permeability zones temporarily, thus diverting fluid to previously unswept areas. Thereby the sweep area of flood water is improved, water cut decreased, and oil production increased.

Polluted Well. Figure 5 showed the performance curves of the polluted well 18–18 through microbial treatment. Before treatment, the resistance coefficient of the

well wall was 0.223, implying that the oil layer near the well bottom was contaminated. After microbial treatment, it was decreased to –0.535, indicating the contamination had been eliminated. Its oil production increased by 746 tons during 612 days.

As Table 9 shows, microbial treatment led to the improvement of the reservoir permeability, reservoir pressure, flowing pressure, and resistance coefficient, resulting in increased oil production. Thus, microbial treatment can eliminate the contamination of the oil layer near the well bottom. That is important evidence of the effect of organic acids produced by injected culture 48.

Conclusions

Our test reservoir at Jilin oil field is a fractured and sand-bedded one with low permeability and production, as well as low carbonate content (only 1.4%). The successful field trials of 44 wells demonstrated the feasibility of the single-well huff 'n' puff process for various wells, such as the thick oil well, scaling well, polluted well, and so on.

Up to the end of 1994, the incremental oil from 42 effective wells was 9,943 tons, namely 226 t/well on average. The average efficiency period of one treatment was as long as 250 days. Daily oil production per well increased by 33.3–733.3%, averaging 204% for 250 days. Thus, the satisfactory results obtained by using single-well bio-huff-'n'-puff process at the Fuyu oil area will carry this process to a new stage of extended application in our country.

In the preceding sections, we have discussed mechanisms of MEOR in the light of specific conditions. We regard the mechanisms of MEOR as combined mechanisms of bacterial activities in a reservoir. Today, the combined mechanism is not very clear. A further study on the combined mechanism of MEOR would make an important contribution not only to our understanding of MEOR but also to the development of MEOR technologies.

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Table 1 Viable Counts of the Gas-Producing Anaerobes during Treatment Processes

In coproduced water before treatment	0–10
Inoculum 48 in tanks	10^5–10^7
In injected fluid mixed with culture 48	10^3–10^4
In coproduced water after treatment	10^2–10^6

Table 2 Changes of CO_2 Content (%) from Wells Treated by MEOR

Well	8-01	42-7	52-6	4-24	40-17	40-24	18-17	18-18	72-13	Average Treatment
Before	2.95	1.36	6.41	0.93	4.67	3.56	3.08	0.63	0.1	2.64
After	13.09	23.58	24.24	32.52	40.86	14.82	14.56	26.47	31.13	25.25
Balance	+10.14	+22.22	+17.83	+31.59	+36.19	+11.26	+11.48	+26.30	+31.03	+22.61

Table 3 Changes of HCO_3 Content (mg/l) from Wells Treated by MEOR

Well	22-26	4-24	18-18	52-6	12-27	56-3	50-3	Average Treatment
Before	1526	1556	1220	2715	1434	2044	1875	1767
After	3234	4302	9230	4027	6865	3661	5217	5219
Balance	+1708	+2746	+8010	+1312	+5431	+1617	+3342	+3452

Table 4 Changes of the Surface Viscosity (MPa) of Oil from MEOR Well

Well	16-18$_2$	18-17$_2$	46-19	18-18	22-26	76-152	Average Treatment
Before	40.2	53.2	40.6	29.3	38.4	68.0	45.0
After	33.8	46.6	33.0	24.3	24.3	58.6	36.8
Balance	-6.4	-6.6	-7.6	-5.0	-14.1	-9.4	-8.2

Table 5 Summary of the Efficiency of MEOR at Fuyu Oil Field

Amount of Treated Wells	44	
Effective wells	42	
Ineffective wells	2	
Before Treatment		
Daily fluid production/well (tons)	0.6–10.7	2.97*
Water cut (%)	14.4–95.4	65.7*
Daily oil production/well (tons)	0.1–1.5	0.64*
After Treatment		
Daily fluid production/well (tons)	1.7–16.3	5.35*
Water cut (%)	11.1–88.2	57.1*
Daily oil production/well (tons)	0.6–5.0	1.83*
Balance		
Daily fluid production/well (tons)	0.2–9.6	2.3*
Water cut (%)		8.6*
Daily oil production/well (tons)	0.2–4.4	1.23*
Period of Efficacy/well (days)	32–612	250*
Total Oil Production Increased (tons)	9943	226*
Daily Oil Production Increased (%)	33.3–733.3	204*

*Indicating average value

Table 6 MEOR Efficiency of the Culture 48 in Thick Oil Wells

Well	Casing Pressure (Mpa) Injection		Oil Viscosity (MPa) Injection		CO_2 Content (%) Injection		Production time	Incremental oil
	Before	After	Before	After	Before	After	(days)	(t/D)
$16-18_2$	0	0.9	40.2	33.8	2.90	27.45	165	0.82
$18-17_2$	0	0.35	53.2	46.6	3.08	14.56	136	0.90
18–18	0	0.5	29.3	24.3	0.63	26.47	247	1.38
22–26	0	0.95	38.4	24.3	2.95	9.15	193	0.28
$76-15_2$	0	0.32	68.0	58.6	2.66	24.15	124	0.76
Average	0	0.6	45.8	37.5	2.44	20.36	179	0.84

Table 7 MEOR Efficiency of the Culture 48 in Scaling Wells

Well	Fluid Production (t/D) Injection		Oil Production (t/D) Injection		Water Cut (%) Injection		Production time	Incremental oil
	Before	After	Before	After	Before	After	(D)	(t/D)
8–0	3.7	6.2	0.5	2.0	86.4	67.0	223	1.23
12–27	2.7	5.7	0.6	1.9	75.9	66.9	236	0.95
40–24	0.9	2.1	0.3	1.3	51.6	45.0	151	0.29
52–6	3.5	10.4	0.3	1.6	92.5	84.2	123	0.37
4–2	3.3	4.8	0.7	3.1	77.6	34.9	141	1.57
+12–2	8.9	11.1	0.5	2.7	94.5	75.8	135	1.53
Average	3.5	6.1	0.5	1.9	77.2	61.2	155	0.92

Table 8 Effect of Water Cut and Permeability on MEOR Efficiency

Well Group	Amount of Well	Fluid Production (t/D Well)	Oil Production (t/D.well)	Water Cut (%)	Oil Increased (t/well)	Duration of Efficacy (D/well)
			Water Cut			
Low Water Cut (<50%)	7				213	241.3
Before treatment		0.86	0.61	29.1		
After treatment		2.04	1.44	29.4		
Balance		+1.18	+0.83	+0.3		
High Water Cut (50–95%)	27				262.6	277.9
Before Treatment		3.43	0.63	81.6		
After treatment		6.04	1.79	70.4		
Balance		+2.61	+1.16	–11.2		
			Permeability			
Low Permeability (<200 md)	10				144	194.
Before Treatment		2.58	0.49	81.0		
After treatment		5.50	1.42	74.0		
Balance		+2.92	+0.93	–7.0		
Medium Permeability (200–300 md)	11				205.0	281.2
Before Treatment		1.71	0.73	57.3		
After treatment		3.05	1.56	49.3		
Balance		+1.34	+0.83	–8.0		
High Permeability (301–355md)	9				452.6	356.7
Before Treatment		4.70	0.57	87.9		
After treatment		7.39	2.04	72.4		
Balance		+2.69	+1.47	–15.5		

Table 9 Effect of Microbial Treatment on Some Characteristics of Reservoir

Well No.	Reservoir Pressure		Flowing Pressure		Resistance Coefficient of Well Wall		Oil Production t/D	
	Before	After	Before	After	Before	After	Before	After
18-18	2.448	2.225	0.079	0.646	0.223	–0.535	0.6	2.2
40-19	1.972	2.063	0.287	0.855	–0.10	–1.091	0.8	1.4
+12-2	2.319	3.513	0.959	1.858	–1.287	–1.757	0.5	2.7
8-21	3.148	3.756	0.178	0.267	–0.999	–1.313	0.4	0.6
56-3	1.962	2.386	0.293	9.683	–0.214	–0.714	0.6	0.9

Preparation of 4–6% molasses and inoculum

↓

Injection with fracturing truck into the well

↓

Shut in the well for 15–21 days

↓

Put well on, observing, and determing the effects of treatment

Figure 1 Procedure of MEOR Treatment

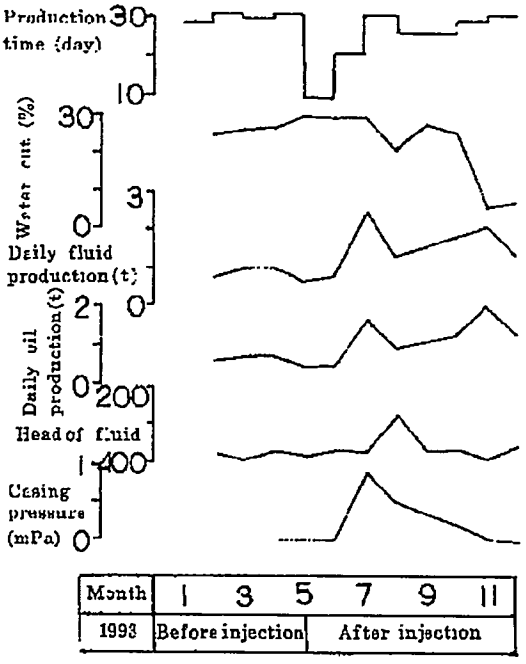

Figure 2 Performance Curves of Enriched Culture 48 Injected into the Well $16–18_2$ (Thick Oil)

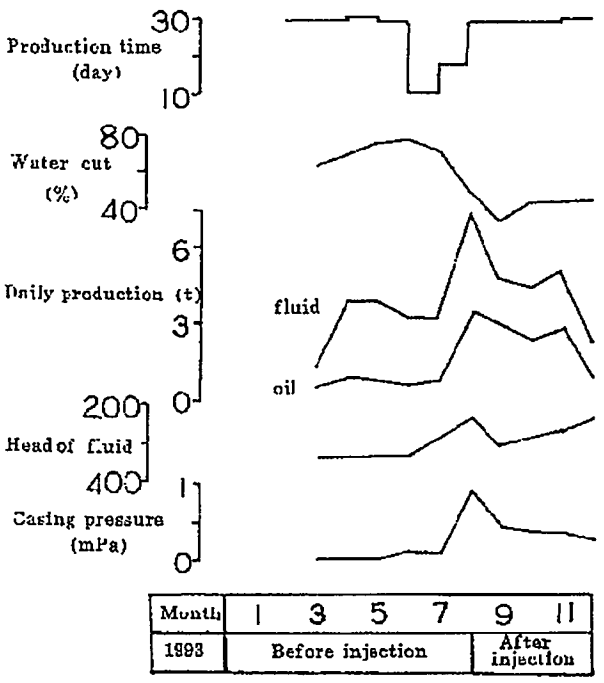

Figure 3 Performance Curves of Enriched Culture 48 Injected into the Well 4-2 (Scaling Well)

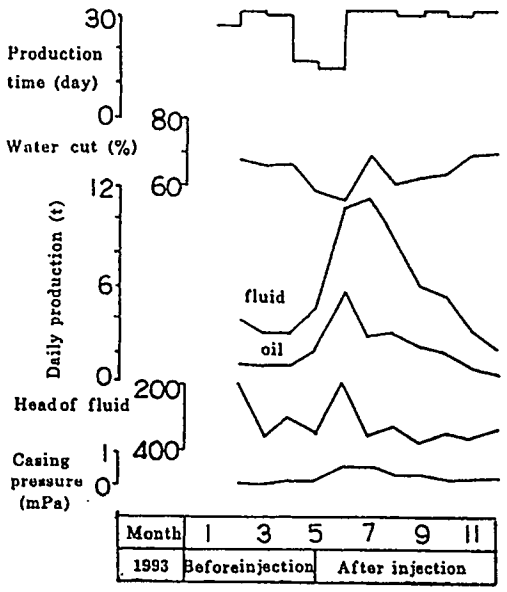

Figure 4 Performance Curves of Enriched Culture 48 Injected into the Well 70-15$_2$

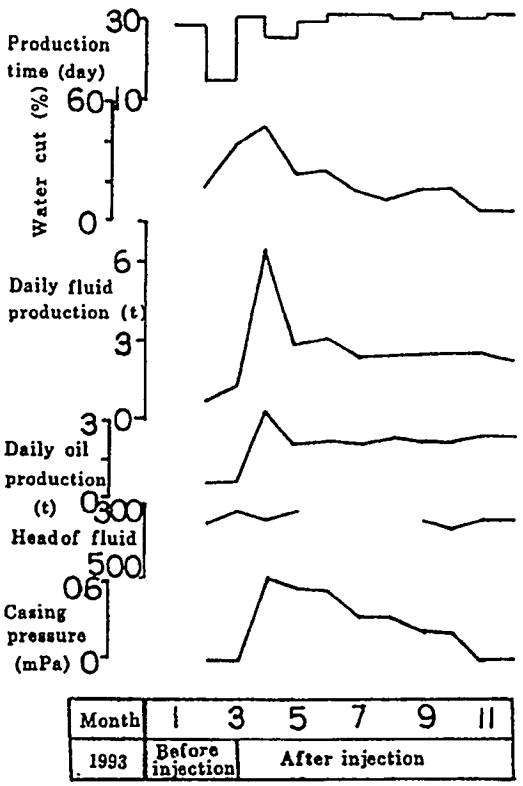

Figure 5 Performance Curves of Enriched Culture 48 Injected into the Well 18-18 (Polluted Well)

Use of Microbes for Paraffin Cleanup at Naval Petroleum Reserve No. 3

Leo Giangiacomo and Alan Khatib
Fluor Daniel (NPOSR), Inc.

Abstract

Naval Petroleum Reserve No. 3 (NPR-3), also known as Teapot Dome, is a government-owned oil field in Natrona County, Wyoming. It is an asymmetrical anticline located on the western edge of the Powder River Basin, just south of the Salt Creek Anticline. Production started in 1922, and today the field is a marginally economic stripper field with average production of less than 3 BOPD (0.5 m^3/D) per well. Total field production is about 1,800 BOPD (286 m^3/D). The Second Wall Creek Formation was waterflooded from 1979 until June 1992 with poor results due to the extensive natural fracture system in this sandstone unit. Since water injection ceased, reservoir pressure has declined to very low levels. Liquids extraction and reinjection of the gas produced from high-GOR wells along the gas-oil contact continues, but the average gas cap pressure has fallen to approximately 150 psi (1.03 MPa) from an original pressure of 1,120 psi (7.72 MPa). Since the oil is highly paraffinic, wax deposition in the hydraulic fractures and the perforations has become a serious production problem. Microbial treatment was considered as a possible low-cost solution.

Four wells were selected in the Second Wall Creek Reservoir with severe paraffin problems and production rates high enough to economically justify the treatment. The expansion of gas produced with the oil in these wells causes a cooling effect that aggravates the paraffin-deposition problem. Treatments with commercially available microbes began in mid-November 1993 with an initial 100 bbl (16 m^3) solution of microbes and nutrients. The wells were shut in for 3 days. Monthly 5 bbl (0.8 m^3) treatments followed until September 1994. Problems were experienced with the production of thick oil after approximately three months. This was interpreted to be a result of previously immobile paraffin being cleaned up. A slight decrease in the decline rate was seen in the wells, although some external factors cloud the interpretation. Microbial treatments were discontinued because of marginal economics. Three of the four wells produced additional oil and had a positive incremental cash flow. Oil viscosity tests did indicate that some positive microbial thinning was occurring, and changes to the treatment procedure may potentially yield more economic results in the future.

Background

Naval Petroleum Reserve No. 3 (NPR-3), also known as Teapot Dome, is a government-owned oil field in Natrona County, Wyoming. Production started in October 1922. In December 1927 the field was shut in due to a leasing scandal. Exploratory and protection drilling was conducted during the 1960s and 1970s. Limited production to prevent drainage to offset leases began in 1964. Full production was authorized in 1976, and reached almost 5,000 BOPD (795 m^3/D) in 1981. In 1995 the field is a marginally economic stripper field with average production of less than 3 BOPD (0.5 m^3/D) per well. Total field production is about 1,800 BOPD (286 m^3/D).

The field is currently operated by Fluor Daniel (NPOSR), Inc. for the U.S. Department of Energy. Since 1976, the mission here was to produce oil at the maximum efficient rate and return a profit to the federal government. A second mission was added in late 1993 in line with the Secretary of Energy's Domestic Natural Gas and Oil Initiative. This mission is to test and evaluate new oil field and environmental technologies and offer training and technology transfer for the benefit of the U.S. petroleum industry, and especially the independent oil operators. This is being carried out by a new organization, the Rocky Mountain Oilfield Testing Center.

Geologically, Teapot Dome is an asymmetrical anticline located on the western edge of the Powder River Basin, just south of the giant Salt Creek Anticline. Production occurs from 10 formations ranging from the Upper Cretaceous Shannon through the Pennsylvanian Tensleep. The Second Wall Creek Member of the Cretaceous Frontier Formation was the major producing reservoir until 1984. It is a naturally fractured, shaly sandstone reservoir with a large gas cap and very little water influx. Porosity ranges from 12 to 17%, and permeability from 1 to 300 md. The reservoir is approximately 2,900 ft (884 m) deep and contains a 35°-API gravity paraffinic crude oil. Sand thicknesses range from 20 to 80 ft (6 to 24 m). Reservoir temperature is 125°F (52°C).

The reservoir was initially produced by pressure depletion, with most wells being rod-pumped. Initial pressure was 1,120 psi (7.72 MPa). Produced gas has been reinjected since 1976 to maintain reservoir pressure. The reservoir was waterflooded from 1979 until June 1992 with poor results due to channeling through the extensive natural fracture system. Cumulative production from the Second Wall Creek reservoir is 9.98 million bbl (1.59×10^6 m^3) oil, 42.4 BCF (1.20×10^9 m^3) gas, and 53.7 million bbl (8.54×10^6 m^3) water. Ultimate recovery is projected to be 17% of the initial oil-in-place. Since water injection ceased, reservoir pressure has declined dramatically. Liquids extraction and reinjection of the gas produced from high-GOR wells along the gas-oil contact

continues, but the average gas cap pressure has fallen to approximately 150 psi (1.03 MPa). Current production is 173 BOPD (27.5 m^3/D), 4,093 MCF/D (116,000 m^3/D), and 2,388 BWPD (380 m^3/D).

Since the crude oil is highly paraffinic, paraffin deposition in the hydraulic fractures and the perforations has always been a serious production problem. Wells are hot-oiled on an "as-needed" basis using the asphaltic-based Tensleep crude oil. When serious production decline is detected, a paraffin solvent treatment using 60% diesel oil and 40% xylene is performed. This procedure ranges from pumping several barrels down tubing to aid pump changes, to mixing the solvent with a crystal modifier and displacing it deep into the formation. Although these treatments have usually been successful, they add significantly to production costs. Microbial treatment was seen as a possible low-cost alternative to these treatments. In 1993, a decision was made to treat several wells and evaluate the effectiveness of this method of treatment.

Well Selection

Well selection for microbial treatments was based on a few simple criteria. The wells should have a documented paraffin problem. They should produce, or have the ability to produce, a relatively high volume of oil to pay out the treatments. And finally, they must produce enough water to have a continuous water phase to enable the microbes to live and move about the rock pores. It was preferred that the wells have pump-off controllers to maximize the benefit of any production increases, and minimize the effect of erratic production rates that are often experienced with paraffin cleanup. Four Second Wall Creek wells were selected on this basis.

Well 51AX15 was drilled in July 1969. The Second Wall Creek was cored and logged, and 7-in. casing was run and cemented above the sand at 2,982 ft (909 m). The completion is open hole. The formation was broken down with HCl acid and hydraulically fractured in January 1970. The well initially flowed oil and was later put on plunger lift. In September 1983 a rod pump was installed. The well was treated for paraffin in March 1989 with 35 bbl (5.6 m^3) diesel-xylene solvent and paraffin inhibitors. This fluid was displaced with 160 bbl (25 m^3) Tensleep crude oil. In April 1991, the well was deepened through the entire Second Wall Creek section. A paraffin treatment similar to the previous one was performed. A single pump serviced the well from June 1991 through December 1994. The well has produced 195,000 STB (31,000 m^3) oil, 1.2 BCF (34×10^6 m^3) gas, and 60,000 bbl (9,500 m^3) water. It produced 29 BOPD (4.6 m^3/D), 26 BWPD (4.1 m^3/D), and 68 MCF/D (1,930 m^3/D) prior to treatment. Its production history is shown in Figure 1.

Well 81AX10 was drilled in November 1977 through the Second Wall Creek, and 5 -in. casing was cemented at 3,035 ft (925 m). It was perforated over 18 ft (5.5 m) with 1 SPF (3 shots/m). The initial stimulation was with 2,500 gal (9.5 m^3) 15% HCl using ball sealers. The well was then put on pump. It was again acidized in February 1979. In June 1984 the well was treated with paraffin solvent and inhibitor, and then displaced with chemicals that generated nitrogen and heat in situ. In December 1985, the well was hydraulically fractured with 20,000 lb (9,100 kg) 12/20 sand. In August 1988, the well was again treated for paraffin with in-situ nitrogen. In January 1991, the well was treated with solvent and inhibitor. The fluid was displaced with asphaltic crude, as described above. Another solvent treatment was performed in September 1993. A single pump serviced the well from February 1993 through March 1995. Cumulative production from the well is 96,000 STB (15,300 m^3) oil, 1.3 BCF (37×10^6 m^3) gas, and 158,000 bbl (25,300 m^3) water. It tested 9.5 BOPD (1.5 m^3/D), 91.5 BWPD (14.6 m^3/D), and 235 MCF/D (6,660 m^3/D) prior to the treatment. Its production history is shown in Figure 2.

Well 62AX3 was drilled in June 1985, and 5-in. casing was set through the Second Wall Creek at 3,217 ft (980.5 m) and cemented. It was perforated over 15 ft (4.6 m) at 2 SPF (6.5 shots/m) and hydraulically fractured with 15,000 lb (6,800 kg) 10/20 sand. In July 1990, the well was treated with solvent and paraffin inhibitor, and displaced with asphaltic crude oil as described for Well 51AX15 above. In September 1993, the well was circulated with 20 bbl (3.2 m^3) solvent for 24 hours. The pump has been in the well since July 1992. Cumulative production is 134,000 STB (21,300 m^3) oil, 32,000 bbl (5,090 m^3) water, and 557 MMCF (15.8×10^6 m^3) gas. The well was producing 12 BOPD (1.9 m^3/D), 12 BWPD (1.9 m^3/D), and 75 MCF/D (2,120 m^3/D) prior to the treatments. Its production history is shown in Figure 3.

Well 12AX11 was drilled in July 1985 and 5-in. casing was set and cemented at 3,163 ft (964 m). The well was perforated over a 38 ft (11.6 m) interval at 2 SPF (6.5 shots/m). It was hydraulically fractured with 25,000 lb (11,400 kg) 20/40 sand. In March 1989, the well was treated with solvent and paraffin inhibitor and displaced with asphaltic crude oil as described for Well 51AX15 above. The well has experienced problems with sand influx. The pump was changed during the microbial treatments in March 1994, when a hole was found in the tubing. Cumulative production was 102,000 STB (16,200 m^3) oil, 38,000 bbl (6,000 m^3) water, 764 MMCF (21.6×10^6 m^3) gas. It tested 7 BOPD (1.1 m^3/D), 13 BWPD (2.1 m^3/D), 87 MCF/D (2,460 m^3/D) prior to treatment. Its production history is shown in Figure 4.

Microbial Treatments

The four Second Wall Creek wells were prepared by performing a 10 bbl (1.6 m^3) treatment with 5% HCl to clean any scale from the pump and tubulars. The acid was dumped down the casing annulus and allowed to soak for one hour before being pumped up the tubing and into the flowline. The acid was not displaced into the reservoir.

Water samples were taken a week after the acid job and prior to microbial treatment, and checked for total chlorides to ensure a habitable range for the microbes. Well 12AX11 had 4,966 ppm, 62AX3 had 4,618 ppm, 51AX15 had 5,837 ppm, and 81AX10 had 6,099 ppm.

The wells were initially treated with a 100 bbl (16 m^3) commercially available microbe solution on November 17, 1993. An oil sample was collected from each well prior to the treatment and analyzed for baseline viscosity characteristics. The microbe solution was mixed with produced Second Wall Creek water to minimize possible formation damage. The solution was dumped down the casing annulus of the wells, and the wells were shut in for three days. The wells were then returned to production, and tested only twice per month, due to the large number of wells producing to the batteries.

The wells were treated with an additional 5 bbl (0.8 m^3) dosage monthly from December 1993 through September 1994. The microbial solution was allowed to soak for 24 hours before returning the well to production. Starting in March 1994, the wells were treated with condensate or hot oil one week prior to treatment with microbes to help remove the paraffin deposits that were being mobilized. These pretreatments continued through September 1994.

Results

Oil samples were gathered at the wellhead before the treatments in November 1993, and then monthly until August 1994. The samples were taken to a laboratory and the oil viscosity was measured as a function of shear rate for each sample. The viscosity data were then normalized to obtain the Newtonian Index and delta viscosity. These parameters are defined as follows:

$$\text{Newtonian Index} = \frac{\mu_i \text{ @ minimum shear rate} - \mu_i \text{ @ maximum shear rate}}{\mu \text{ @ minimum shear rate} - \mu \text{ @ maximum shear rate}} \quad (1)$$

$$\text{delta viscosity} = \frac{\Sigma\mu_i - \Sigma\mu}{\Sigma\mu_i} \quad (2)$$

where: μ_i is the initial oil viscosity prior to inoculation
μ is the oil viscosity following inoculation

Newtonian fluids exhibit constant viscosity as shear rate is varied.[1] Pseudoplastic fluids exhibit decreasing viscosity with increasing shear rate. Newtonian fluids have molecular compositions of a single size or a small range of sizes of molecules, like diesel oil and thin motor oil. Pseudoplastic fluids have a wide range of molecular sizes, such as colloidal particles and wax crystals. Most crude oils behave like pseudoplastic fluids. If microbes were improving paraffin control by metabolizing carbon atoms from the long-chained molecules, a decrease in pseudoplastic behavior should be expected. A positive increasing delta viscosity trend and an increasing Newtonian Index trend above 1.0 should be seen to verify favorable microbial action. Figures 5 and 6 summarize the results of these laboratory tests.

Well 81AX10 was on a sharp decline through 1992. The decline moderated in 1993, and only the 1993 data were used to determine a baseline prior to treatment. This may have caused a more conservative determination of the effects of microbe treatments. This well had a higher water cut than the other wells (approximately 90%). Following treatment with microbes, the well did show a definite and consistent increase in oil production, as shown in Figure 7. No conclusive effects on water or gas production were noted. The oil sample analysis showed a positive delta viscosity and an increasing Newtonian Index throughout the treatments. The incremental oil production is interpreted to result from microbial thinning of the oil causing a viscosity reduction and an increased Newtonian behavior. The well is estimated to have produced 834 bbl (133 m^3) of incremental oil above its previously established baseline. After subtracting the cost of $3,373 for the microbial treatments, and $4,200 for hot oil treatments, a profit of $6,084 was realized.

Well 62AX3 has been on a fairly steady decline throughout its history. Starting in late 1993, a steep decline in production was experienced. In September 1993, the well was circulated with solvent to remedy paraffin plugging. The treatment was only slightly successful in restoring production. This history made it difficult to establish a baseline of microbial treatments. The steep decline immediately prior to microbial treatments was used as a baseline. Production continued to follow this steep decline for seven months following the treatment, as shown in Figure 8. This production trend corresponds to the unfavorable behavior of the delta viscosity and Newtonian Index. This effect is attributed to the action of microbes mobilizing paraffin deposits that were previously immobile and causing a thickening of the oil and an increased pseudoplastic behavior. Starting in June 1994, a decrease in the decline rate is seen, accompanied by a reversal in the trend of increasing water production. Much of the success of this well is believed to be due to cleanup of paraffin deposits, resulting in a long-term decline curve shift. Gas production from

this well ceased after microbial treatments began. The reasons for the gas and water production decreases are not clear. It is possible that this was due to plugging of individual perforations contributing water and gas phases to production. This well is estimated to have produced 1,277 bbl (203 m^3) of incremental oil above its previously established baseline. After subtracting the cost of $3,373 for the microbial treatments, and $4,200 for hot oil treatments, a profit of $11,806 was realized.

Well 12AX11 experienced a sharp drop in production just prior to the microbial treatment. It was difficult to establish a baseline for this well due to erratic production. Since the sharp drop was suspected to be caused by paraffin, it was weighted heavily when establishing the baseline for the microbial treatments. The detailed production decline around the period of microbial treatments is shown in Figure 9. Production decreased for one to two months following the initial treatment, then started an increasing trend through August 1994. A sudden decrease in oil production was experienced in August 1994, which appeared to be due to paraffin plugging. The Newtonian Index and delta viscosity data remain unfavorable throughout this period. In this well, incremental production seems to be the result of the cleanup of paraffin deposits and the restoration of well productivity. This caused a decrease in the decline rate and yielded long-term incremental oil production. This well also exhibited a significant drop in gas production concurrent with the initial microbial treatment. No change in water production was seen. This well is estimated to have produced 1,092 bbl (174 m^3) of incremental oil above its previously established baseline. After subtracting the cost of $3,373 for the microbial treatments, and $4,200 for hot oil treatments, a profit of $9,322 was realized.

Well 51AX15 operations were altered from a nonvented casing annulus to a vented annulus shortly before the initial microbial treatment. This lowered the producing bottom-hole pressure and caused a significant increase in oil production. It was also affected by natural gas huff-'n' puff injection in an offset well several months into the treatments. These effects seriously impaired the ability to establish a baseline and quantify the effects of the microbial treatments. The production data for this time period are shown in Figure 10. Positive results are seen in the laboratory data in Figures 5 and 6. These data indicate a dominant thinning mechanism was occurring, rather than the damage cleanup mechanism seen in Wells 12AX11 and 62AX3.

One major problem encountered to some degree in all the wells was the production of very thick oil, starting approximately two months into the treatments. This is interpreted to be the product of microbial action breaking down the immobile paraffin depositions caused by the distillation effect of frequent hot oil treatments. This thick crude caused very erratic production tests, and required, at minimum,

monthly hot oil and/or condensate treatments to keep the perforations and pump intakes from plugging.

The pump-off controllers on the wells were invaluable in identifying when the wells were plugging and in need of hot oiling. Due to the added attention these wells were receiving, production was maximized. However, this thick fluid production could have had a devastating effect on oil production, if poor inflow performance had not been quickly identified and remedied. The cost of the added attention given these wells by the pumpers could not be quantified and is not included in the economic analysis of these treatments.

The treatments were discontinued in September 1994 due to the marginal economics realized at that time and the higher level of attention required. The beneficial effects of the treatments are still ongoing, improving the economics significantly from when the decision was made to discontinue treatments. Production decline rates have been lowered as shown in Figures 7 through 10. It is still not known how long these effects will persist. The most incremental oil was obtained from wells where paraffin damage cleanup occurred, rather than from oil thinning.

Microbes have potential as an economic treatment for wells with paraffin damage. Another program is currently being considered and some variations on the application may be used. One such variation would be to use a larger treatment to inoculate more of the reservoir surrounding the well and allow a longer soak time. Another variation is to inoculate wells prior to natural gas or air huff-'n'-puff treatments. This combination of microbial viscosity reduction and increased wellbore area pressure may cause more incremental oil production than the sum of incremental production of both processes employed separately.

Conclusions

1. Positive economic results can be seen in the field from using microbes to treat wells with paraffin problems.

2. Wells with significant paraffin deposits will go through a production phase of heavy, thick fluids as these deposits are cleaned up. Additional hot oiling is required during this period to aid the cleanup process. These types of wells yielded the highest incremental oil production.

3. Monitoring produced oil for changes in Newtonian Index and delta viscosity is a good indicator of microbial action and the mechanism causing incremental oil production.

4. Pump-off controllers are very useful during microbial treatments on intermittently rod-pumped wells to facilitate production during periods of variable fluid inflow, and also to help identify downhole plugging conditions.

Acknowledgments

The authors wish to thank the U.S. DOE, Fluor Daniel (NPOSR), Inc., and Fred Brown of Microbes, Inc. for their permission to publish this paper and their help in doing so.

References

1. Brown, F. G. Personal communication. May 24, 1994.

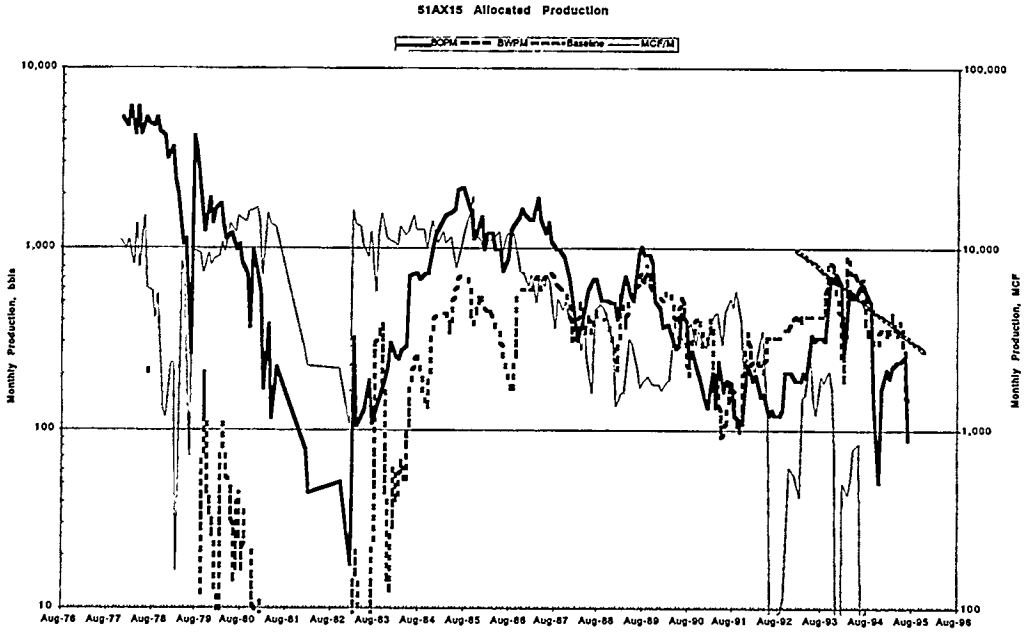

Figure 1 Well 51AX15 History

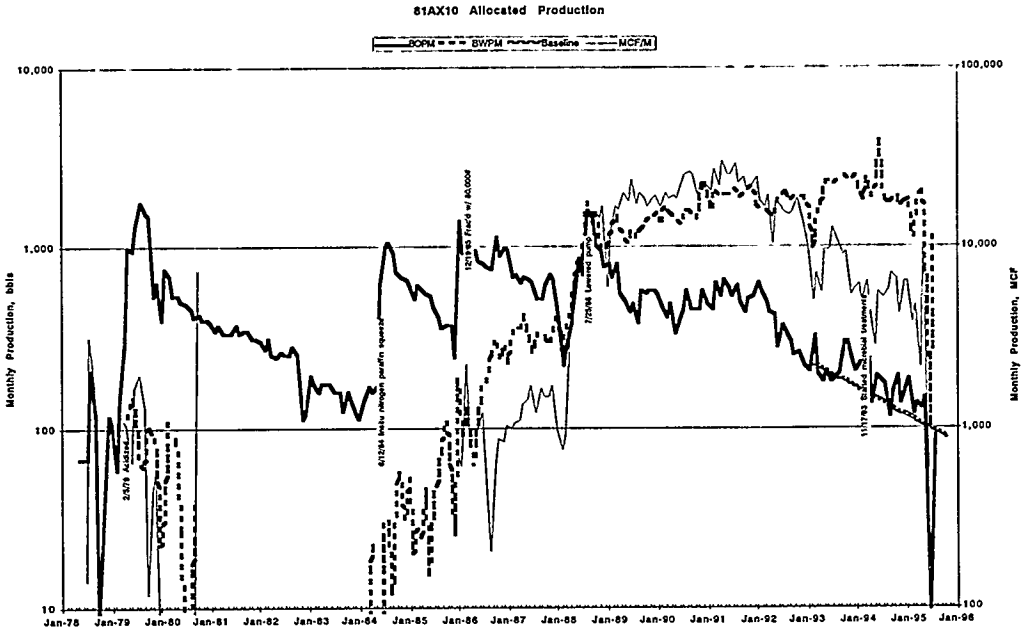

Figure 2 Well 81AX10 History

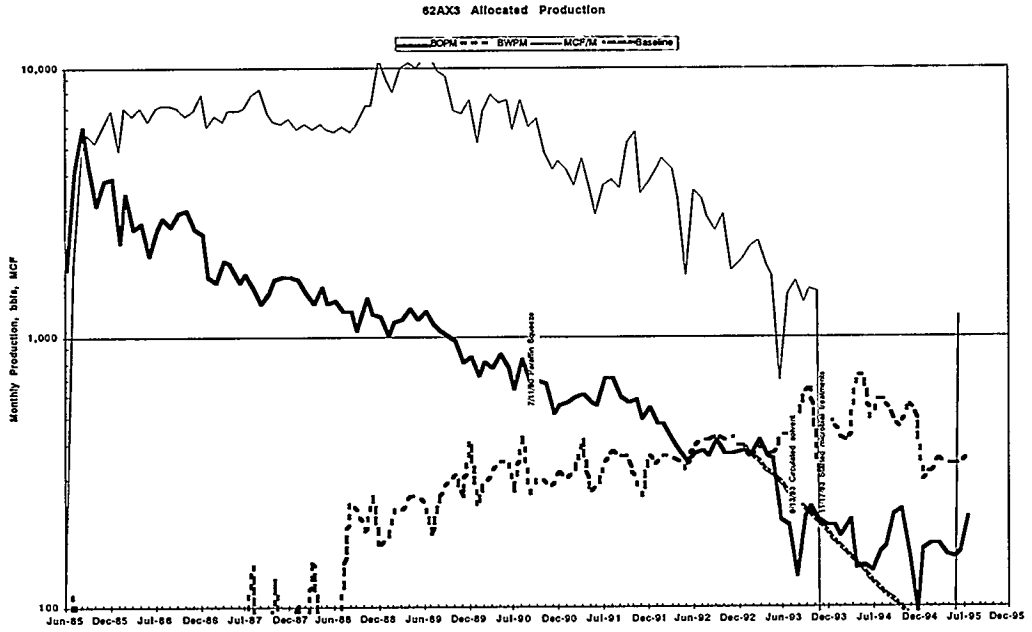

Figure 3 Well 62AX3 History

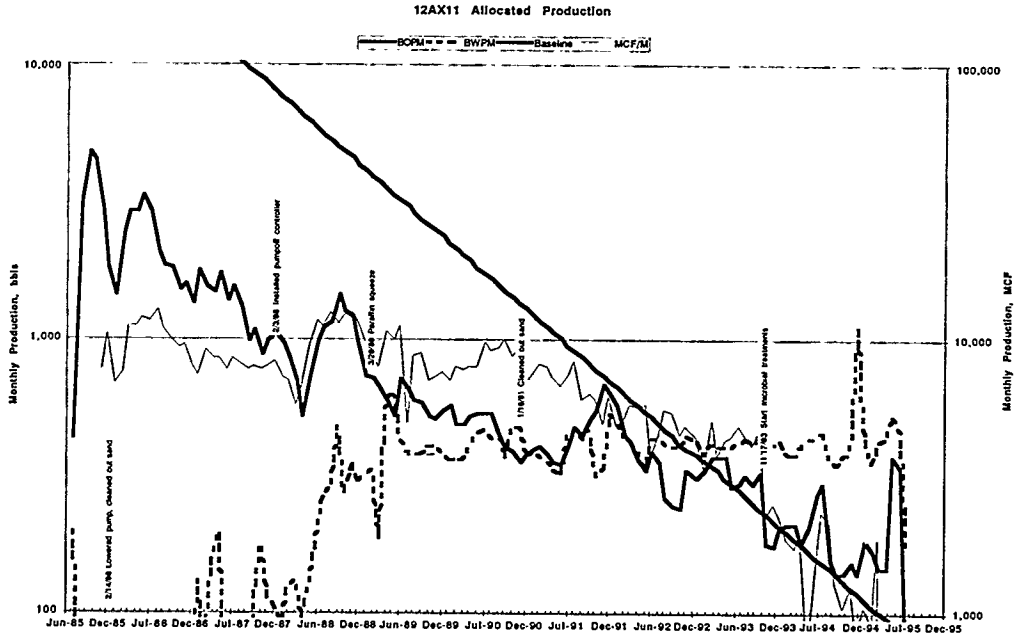

Figure 4 Well 12AX11 History

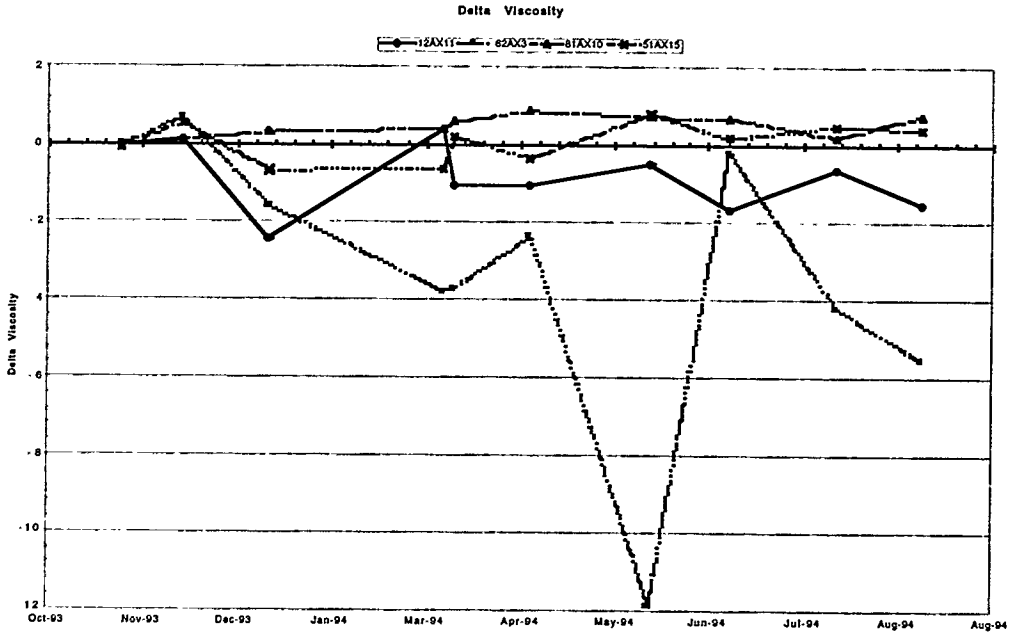

Figure 5 Delta Viscosity

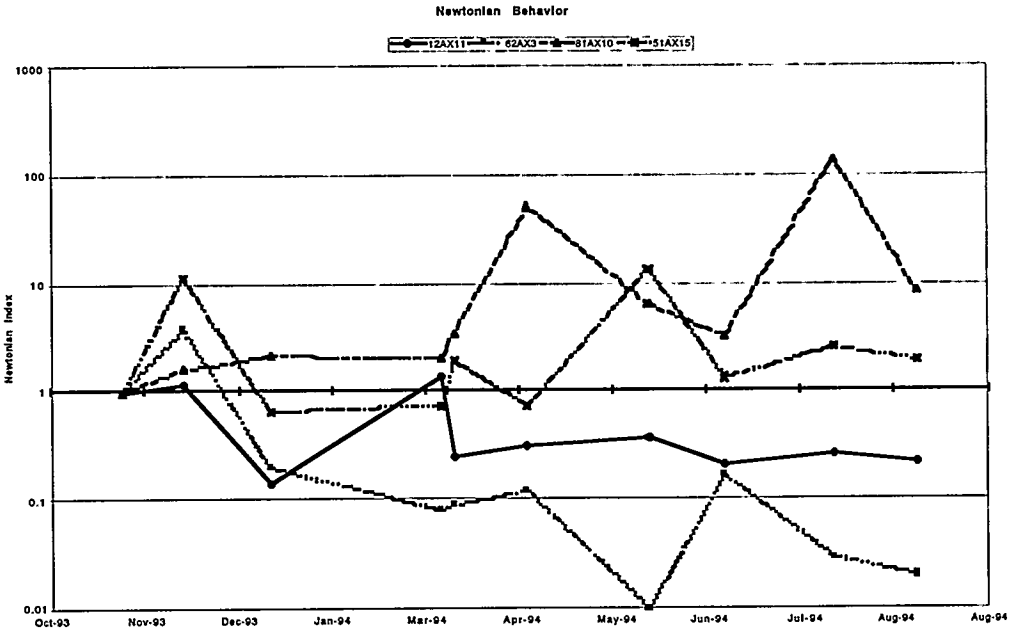

Figure 6 Newtonian Behavior

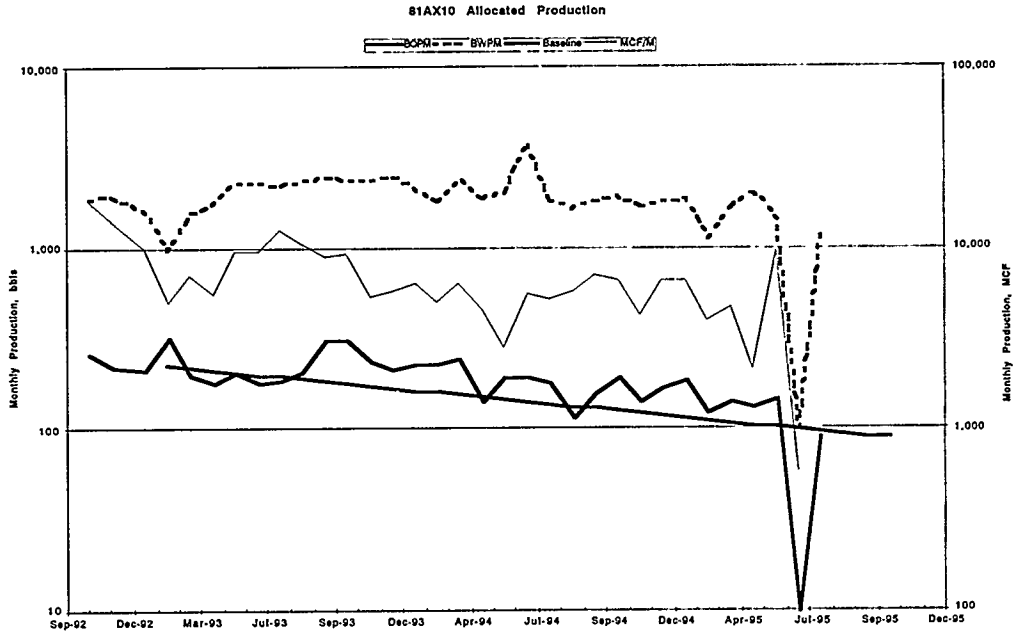

Figure 7 Well 81AX10 Results

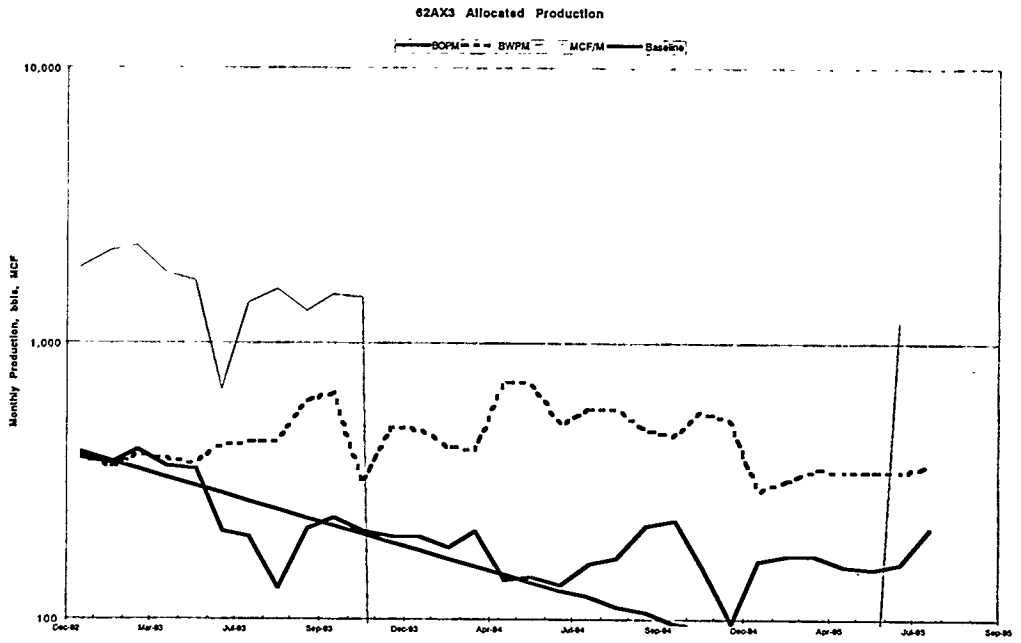

Figure 8 Well 62AX3 Results

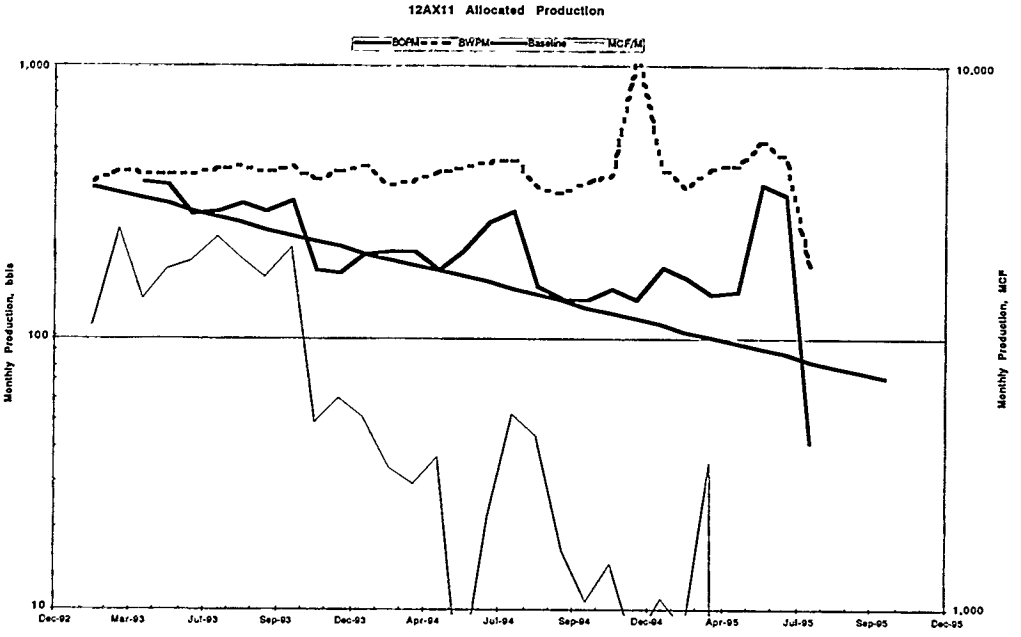

Figure 9 Well 12AX11 Results

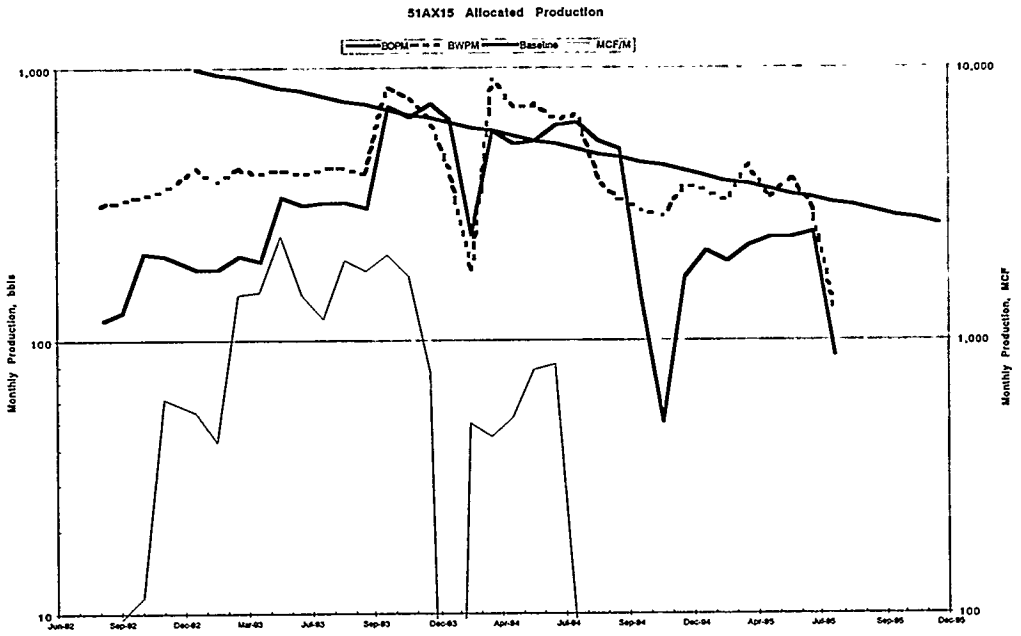

Figure 10 Well 51AX15 Results

EOR by Stimulated Microflora

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Abstract

A combined microbiological and physico-chemical method for EOR has been developed for flooded West Siberia oil fields with formation temperature of 45°–95°C (318–365K). Formation water includes rich and various biocenoses numbering up to 2×10^7 cells per ml. Representatives of genera, i.e. *Pseudomonas, Bacillus, Actinomyces, Micrococcus, Mycobacterium, Sarcina,* etc. were found to be the most widely distributed microorganisms. The method is based on injection of systems exhibiting high oil displacing capacity and at the same time being an additional nitrous nutrient for endemic populations of microorganisms. Their injection into formation water favors biomass growth by 4–6 orders and promotes syntheses of biosurfactants, biopolymers, acids, etc., and gaseous products.

The features of residual oil displacement have been studied on laboratory models using a combined microbiological and physico-chemical method. A curve for the yield of residual oil is presented by two peaks. The first peak is stipulated by the washing action of oil displacement system, and the second one by the effect of metabolites produced at stimulation of biogenic processes. Oil displacement index increases by 15%–30%.

Introduction

Stimulation of endemic populations of microorganisms is one of the main trends in the development of microbiological methods for EOR employed in the oil fields at later stages of their development by waterflooding. The injection of nutrient substances promotes growth and vital activity of reservoir microflora and the formation of metabolites promoting oil displacement.

Major oil fields of West Siberia are being developed by waterflooding. An aqueous phase containing dissolved salts and organic compounds promotes the growth of reservoir microflora. The saturation of reservoir biocenoses, temperature regime, low salinity, and reservoir heterogeneity create favorable conditions for the application of microbiological methods for EOR.

Therefore the development of a combined microbiological and physico-chemical method is a promising solution for EOR problems. The method is based on the injection of systems exhibiting high oil displacing capacity and at the same time being a multicomponent nitrous nutrient substrate that stimulates reservoir biocenoses. Oil displacement is performed by the systems themselves and by the stimulated microflora.

Results and Discussion

Microflora of West Siberia Oil Fields

Studied were physico-chemical properties and composition of microbial associations of the formation waters obtained for the following oil fields of West Siberia: Sovetskoye, AV_1 formation, temperature 45–55°C (318–328K); Vakhskoye, JV_1, 90–95°C (363–368K); Lor-Yeganskoye, BV_{10}, 80°C (353K), and Samotlorskoye, AV_1, AV_{2-3}, and BV_{10} formations, 50–80°C (323–353K). The samples from 160 injection and producing wells have been studied. The formation waters of the investigated horizons are classified as a chlorine-calcic type including sulfates, their salinity being in the range of 10 to 40 g/l. The formation pressure was 18–24 MPa. It was maintained by the flooding with surface, fresh, Senoman, and brine waters or their mixtures. Redox potential was 90–200 mV. Aqueous phase of low salinity with dissolved organic additions and other natural factors create the conditions suitable for the development of the reservoir microflora. Flooding recovery with surface and fresh waters decreases the salinity of the formation water favoring the growth of microbial association including aerobic and anaerobic organisms.[1, 2]

The composition of the reservoir microflora has been analyzed by a routine sowing of aqueous and oil samples on nutrient media to detect several species and physiological groups of microorganisms. The absence of sulfates in the formation waters maintains a low level of sulfate-reducing bacteria (SRB), the number of which was below 2,500 cells/ml. Thermophilic microorganisms of *Desulfotomaculum* and *Thermodesulfobacterium* genera have been determined to predominate in SRB.

A wide distribution of thermophilic spore-forming bacteria is typical for hydrocarbon oxidizing microflora from high temperature reservoirs of West Siberia oil fields. The probably high formation temperature of Jurassic and Cretaceous deposits (70–95°C, or 343–368K) in Samotlorskoye, Vakhskoye, and Lor-Yeganskoye oil fields is a selective factor favoring the selection of stable thermophilic spore-forming and vegetative microorganisms. Periodic cultivation of the reservoir microflora in pentane, hexane, heptane, octane, hexadecane, and aromatic compounds—benzene and toluene—is characterized by the accumulation of acid products, decrease in redox potential from +250 to +30 mV and by a synthesis of fluorescing yellow-green pigment. The number of hydrocarbon oxidizing group was in the range of 0.5 to 3.0×10^6 cells/ml.

A denitrifying group of microorganisms is the most widely spread in the oil fields of West Siberia. It includes representatives of these genera: *Pseudomonas, Actinomyces, Micrococcus, Bacillus, Mycobacterium,* and others. The number of the denitrifying group exceeds 2×10^6 cells/ml. Biogenic processes of denitrification were studied under aerobic and anaerobic conditions. In the absence of oxygen, the second fermentation system of bacteria functions. It allows using nitrate oxygen as a hydrogen acceptor in oxidation of organic substances. Under reservoir conditions where the oxygen gap occurs, denitrification proceeded, reducing nitrates to nitrites and N_2 with free nitrogen and carbonic gas evolution.[1,2]

The activity of denitrification processes was determined by the change in redox potential. Reservoir microflora from Sovetskoye oil field, AV_1 formation, 45°–55°C (318–328K), decreased the potential from +210 to –150 mV. In other oil fields with formation temperature of 70°–95°C (343–368K) the redox potential decreased up to –70 mV. The highest microflora activity was observed at injection of surface waters with a decreased redox potential up to 600 mV.

The total amount of heterotrophic microflora determined by growing on beef-extract agar varied in the range of 4×10^5 to 1×10^7 cells/ml. The number of tionic [sic] and methane-oxidizing organisms in the formation waters was in the range of 1 to 10^5 cells/ml. Biocenose from AV_1 formation, Sovetskoye oil field, had the most various genus composition. Microorganisms of the genera *Pseudomonas, Actinomyces, Micrococcus (Phodococcus), Bacterium, Micobacterium, Sarcina,*

Mycoccus, Pseudobacterium, and *Thiobacillus* were the most widely distributed heterotrophs in this oil field, where *Pseudomonas* predominated. They constituted about 70% of the reservoir microflora.

Spore-forming *Bacillus* bacteria are the most distributed microorganisms in the formation waters from Vakhskoye oil field with formation temperature of 90°–95°C (363–368K). They have various specific composition. We have determined more than 12 species of *Bacillus* bacteria. In Talinskoye oil field, JK_{10-11} formation, 90°–100°C (363–373K), *Bacillus* constituted up to 80% of the microflora.

Thus, formation waters from Cretaceous and Jurassic deposits in West Siberia oil fields, with temperature of 45–95°C (318–368K), salinity 10–40 g/l, and water cut up to 90%, are characterized by a rich and various biocenoses. Natural conditions in oil fields are favorable for the application of microbial methods for EOR.

Oil Displacing Systems As a Nutrient Substrate for Reservoir Microflora

Pilot operation testing of EOR technologies for low-permeability formations has been carried out in the oil fields of West Siberia using the systems based on surfactants and ammonia buffer system (IKhN and IKhN–KA systems). They may be used in a wide range of formation temperatures and salinity for low permeable and heterogeneous reservoirs. The components of the buffer system are a constituent part of nitrogen geochemical cycle and serve as an additional nitrous substrate for the reservoir microflora.[1, 3, 4]

Systematic study of the formation biocenoses in the areas of IKhN and IKhN–KA system injections showed a significant growth of heterotrophic microflora, a denitrifying group included. In the recovered waters from the wells of the test area, the total amount of heterotrophes increased by 5–10 million cells/ml. The average amount of microorganisms of genera *Pseudomonas* was 6 million cells/ml, of *Actinomyces*—6–10 million cells/ml, of hydrocarbon oxidizing group of bacteria—2.5 million cells/ml, and of a denitrifying group—5–11 million cells/ml. Usually the cultures constitute associations under the formation conditions and sorb on the formation rocks, impeding their measurement. Pilot operation testing, carried out in the oil fields of West Siberia using IKhN and IKhN–KA systems, showed the feasibility for a directed stimulation of the endemic reservoir microflora.

Based on oil displacing IKhN–KA systems, substrates have been developed providing the reservoir microflora with an additional multicomponent nitrous nutrition. Normally, for the formation fluids of the chlorine-calcic type, nitrous compounds are a limiting substrate. The addition of these compounds to the

formation water significantly stimulates microbial growth and development. Sterile formation water from Sovetskoye oil field containing 0.1%–10% solution of IKhN–KA system was used as a cultural medium.

Associations and pure cultures of microorganisms, separated from the formation water, served as inoculate. The addition of 0.1%–10% solution of IKhN–KA system promotes vital processes of microflora, i.e., its growth and development. Maximum growth of microbial populations of *Bacillus* and *Pseudomonas* genera from 10^3 to 10^8–10^{10} cells/ml was observed at the concentration of 0.1%–0.5%. The study of growth kinetics of spore-forming *Bacillus* bacteria in the formation water containing 0.1% solution of IKhN–KA system showed the increase of cell numbers by 3–4 orders on the third day of cultivation. When we added only system components the number of hydrocarbon oxidizing populations increased by 4–6 orders on the fifth day. The system solution, used as a multicomponent nutrient substrate, not only stimulates the growth and development but also increases biochemical activity of microflora, as well as the processes of denitrification and oxidation of oil hydrocarbons. The deepest destruction of n–paraffins with a degradation index of 0.23–0.27 was observed by the bacteria stimulation of *Pseudomonas*, *Bacillus*, *Micrococcus,* and *Micobacterium* genera. A consortium culture contacted oil during 30 days, but already on the fifth day chromatographic analysis showed oxidation of heavy n-alkanes C_{21}–C_{24}. Oil oxidation was accompanied by a decrease in the interfacial tension.

Directed effect on the endemic microflora by the injection of oil-displacing IKhN–KA system stimulates the growth and development of microorganisms, oil oxidation, and synthesis of metabolites. The result is increased conformance control and enhanced oil recovery.

The Study of Residual Oil Displacement by Stimulated Microflora

The processes of oil displacement by a combined microbiological and physico-chemical method for EOR have been studied under laboratory conditions. Reservoir microflora was stimulated by an IKhN–KA system that exhibited high oil displacing capacity and was a source of multicomponent nitrous nutrition for microorganisms.

The displacement of residual oil has been studied under the simulating reservoir conditions. Studied were the formations from Cretaceous deposits (group A) of Sovetskoye oil field, typical for West Siberia. Linear bulk models of the reservoir were used. Their permeability was in the range of 2 to 10 mkm, and pore volume was 38–45 cm. Dead oil from Sovetskoye oil field, AV_1 formation, was used for

saturation at a density of 0.8725 g/cm and a viscosity of 11.38 mPa·c at 25°C (298K).

A model of the formation water from Sovetskoye oil field was used for oil displacement, its salinity being 21.06 g/l, density 1.01 g/cm, and viscosity 0.97 mPa·c at 25°C (298K). Its composition was as follows, g/l: 16.3 NaCl; 3.9 $CaCl_2$, 0.66 $MgCl_2$, 0.15 $KHCO_3$, and 0.004 Na_2SO_4. The oil displacement was carried out until water cut reached 100%. Indexes of oil displacement by water were in the range of 31% to 47%.

An aerobic hydrocarbon oxidizing group of microorganisms was separated from the formation water to simulate a process of oil displacement by stimulated microflora. The group contained thermal heterotrophes of *Pseudomonas*, *Bacillus*, *Micrococcus*, and *Actinomyces* genera. Reservoir models containing residual oil were treated with a pore volume of nutrient medium including 0.5%–1.0% solution of IKhN–KA system to stimulate microflora and microbial suspension (200–600 million cells/ml). After that the models were shut in and incubated for 5–14 days at 45°C (318K). After incubation the displacement was carried out with 5–10 PV of sterile formation water. Outlet contents of oil, microorganisms, and IKhN–KA system components were determined. The amount of microorganisms was analyzed by the method of maximum dilution and growing on beef-extract agar. Microbial cells sorbed on hard porous model surface were scraped by Tween-80 and analysed by the same method. During incubation one can observe intensified growth and development of microflora and the accumulation of the metabolic products. After incubating the rate of liquid filtration through the model decreased 5–30 times.

At the displacement of residual oil by the reservoir microflora containing no stimulating substrate, the increase in oil displacement index was in the range of 2.6% to 6.5%, and absolute displacement index did not exceed 43%. Figure 1 presents the dynamics of residual oil yield (in wt% relative to total displaced oil). Maximal yield of microorganisms was registered after the yield of residual oil.

In the use of stimulated microflora, the absolute displacement index was 63% to 72%, and the increase in displacement index was in the range of 15% to 30%. Kinetics of residual oil displacement by stimulated microflora is presented in Figure 2. The yield of residual oil (in wt% relative to total displaced oil) is presented by two peaks. The formation of the first peak is likely connected with oil displacing capacity of IKhN–KA system, while the second one is oil displacement by stimulated microflora. The concentration of major microorganisms was observed after oil yield (see Fig. 2), their maximal amount in the samples was 5×10^8 cells/ml. It indicates intensive growth and development of microflora.

Thus high efficiency of a combined physico-chemical and microbiological method for EOR is realized due to the oil-displacing capacity of the IKhN–KA system itself and stimulated reservoir microflora.

Conclusion

A combined physico-chemical and microbiological method for EOR has been proposed for oil fields developed by waterflooding. The method is based on injection of systems exhibiting high oil displacing capacity and at the same time being an additional nutrition source intended to activate reservoir microflora.

Formation waters from Cretaceous and Jurassic deposits of West Siberia contain rich and various biocenoses numbering up to 20 million cells per ml. Bacteria of the *Pseudomonas, Bacillus, Actinomyces, Micrococcus, Mycobacterium, Sarcina,* etc., genera were found to be the most distributed microorganisms. *Pseudomonas* predominate in Cretaceous deposits (about 70%). Hydrocarbon-oxidizing microflora of high temperature formations is predominantly presented by thermophilic spore-forming bacteria (*Bacillus* genera). A denitrifying group of microorganisms is widely distributed. It creates favorable conditions to use microbiological methods to enhance oil recovery.

The study of residual oil displacement by stimulated microflora under the conditions simulating that of the formation showed that the yield of residual oil occurs in the form of two peaks: the first peak corresponds to oil displacement by IKhN–KA system, while the second one is by stimulated microflora. Absolute displacement index is 63%–72%, and the increase of displacement index relative to waterflooding is 15%–30%.

The method is ecologically harmless, easily producible, and can be realized by standard equipment. It is promising for oil fields developed by waterflooding especially at later stages.

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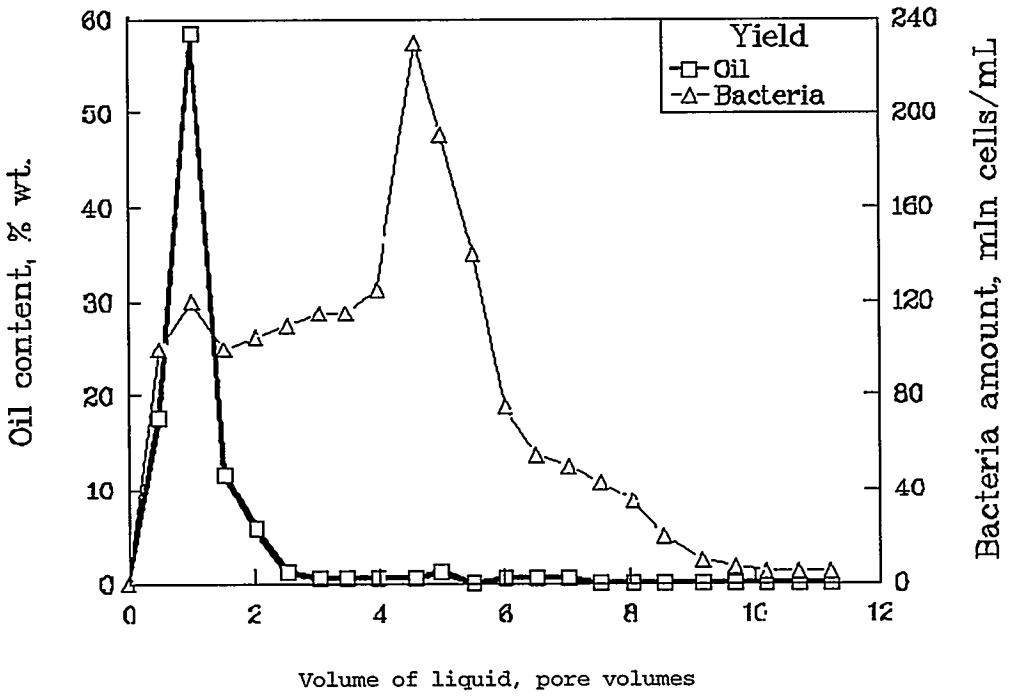

Figure 1 Dynamics of Residual Oil Displacement by Formation Water Containing Microflora

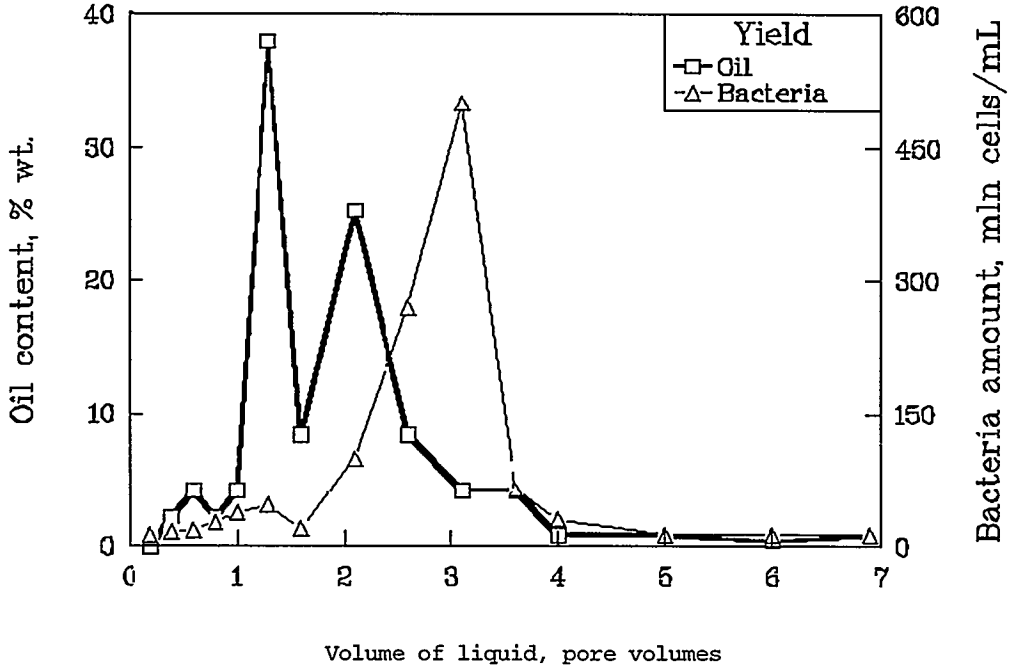

Figure 2 Dynamics of Residual Oil Displacement by Microflora Activated with IKhN–KA System

Development and Application of a New Biotechnology of the Molasses In-Situ Method; Detailed Evaluation for Selected Wells in the Romashkino Carbonate Reservoir

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Abstract

On the basis of different laboratory studies, by which special strains of the type *Clostridium tyrobutyricum* were found, the application of molasses in-situ method for the enhanced recovery of oil in Romashkino oil field was executed. In an anaerobic, 6%–molasses medium the strains produce about 11,400 mg/l of organic acids (especially butyric acid), 3,200 mg/l ethanol, butanol, etc., and more than 350 ml/g of molasses biogas with a content of 80% CO_2 and 20% H_2. The metabolics of *Clostridium tyrobutyricum* depress the growth of SRB, whereas methanogenic bacteria grow in an undiluted fermented molasses medium very well. In this way the dominant final fermentation process is methanogenesis.

By laboratory studies with original cores under the conditions of the carbonate reservoir in Bashkir, the recovery of oil increased from 15% after waterflooding to 29% OOIP during the treatment with molasses and bacteria.

We developed a new biotechnological method for a self-regulated, automatic continuous culture and constructed a special pilot plant with a high technical standard. The plant produced during the pilot on Romashkino field (September 1992 to August 1994) about 1,000 m^3 of clean inoculum with a content of 3–4 billion cells per ml. This inoculum was injected in slugs together with 15,000 m^3 of molasses medium, first in one, later in five wells.

We will demonstrate for two example wells the complex microbiological and chemical changes in the oil, gas, and water phases, and their influences on the recovery of oil.

The highlights of the pilot results are:

- Decrease of water cut in the field by about 20%, from 80% to 60%
- Increase of well production rate between 50 and 65
- A higher gas/oil ratio
- Desorption of oil from the carbonate rock
- Enlargement of injection in corresponding wells

Introduction

EOR allows the world's oil reserves (140 billion tons) to increase by approximately 70 billion tons, an additional recovery of 10% of the OOIP (original oil in place). With a current production level of approximately 3.2 billion t/a, this represents an additional production of at least 20 years. About 50% of the world's oil reserves lie in carbonate reservoir beds. The problem is that the EOR technologies, such as polymer, surfactant, and alkali flooding, cannot be applied to carbonate rock, or at the most provide a minimum of efficiency.

As a part of the EOR technologies, and as the molasses in-situ process, MEOR is particularly suited for application in carbonate oil reservoirs. In contrast to sandstone reservoirs, the injectivity and spread of the bacteria within the layer are more favorable in fissured porous carbonate rocks. There are no problems with blocking of the injection well or the oil-bearing layer as a result of filtration, adsorption, or bacterial growth.

The organic acids which are formed through fermentation of the molasses by the bacteria in the reservoir do cause a rock-dissolving process. An increase in permeability is arrived at, and fresh sections of the matrix which are not quite drained will be pulled into the oil extraction.

Erdöl-Erdgas Gommern GmbH has concerned itself with molasses in-situ processes since 1967, and has carried out field experiments in a carbonate reservoir with formation water of high mineral content. Huff 'n' puff as well as microbial flooding throughout the field was successfully applied.[2]

In order to pursue these matters further, we carried out preparatory microbiological and technical investigations for a pilot experiment in the Bashkir horizon of the Romashkino reservoir on behalf of the Russian Science Academy between 1989 and 1991. Reports on these investigations were made at the Biohydrometallurgy Congress, Jackson Hole, Wyoming, in 1993, as well as at the 7th European IOR Symposium, Moscow, in 1993.[3,4]

Pilot Field 302 of the Romashkino Reservoir

The Romashkino reservoir lies in the Republic of Tatarstan, CIS (see Fig. 1). MIOR is to be carried out in the Bashkir horizon, a dual porosity fractured reservoir with organic limestone of lower Middle Carboniferous age. The top of the Bashkir lies at a depth of between 493 and 515 m. The average thickness of the layer is 5.7 m. The Bashkir is very heterogeneous with alternating zones of low (1%–3%) and

high (13%) porosity. The pore radius is from 2.4 to 3.8 μm. A distinct secondary overlaid (frac) major fissure system exists, stretching from NE to SW, as well as a vertically standing microfissure system.

Concordant with the shallow depth, the layer temperature is low (21°C) as is the rate of mineralization, 30–40 g/l (see Table 1).

The Bahskir has a medium oil viscosity of 50–80 mPa and an oil density of 0.903 g/cm^3.

Production in the pilot zone of the Bashkir began in 1975 with well 512. Between 1980 and 1982, 29 wells were drilled (see Fig. 2), of which 6 were injection wells and 23 production wells. Water injection began in 1981, as a measure to maintain pressure. Up until 1991 a total of 244,615 m^3 of water was injected; to begin with at a constant rate, and then in cycles. River water was used for flooding purposes. This, along with the mild layer conditions, led to an intense bacterial settlement of the Bashkir (see Table 2).

Preparation for the Pilot Experiment

The target of preparatory work was to find suitable production strains for the molasses in-situ process and to develop a biological process technique, which would make possible, in spite of the high heterogeneity in the Bashkir, the success of a guided and dominant fermentation process which guarantees an improved oil production.

Screening

For a dominant fermentation process, the production strain must have the ability to suppress the existing bacteria in Bashkir. The requirements for this are a rapid growth and a high rate of product formation under layer conditions.

In one screening, hundreds of strains of anaerobic fermenting bacteria were isolated. Only three strains of the *Clostridium tyrobutyricum* genus fulfilled the requirements. At a reservoir temperature of 20°C they grew in a 4% molasses medium with a generation time of 4 hours and reached a maximum concentration of 3–4 billion cells/ml. The principal metabolism products are shown in Table 3.

Change in Effect of Layer Microflora

Extensive work was carried out in order to investigate the change in effect of the *Clostridium tyrobutyricum* with microorganisms of the Bashkir, of floodwater used for flooding purposes, and of molasses. It was maintained that the dominant fermentation process with ≥90% of the product yield of the pure culture can be utilized, if:

- The injection medium is inoculated so that $\geq 10^7$ young cells of *Clostridium tyrobutyricum* is contained.
- In feeding media with >2 wt% of molasses, there exists such a content of metabolism products, in particular alcohols, that sulfate-reducing bacteria (SRB) are killed off or have their growth repressed (see Fig. 3).
- As a result of specific inhibition of SRB, the final anaerobic formation of organic acids, alcohols, and hydrogen occurs in the presence of sulfate by methanogenesis.

In Figure 3, *Clostridium tyrobutyricum* and a mixed culture of SRB (see Fig. 3) are cultivated together in a medium containing sulfate (1 wt%) and molasses (2 or 6 wt%). Independently of the starting cell content of the SRB, 10^2 or 10^6 cells/ml in the medium with ≥2 wt% of molasses produces a multiplication of the SRB. Whereas in the medium with 6 wt% molasses, the SRB, after product formation due to the Clostridia, have been killed off by the third day. The remaining cell content is formed by inactive SRB spores, H_2S does not appear in media ≥4 wt% of molasses.[5]

Oil Recovery Experimental Model

In more than 30 laboratory model experiments, the best strains were selected according to their effect on improvement to oil production. Static (imbibition test) and dynamic attempts (flood experiments) were carried out under the temperature and pressure conditions of the Bashkir. An example of this is illustrated in Figure 4. An original core of the Bashkir (12 cm long, 6 cm diameter) was flooded to an irreducible water saturation of 21% which was saturated with dead crude of Romashkino (58 mPa). In the first phase of waterflooding over 83 days, an output of 15% OOIP was attained. The injection of a molasses nutrient solution and *Clostridium tyrobutyricum* led to a total oil recovery of 28% OOIP. In the core container during the MIOR a pressure increase from 70 to 90 bar was measured. This was caused by the formation of biogas. The organic acids which were caused

by the molasses fermentation released carbonate solids (approximately 5 t molasses released 1 t of carbonate), which led to an increase in porosity from 10% to 11.9%.

The greater release of oil due to MIOR can be explained by:

- Energy due to in-situ biogas formation
- Change in pressure potential in fissures and pores
- Decrease in viscosity of oil by increase in volume and hydrogenization
- Change in border surface tension oil/water
- Improvement of capilliary absorption of medium—imbibition
- Increase of permeability; bringing in of fresh matrix by rock solution

Operation of Pilot Project

In the period between 1992 and February 1995 the pilot experiment of a German-Russian research and development project was realized.

The partners on the Russian side were:

- Institute for Microbiology of the Russian Academy for Science, Moscow
- Proizvodstrennoe obedinenie "Tatneft," Altmetjewsk

and on the German side:

- Erdöl-Erdgas Gommem GmbH
- VEBA OEL AG, Gelsenkirchen

The project was promoted on the Russian side by the Ministry for Science, and on the German side by the Ministry for Education, Culture, Science, and Technology (MBKWT).

Biotechnological Plant and Field Equipment

As described previously, one of the requirements for the realization of a dominant fermentation process in a reservoir of high settlements is the extended entry of sufficient quantities of inoculum over the entire injection of nutrient medium. An inoculation ratio of 1: 10 to 1: 50 is the target. To guarantee this in the field, modern biotechnological equipment is essential. As a result of specific processing technological experiments by Erdöl-Erdgas Gommem GmbH, a biotechnological plant for the inoculation process of the pilot field was developed (see Fig. 5).

By using the process of a self-regulating, continuous culture of *Clostridium tyyrobutyricum,* it was possible to reduce the container volume to such an extent that a mobile plant could be completed in Germany and installed in the pilot region. It has a performance level of 10 to 15 m^3 inoculation material per day, which is sufficient for an injection volume of 100 to 500 m^3 per day.

Figure 6 shows by the use of examples the cultivation of a pure culture of *Clostridium tyrobutyricum* in a sterile 2.5 m^3 field fermenter. The cultivation of the inoculation material takes place, by the use of lyophilisates, in the field laboratory up to the 10 liter level. In the 2.5 m^3 production fermenter 900 liters of nutrient medium was first of all sterilized with a content of 6 wt% molasses; 0.3% NH_4CI, and 0.3% polyphosphate. The inoculation took place with 100 liter preculture, which was prepared in a special fermenter in the biotechnological plant. The initial cell content was 2×10^8 cells/ml. Within the first 12 hours, a cell content of 1.2×10^9 cells/ml under automatic pH regulations will be reached. After this, the switchover will be made to self-regulating automatic nutrient solution application. The culture demands, corresponding to its activity (acid production), a strong alkaline molasses medium (pH >11), which maintains the pH value in the fermenter at a constant level. After about 22 hours, the maximum fermenter capacity has been reached, and the inoculation fluid overflows in the container tank. After about 28 hours, the fermenter continuously produces 700 liter/hr of inoculation fluid with a content of 2.8×10^9 cells/ml. From the storage tank the inoculation fluid is automatically pumped off in slugs of about 2 m^3 and then injected with the molasses medium.

Injection

At the end of September 1992, a test injection in the central well 427 was started. Until the end of October 1992, a nutrient solution of 612 m^3 with 56 tons molasses and 225 m^3 inoculum was injected. On completion of injection the well was closed. At the end of May 1993 injection in well 427 continued. Four injectors (423, 434,

418, and 421) were gradually converted from water injection to inoculated molasses solution (see Table 4).

MIOR treatment was continued until October 1993 and was resumed after the necessary winter break in a third phase from June 1994 through August 1994. Figure 7 illustrates the cumulative injection procedure. A total of 15,225 m^3 nutrient medium with 1.023 tons molasses and 1,205 m^3 inoculum had been injected. This volume equalled approximately 5% of the pore space of the pilot field.

Observations Made on Producing Wells

In the field laboratory installed on site, EEG performed comprehensive analytical studies on all 23 producers of the pilot field. In addition, INMI and Tatneft regularly performed testing. These studies and tests covered gas analyses (C_1 to C_4 hydrocarbons, carbon dioxide, hydrogen, H_2S), analysis of stratum water, especially organic acids, alcohols, pH, density, cations (Ca^{2+}, Mg, NH_4^+), anions Cl^-, HCO_3^-, SO_4^-), as well as the melanin dyes of molasses. The latter agents had proved to be good tracers of MIOR fluids. In the period under review, the injected MIOR fluids or metabolites of bacteria had not yet arrived at all wells in the test field.

Modifications to the stratum are demonstrated especially on well 437 and 432. Well 437 is located approximately 580 m southeast of the central injector 427; its production behavior strongly depends on the latter. In a tritium tracer test a good flow link with 427 was observed already after 10 days. Well 432 is located in the central field and is influenced both by injectors 427 and 431.

Melanin indications (see Fig. 8) show that MIOR fluids arrive, as early as 13 days after injection, at well 437 (150 color units) and to a less extent at well 432 (40–80 color units). After 19 days the first individual Clostridia were observed on well 437, i.e., other observations made on sandstone fields show an extension of the media front also for fissure reservoirs. The propagation of *Clostridium* had grown to 1×10^4 cells/ml by the end of injection in 1994.

CO_2 Balance

The determination of the CO_2 content of the accompanying gas and of the HCO_3^- content of stratum water provided strong indications of the fermentation process in the reservoir (see Fig. 9).

At well 437 the CO_2 content increased as early as 1992 from 3.5 I/O to 10% within 12 days, an equivalent development was observed on well 432. As injection was continued, the CO_2 content increased to 39.4% on well 437 in 1993 and on well 432 to 15.8% in 1994.

The observation of the HCO_3^- content of stratum water revealed, after an initial slight increase in the 1992 winter season, a rise on all wells in the test field from an average rate of 0.1 to 0.4 g/liter to 0.7 to 0.9 g/liter. This is proved by the rapid propagation of CO_2 generated during the fermentation process, across the total test field. In the further course of the test, an increase of the HCO_3^- content to 300%–400% was recorded, for some wells even to 80%–100%, on well 437 the content increased, for example, to 3.5 g/liter. At an average increase of 1 kg HCO_3^-/m^3 of stratum water and a water of 350,000 m^3, a CO_2 volume of 128,450 m^3 was bound.

The CO_2 balance of natural gas was increased by approximately 4% for the entire field. Considering also CO_2 dissolved in stratum water, a CO_2 increase of approximately 350,000 m^3 was calculated for the whole field. This value corresponds to the formation rate of approximately 1,000 tons molasses for fermentation by *Cl. tyrobutyricum.*

Organic Acids—Solution of Rock

An important indicator for the dominant fermentation process in the reservoir was the organic acids identified in the stratum water extracts (see Fig. 10). Table 5 summarizes the organic acid contents of some wells. With the dominating position of butyric acid, these values correspond to the fermentation type of *Clostridia.*

In the stratum water extracted, a maximum content of 6 g organic acid/liter was determined, corresponding to approximately 50% of the generation potential of a 6% by weight molasses solution.

The pH behavior (see Fig. 8) of the water extracted from some wells with good fissure links with the injectors shows that the flow time is not long enough to ensure a complete reaction with the reservoir rock. On producer 437/432 complete neutralization was reached, therefore, only during the winter season. Hence, it can be concluded that the rock dissolving process is accomplished not only in the injector areas located in the vicinity of the wells, but improvements in permeability are also recorded in regions further away from the wells.

Due to the carbonate solution, an improvement of the injection of the injectors was observed on the reservoir. The conclusion that a rock dissolving process takes place

in the reservoir is supported by a three to fourfold increase of the Ca^{2+} content of stratum water compared to the initial value, while the Cl^- content is decreasing. Limestone is not only dissolved and transferred in the field but also extracted in the form of soluble Ca^{2+} salts of organic acids. The calculations of the molar relation for the organic acids extracted and the Ca^{2+} content corresponded.

By December 1994, 1.9 tons of organic acids were extracted from well 437. The alkaline earth ion balance showed an additional yield of 210 kg calcium and 97 kg magnesium, corresponding to solved amounts of 525 kg $CaCO_3$ and 336 kg $MgCO_3$. The 861 kg of solved reservoir rock are equivalent to an acid yield of 1.53 tons. Considering that the organic acids have not yet fully reacted, this amount corresponds to the yield of 1.9 tons recorded. For well 432, the balance showed a yield of 1.95 tons of organic acids and approximately 0.9 tons reservoir rock dissolved (through the Ca^{2+} and Mg^{2+} contents in stratum water).

Final Decomposition of Metabolites

To evaluate the final decomposition process of the produced organic acids, alcohols, and hydrogen in the reservoir, balances were performed. As stated earlier, the presence of sulfate-containing stratum water can lead to the generation of H_2S and/or methane. The determined sulfide contents of stratum water and H_2S contents of the accompanying gas were subject to time and well-dependent variations.

It can be concluded that during the pilot test no mass generation of SRB with a bacterial count $\geq 10^6$ cells/ml was observed in the reservoir. As in the preliminary studies, the bacterial count varied between 10 and 10^4 (in few cases 10^5) cells/ml while for 1993 a slight increase was observed and for 1994 a decline of the cell content.

The sulfide and H_2S concentrations have been determined in more than 400 measurements. The sulfide content of the entire reservoir fell from 0.2 g/liter in 1992 to 0.15 g/liter in 1994. The H_2S content of the accompanying gas was reduced on average from 56.5 g/m^3 in 1992 to 41.4 g/m^3 in 1994.

The tests showed an enhanced methanogenesis in the pilot field. The bacterial count of the methane generating bacteria rose from an average rate of 1–10 cells/ml to 100–1,000 cells/ml in 1994. On all wells with increased CO_2 content the methane/ethane ratio went up considerably. The absolute methane content increased by 1% to 3%. In the period under review, the balance of the oil accompanying gas shows a methane surplus production of 71 m^3 and 82 m^3 for wells 437 and 432 respectively, i.e., 15% of the methane extracted is due to methanogenesis. Another proof of methanogenesis is changes in the isotope ratios. At wells influenced by MIOR, lighter methane with $\delta^{13} C_{CH_4}$ of –50 to –54 compared to initial values of

–45.5 to –46, was identified, while the physical atomic weight of ethane with $\delta^{13}C_{C_2H_6}$ of -40.3 and propane $\delta^{13}C_{C_2H_8}$ of –34.7 was not changed.

Production Results

Methanogenesis and the generation of biogas during molasses fermentation contributed considerably to an increased gas/oil ratio. For the entire field the ratio had increased from an average value between 3.6 and 4 to 7.8 in 1993 and 8.5 m^3 gas/m^3 oil in 1994. Wells which were especially influenced by MIOR revealed ratios between 16 and 20 m^3 gas /m^3 oil.

Improved flow conditions in the reservoir and increased gas/oil ratios led to an increase of net oil production by 50% to 65% without changing the production regime. The water content of the entire field was reduced from 74% to 57%; the wells influenced by injector 427 revealed a decrease from 89% to 59%.

Due to the reduced water content of the production medium and increased net oil production, a surplus of 2,200 tons of crude oil was produced by December 1994 in 16 production months (winter breaks). Figure 11 shows the production behavior of 14 wells with pronounced MIOR indication. Assuming a mean production (1/90 until 9/92) of 6.7 tons/D which fell to 6 tons/D during the test period, an increase of the production rate to 14 tons/D can be recorded for 1993. Production in 1994 is affected by an extremely long winter break so that the operations were restarted only at the end of June. Still, the production rate of 12 tons/D was twice that of the figure forecasted.

The production output for the example wells 420 and 432 is shown in Figure 12. Well 432 is considerably influenced by the injection in the central well 427 and well 431. Before MIOR treatment this well had a very high water content that was considerably reduced to approximately 80% and the oil production remarkably increased. This tendency continued throughout 1993 and was enhanced in 1994 especially by the carry on of the MIOR treatment. By December 1994 the water content had fallen to 68%, while oil production of well 432 had grown from 0.1 tons/D to 1.5 tons/D.

The marginal well 420 had a very high water content for many years. Due to an underdimensioned injection in 1991, it received oil from the more pressurized marginal area causing a temporary decline in the water content. Well 420 is influenced by injector 421. MIOR treatment of this well started only in June 1993 and showed minor response during the initial year. The water content was reduced from 94% to 90%. In 1994, when water content dropped considerably to approximately 64%, the gas/oil ratio increased remarkably to 16 m^3/m^3 and the net

oil rate increased from approximately 1 to 4.6 m^3/D. The surplus production from this well was equivalent to that of well 432.

Summary

- In the framework of a German-Russian R&D project, a pilot test of the molasses in-situ method was performed in the Bashkir fissure porous carbonate rock in the Romashkino reservoir.

- The use of modern biotechnology ensures a well-targeted dominant fermentation process in a densely populated reservoir, caused by a pure culture of *Clostridium tyrobutyricum.*

- Comprehensive analytical studies performed in the production field allowed the balancing of the metabolites generated and identification of major geochemical reaction mechanisms.

- MIOR caused a remarkable reduction of the water volume extracted, an increase of the gas/oil ratio, and an increase of net oil production leading to a considerable surplus production of crude oil. As the MIOR reactions still continue, a final evaluation cannot yet be made.

- Final biological degradation of metabolites generated tends to be accomplished mainly in the form of methanogenesis.

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Table 1 Characteristics of Bashkirian Reservoirs

Top Reservoir	493–515 m (selected)
Productive Area	1.070,000 m^2
Reservoir Rock	Organic limestone fissured porous
Average Porosity	9.8 %
Average Net Thlckness	5.7 m
Current Reservoir Pressure	6–7 MPa
Reservoir Temperature	21°C
Oil Density	0,903 g/cm^3 (25° API)
Oil Viscosity	50–80 mPas
Gas-Oil-Ratio	3.2 Nm^3/m^3
Brine Salinity	30–40 g/l
Daily Oil Production	16.5 m^3/operating day
Daily Fluid Production	38.0 m^3/operating day
Average Fluid Rate	1.6 m^3/well+operating day
Producers Operating	24
Injectors Operating	6

Table 2 Initial Microbial Population, Bashkir

Physiologic Group	Injection Well	Production Well	Core
Heterotrophic Aerobic Bacteria	10^3	1	10^2
Hydrocarbon-Oxidizing Bacteria	10^2	1	10^3
Methylotrophic Bacteria	10^3	0	10^2
Thiobacteria	1	0	10^2
Heterotrophic Anaerobic Bacteria	10^4	10	10
Fermentation Bacteria	10^5	1	10^3
SRB	10^5	10^2	10^2
Methanogenic Bacteria	1	1	10

Table 3 Cellular Byproducts

Methyl Alcohol	213 mg/l	Acetic Acid	1.996 mg/l
Ethyl Alcohol	2.378 mg/l	Propionic Acid	909 mg/l
Acetone	124 mg/l	I-Butyric Acid	413 mg/l
Propyl Alcohol	402 mg/l	n-Butyric Acid	7.762 mg/l
Butyl Alcohol	393 mg/l	Hydrogen	70–40 ml/g molasses
i-Amyl Alcohol	673 mg/l	Carbon Dioxide	300–330 ml/g molasses

Table 4 Distribution of the MIOR Fluids to the Five Injectors

Well	Total Injection, m^3	Inoculum, m^3	Molasses, t
427	3.798	441	245
423	3.138	192	195
434	3.446	261	213
418	3.658	188	226
421	2.390	123	144
Summary	16.430	1.205	1.023

To maintain the reservoir pressure, all treated wells had been reflooded with river water since September 1994.

Table 5 Organic Acids in Brine of Some Producing Wells (mg/liter)

	422	429	432	437
Acetic Acid	1716	1849	1026	1240
Propionic Acid	800	820	501	648
i-Butyric Acid	29	82	21	30
n-Butyric	2652	2979	1359	2750
i-Valeric Acid	39	18	13	31
n-Valeric Acid	125	70	50	159
i-Capronic Acid	9.8	3	0	0
n-Capronic Acid	453	132	48	194

Figure 1 Location Map

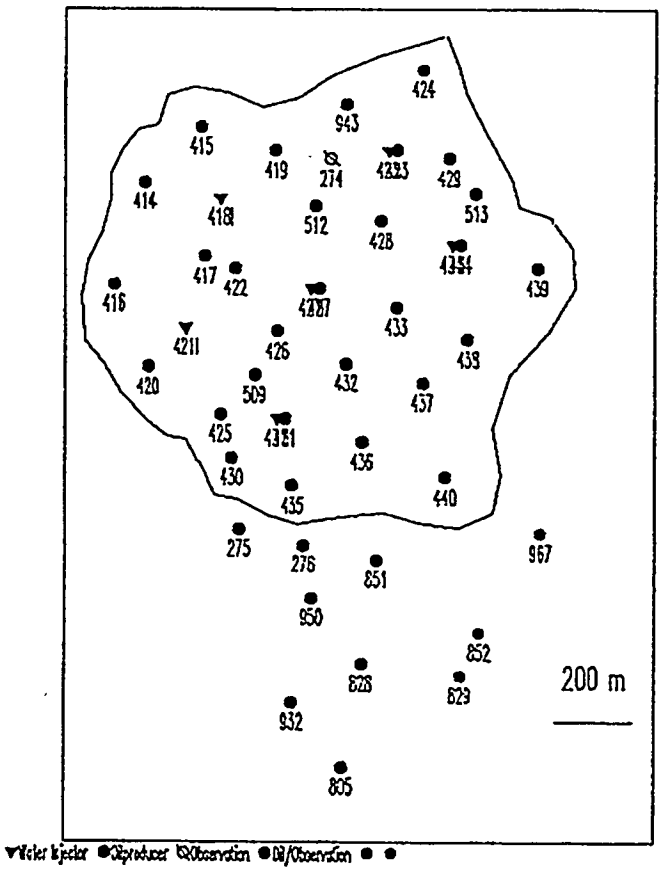


Figure 2 Wells, Bashkir

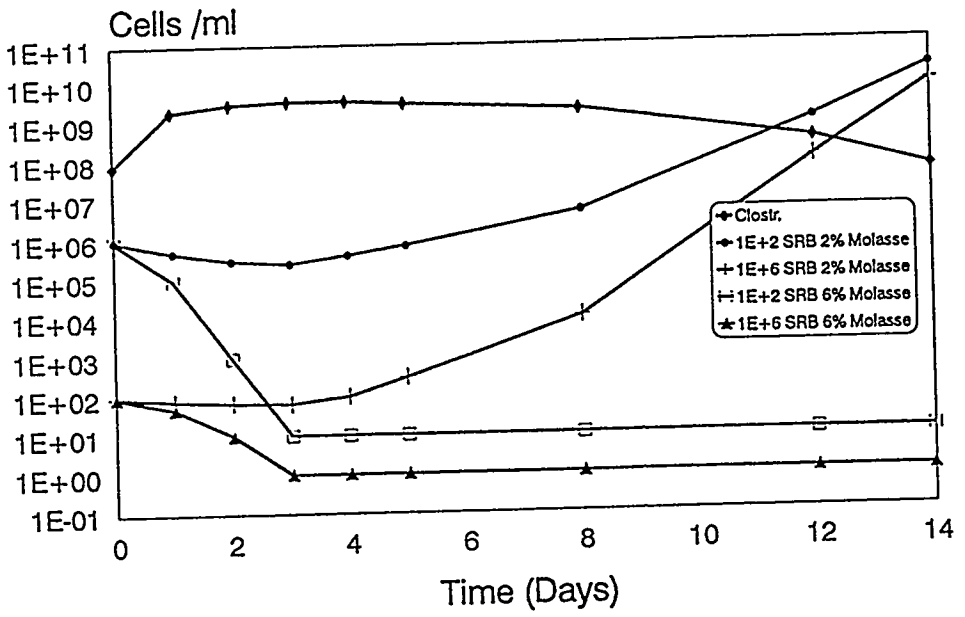
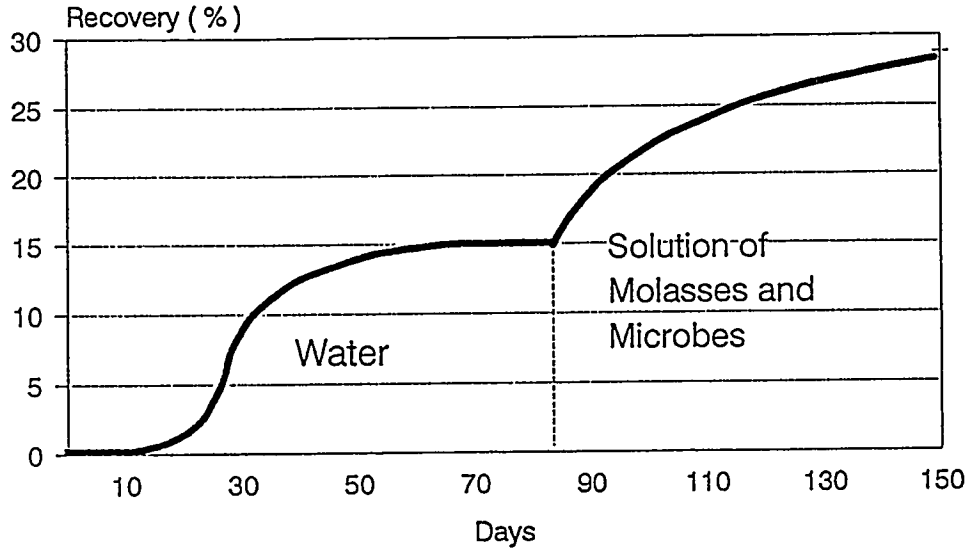


Figure 3 Growth of a Mixed Culture from Clostridiac and SRB by Various Concentrations of Molasses



Porosity=10 %, Oilsaturation=79 %, Viscosity=58 mPas, PV flooded= 0,25/d

Figure 4 Coreflood Experiment

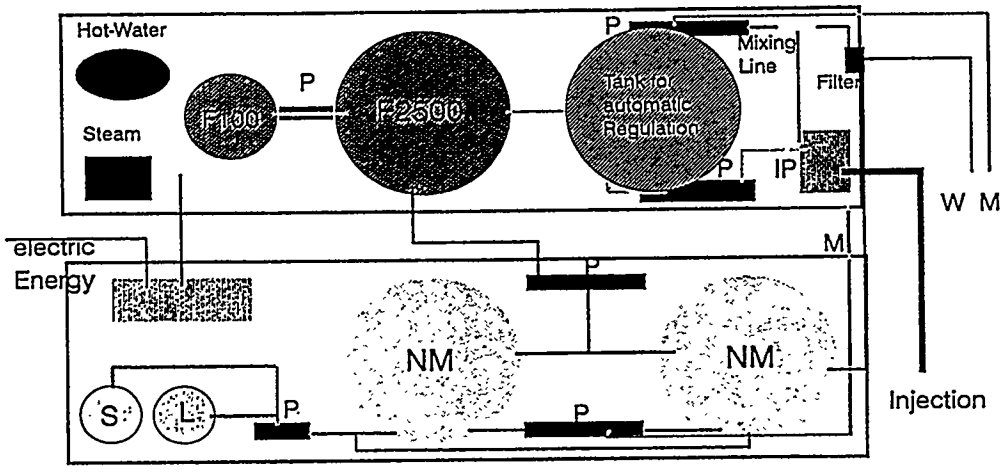


Figure 5 Biotechnological Plant (Schematic)

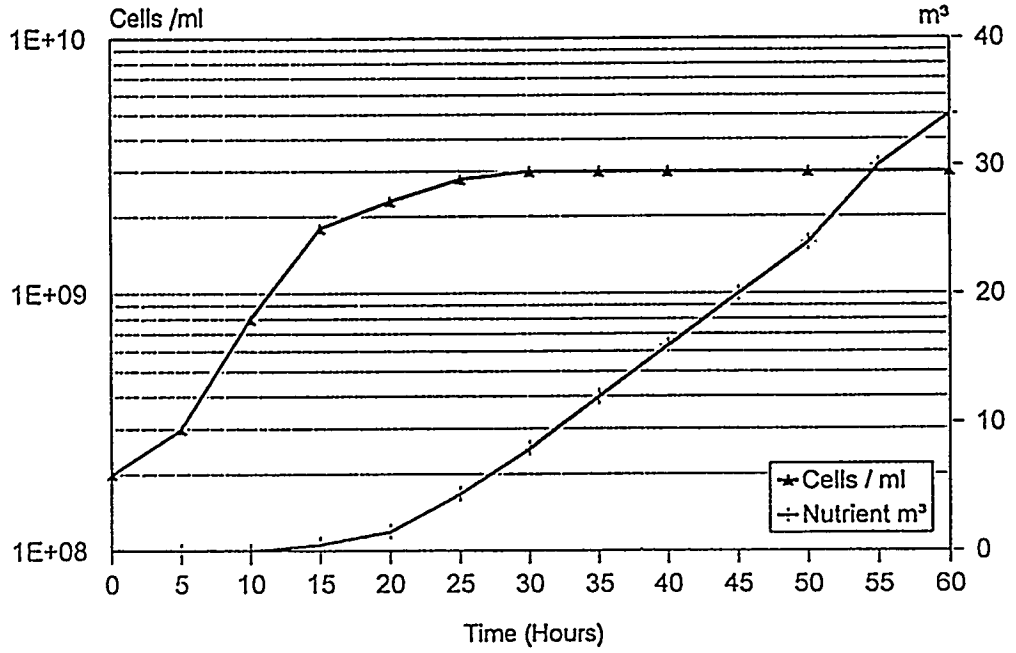


Figure 6 Continous Culture of Clostridium in a 2.5 m^3 Liter Fermenter

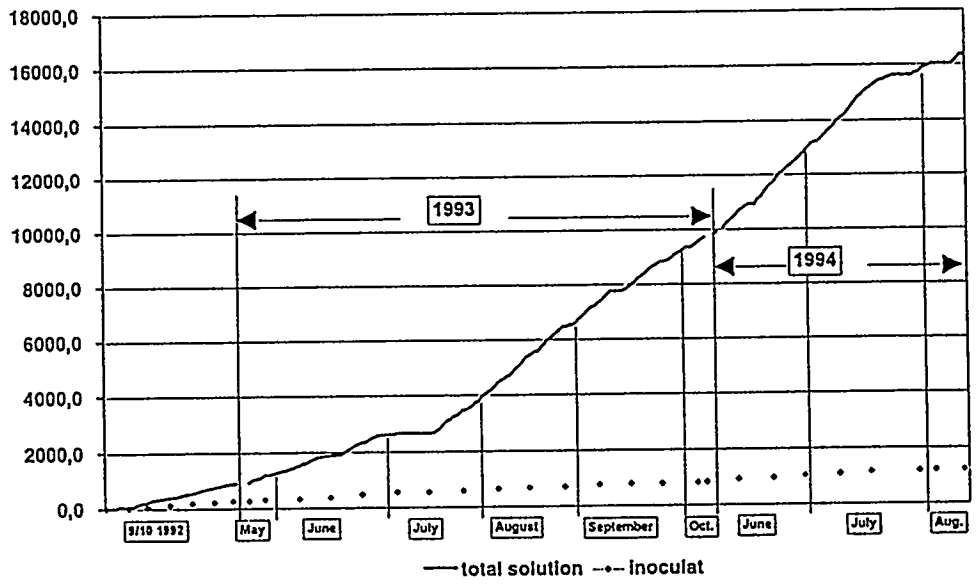


Figure 7 Cumulative Injection Procedure (m^3)

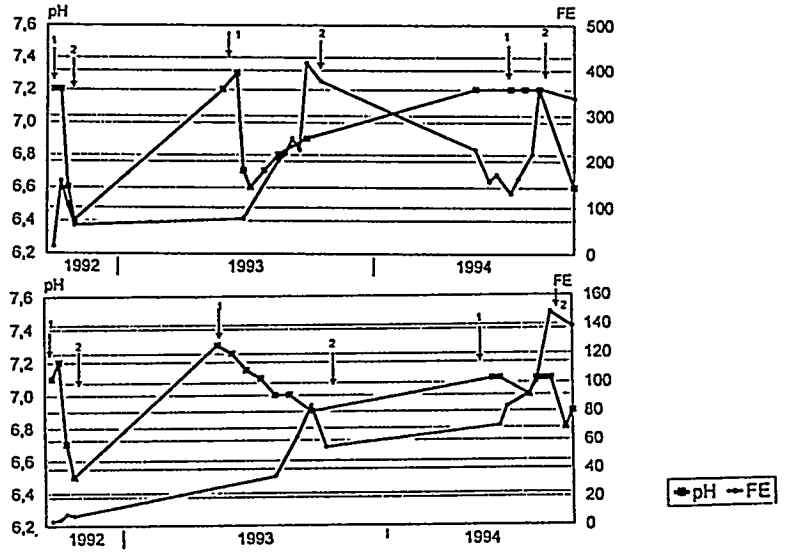


Figure 8 Well 437 (above) and Well 432 (below) pH and Melanin (FE) Indications-Injection (1 Start, 2 End of the Injection)

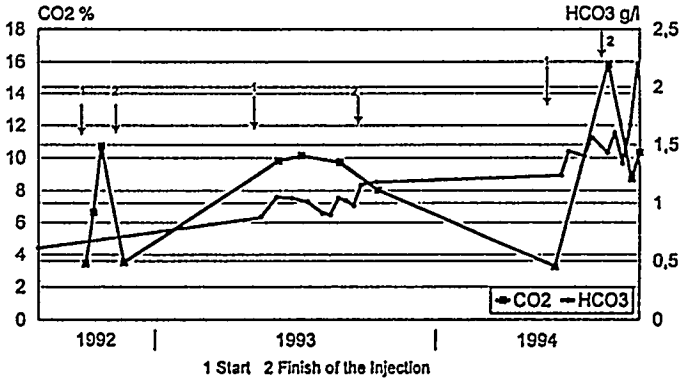


Figure 9 Development of CO_2 and $HC0_3$- Content on Well 432

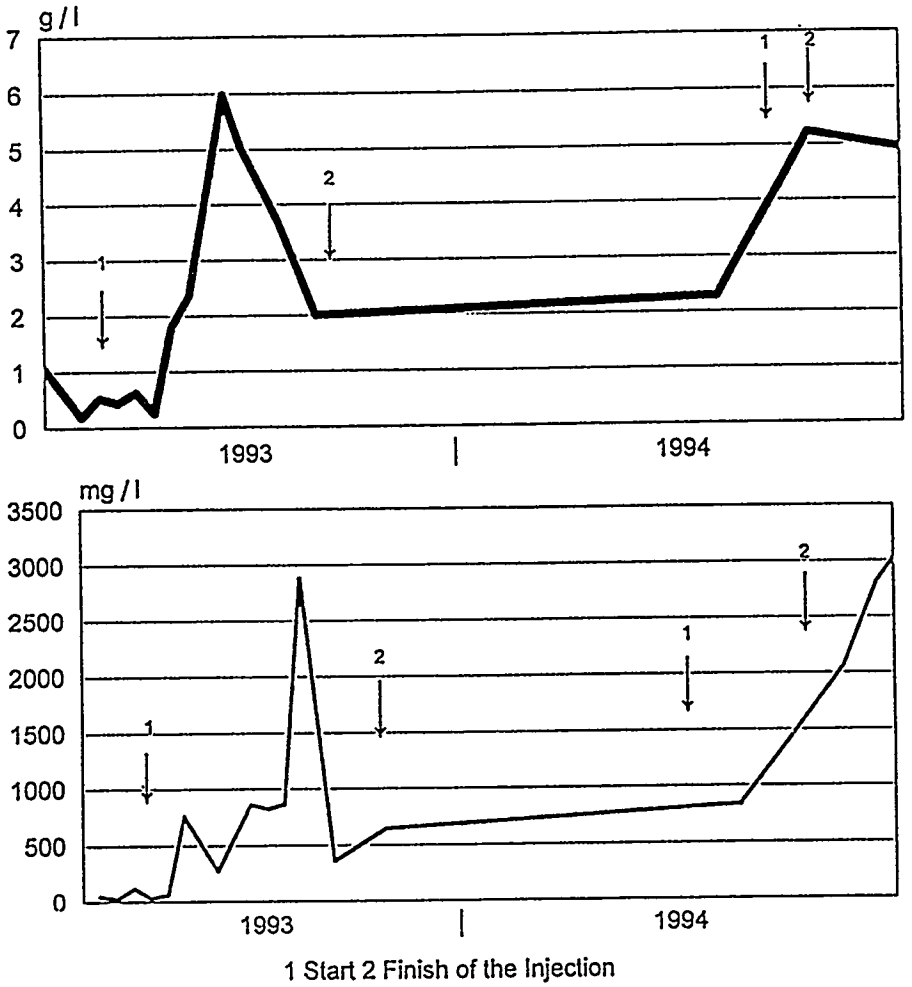


Figure 10 Organic Acids on the Producers 437 (above) and 432 (below)

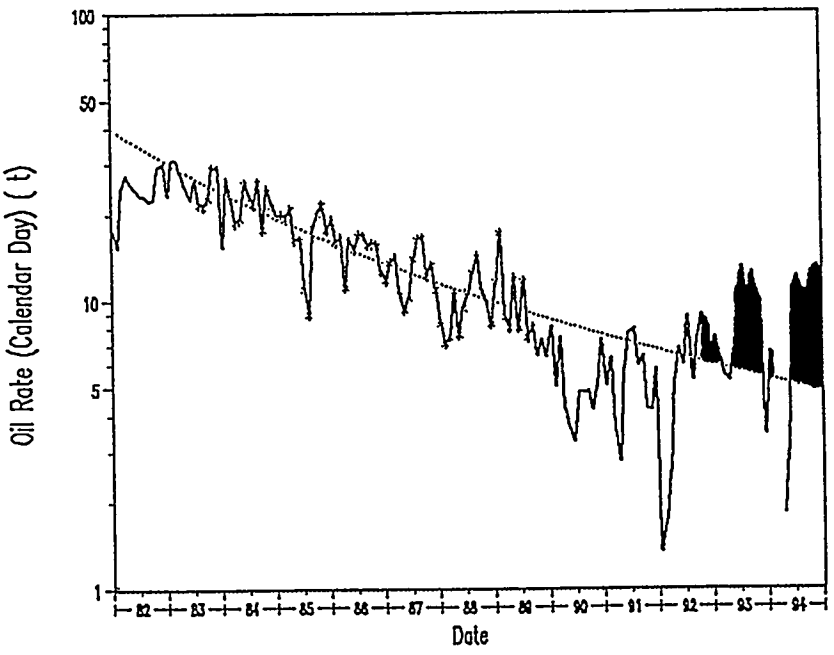


Figure 11 Total for 14 Wells

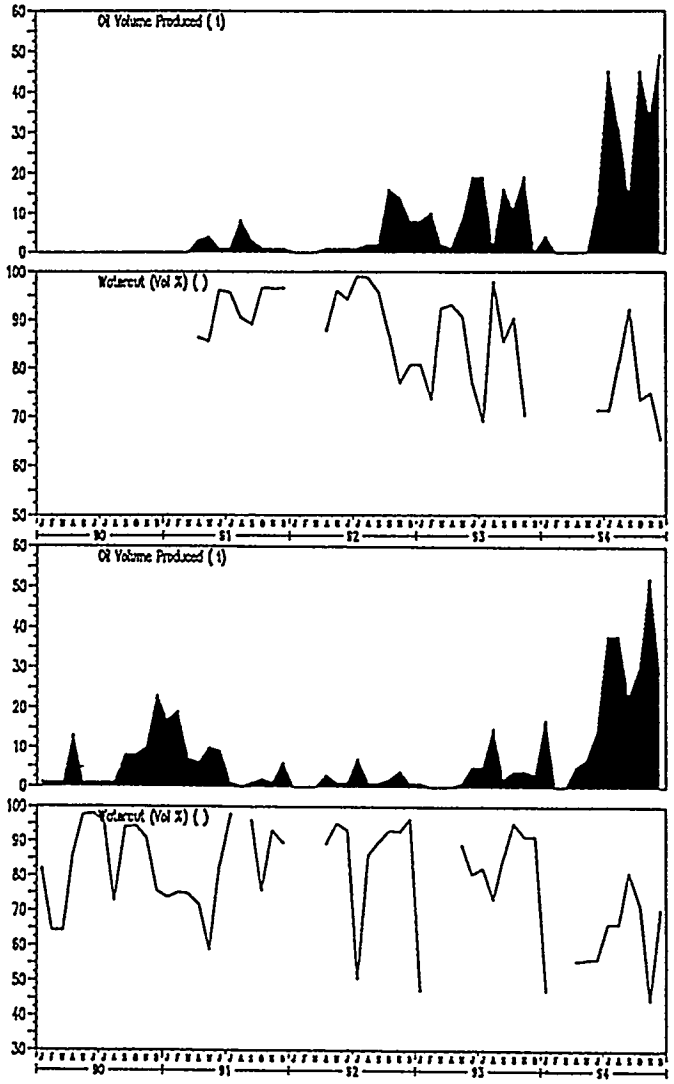


Figure 12 Oil Production per Month (t) and Watercut (vol%) for the Wells 432 (above) and 420 (below)

A Field Demonstration of the Microbial Treatment of Sour Produced Water

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Abstract

The potential for detoxification and deodorization of sulfide-laden water (sour water) by microbial treatment was evaluated at a petroleum production site under field conditions. A sulfide-tolerant strain of the chemautotroph and facultative anaerobe, *Thiobacillus denitrificans*, was introduced into an oil-skimming pit of the Amoco Production Company LACT 10 Unit of the Salt Creek Field, Wyoming. Field-produced water enters this pit from the oil/water separation treatment train at an average flowrate of 5,000 bbl/D (795 m^3/D) with a potential maximum of 98,000 bbl/D (15,580 m^3/D). Water conditions at the pit inlet are 4,800 mg/l TDS, 100 mg/l sulfide, pH 7.8, and 107°F. To this water an aqueous solution of ammonium nitrate and diphosphorous pentoxide was added to provide required nutrients for the bacteria. The first 20% of the pit was aerated to a maximum depth of 5 ft (1.5 m) to facilitate the aerobic oxidation of sulfide. No provisions for pH control or biomass recovery and recycle were made.

Pilot operations were initiated in October 1992 with the inoculation of the 19,000 bbl (3,020 m^3) pit with 40 lb (18.1 kg) of dry weight biomass. After a brief acclimation period, a nearly constant mass flux of 175 lb/D (80 kg/D) sulfide was established to the pit. Bio-oxidation of sulfide to elemental sulfur and sulfate was immediate and complete. Subsequent pilot operations focused upon process optimization and process sensitivity to system upsets. The process appeared most sensitive to large variations in sulfide loading due to maximum water discharge events. However, recoveries from such events could be accomplished within hours.

This paper details all pertinent aspects of pilot operation, performance, and economics. Based on this body of evidence, it is suggested that the oxidation of inorganic sulfides by *T. denitrificans* represents a viable concept for the treatment of sour water coproduced with oil and gas.

Introduction

The Salt Creek Field is located 40 miles north of Casper, Wyoming, and is one of the oldest oil fields in the region. Currently, it is operated as a mature waterflood with approximately 1,000 production and 950 injection wells. Production averages 10,000 bbl of oil and 750,000 bbl of water per day. Of the total water production, nearly 95% is reinjected while the remainder is discharged to the surface. These discharges are termed "beneficial" in that they represent an important source of fresh water for the support of local livestock and wildlife in this predominantly arid region.

Over time, produced fluids from the field have become progressively more sour due to microbial reduction of sulfate which was introduced into the reservoir during waterflooding. Because potential odor and toxicity issues are associated with sour water discharges, the merits of this practice are under active evaluation.[1] If complete water reinjection is to be avoided, a cost-effective treatment option which allows the continued discharge of water while eliminating the odor and toxicity attributed to H_2S must be found.

Several remediation options exist for the control of soluble inorganic sulfides in water, such as chemical oxidation, air stripping, precipitation, air oxidation, and biological treatment. For Salt Creek, the choice of a particular treatment option is severely restricted by the operating environment. Beyond obvious technical issues, the most significant restrictions are associated with stringent capital and operating expense limits. Of necessity these limits mandate the use of existing facilities (production pits) and manpower. Of the accepted chemical or physical treatment options available, most can be rejected out-of-hand because of their high operating expenses, capital costs, or inability to effect immediate H_2S odor control in an unconfined production pit. From the remaining options only biological treatment appears to offer sufficient process flexibility to achieve effective control at low costs and under the imposed operating restrictions.

The use of biological treatment for H_2S abatement in produced water is not new to the industry. In the recent past several Wyoming operators have installed land treatment cells for this purpose. These installations purportedly achieve H_2S control by a combination of biological treatment, precipitation, aeration, and oxidation in a passive environment.[2,3] The use of such an installation at Salt Creek, however, was rejected because of the need for extensive facilities modifications and the uncertainty as to whether H_2S odors could be effectively controlled by a passive open-air process. As an alternative, the potential for active biological treatment was explored. This approach was predicated upon augmenting the existing pits and lagoons of the Salt Creek discharge system with a self-sustaining bacterial

population capable of efficient sulfide oxidation at prevailing conditions. Specifically, the use of a sulfide-tolerant strain of *Thiobacillus denitrificans* (strain F) was examined because of its demonstrated ability to efficiently oxidize sulfide both aerobically and anaerobically.[4–8] *T. denitrificans* is a strict autotroph and facultative anaerobe capable of utilizing sulfide, elemental sulfur, and thiosulfate as energy sources with oxidation to sulfate. Although aerobic oxidation is kinetically faster, nitrate may be used in lieu of oxygen as a terminal electron acceptor in the biological oxidation pathway under anoxic conditions (which likely prevail at Salt Creek). Hence, the opportunity to treat sour water in existing anoxic pits is offered by the addition of ammonium nitrate to the discharge. Ammonium, as well as phosphate, are required in small quantities as nutrients to sustain active bacterial growth. Aqueous carbonate can be used as the sole carbon source.

Prior to field application, the feasibility for *T. denitrificans* strain F to remove 10 mg/l of total inorganic sulfides from a simulated Salt Creek brine was assessed.[9] The brine was treated continuously in a plug flow reactor scaled to the dimensions of a field production pit. Operating conditions for pH (7.0), temperature (105°F), and hydraulic residence time (0.3–6 days) were chosen to mimic field conditions. The reactor was operated anaerobically to reflect the anoxic conditions within field pits. At scaled flow rates of 60,000 bbl/D, complete removal of sulfide from the feed was achieved without detectable H_2S emissions in the reactor offgas. Process performance, nutrient requirements, and sensitivities to hydrocarbon and water treatment chemicals were determined to be within acceptable ranges to warrant field testing.

In this paper, the results of process field testing are reported for a pilot initiated at the LACT 10 unit of the Salt Creek Field in October 1992. Aspects of pilot construction, operation, and performance are detailed. Process performance was specifically monitored to determine sulfide removal efficiencies, sensitivities to upsets, minimum nutrient requirements, and overall operational expenses.

Site Description

The LACT 10 unit of the Salt Creek Field is an oil/water separation facility wherein approximately 1,000 bbl/D oil and 98,000 bbl/D water are processed. Upon separation, water is diverted from the treatment train to a large transfer tank from which all but 5,000 bbl/D is routinely utilized for waterflood reinjection. The 5,000 bbl/D overflow is shunted to the surface discharge system in order to maintain the associate pits, lagoons, and wetlands (see Fig. 1). On occasion, injection plant failures necessitate full discharge to the surface system. Thus, large flowrate variations are inherent to the system.

Water enters the surface discharge system at Pit 1 through a submerged 16-in. line. Pit 1 is an earthen lagoon which serves as an oil-skimming pond. Its approximate dimensions are 260 ft × 110 ft with a variable depth from 1 to 20 ft. The estimated volume of the pit is 19,000 bbl. From Pit 1 water flows to a series of smaller detention pits, over a cascade, through a meandering lagoon, and eventually to Salt Creek. The point of confluence is within one-eighth mile of Midwest, Wyoming. The total extent of the system is nearly one-third mile and the average hydraulic detention time within the system is somewhere between 3 to 10 days. Under full discharge conditions, much of the system is bypassed and the hydraulic residence time is on the order of tens of minutes.

The average water temperature entering Pit 1 is 107°F, and the pH is slightly alkaline (7.8). The brine is relatively fresh with a Total Dissolved Solids (TDS) content of 4,800 mg/l. The brine composition is reported in Table 1. The sulfide concentration is 100 mg/l. Based on an average daily flowrate of 5,000 bbl, sulfide influx to the pit is about 80 kg/D. Dissolved oxygen (DO) levels within the pit are less than 1 mg/l and, therefore, the pit is largely anoxic.

Pilot Design/Operation

Because of the aforementioned capital limitations, Pit 1 was chosen as the primary treatment facility for the bioaugmentation pilot. Conceptually, the piloting program sought to establish the efficacy of "seeding" Pit 1 with a self-sustaining population of *Thiobacillus denitrificans* strain F capable of sulfide oxidation under prevailing conditions of flowrate, temperature, and sulfide flux. Contrary to the laboratory prototype, the facility was designed as a partially aerated basin to facilitate mixing and to take advantage of higher reaction rates associated with the aerobic bio-oxidation of sulfide. The collection and recycle of biomass from pit effluents were not attempted because of the extensive facilities modifications required. Carbon for cell growth was obtained from the field brine as carbonate (see Table1). Additional nutrients were limited to ammonia, which was added as ammonium nitrate. The nitrate serves as a potential terminal electron acceptor in anoxic portions of the pit. In what follows the construction, startup, operation, and monitoring of the pilot are detailed.

Construction

Two modifications to the existing LACT 10 facility were required to conduct the pilot; specifically, providing the means to add nutrients to the discharge water prior to its entering the pit and aerating approximately one-fourth the pit volume.

Because of cost and availability, the primary nutrient and electron acceptor source, ammonium nitrate, was obtained as a prilled solid in 40 lb bags. This necessitated the construction of a batch-mixing facility to deliver the nutrient in liquid form. The mixing facility consisted of two 750 gal fiberglass tanks each outfitted with a centrifugal pump for solids circulation. The first tank served as a primary mixing tank. The second tank served as a holding and delivery vessel. Nutrient solution was metered to the produced water discharge line by means of a variable stroke chemical metering pump. All materials used in the construction of the system were compatible with corrosive liquids. The entire mixing facility was enclosed within an earthen berm for spill containment. Drainage was provided to the pit.

To completely aerate the first 30 ft of the pit, an air manifold was constructed and connected to an existing 1,000 SCFM blower (Sutorbilt 7 MF). The manifold was made from 5 in. PVC pipe and extended down either side of the pit. Three-inch PVC laterals were spaced at 5 ft intervals on each leg of the manifold. The laterals were 60 ft in length. Quarter inch holes spaced on 1 ft centers were drilled in the last 30 ft of each capped lateral. To provide aeration at depth, the laterals were weighted with several 20 ft lengths of 1/2-in. iron rod. The approximate average depth of aeration was 5 ft. A valve was placed on each lateral to control and balance air rates.

A schematic of facility modifications is provided in Figure 2. With the exception of the aeration manifold, all major capital items were obtained from field surplus equipment. Field personnel were utilized for construction at an estimated cost of material and labor of $5,000.

Pilot Start-Up

Prior to physically operating the pilot, a sufficient amount of biomass had to be cultured in order to adequately "seed" the pit. Based upon the laboratory study it was estimated that 50 mg/l of dry weight biomass would be required to successfully inoculate the pit volume under an average sulfide influx.[9] The estimate was predicated upon the amount of *T. denitrificans* necessary to oxidize enough sulfide such that their toxicity threshold (80 mg/l) to sulfide was not exceeded.[8] The calculated biomass weight to "seed" Pit 1 was about 350 lb.

A special fermentation facility was constructed at the University of Tulsa to grow biomass. The facility consisted of a 1,000 gal aerated batch fermentor and the necessary equipment for pH and temperature control. *T. denitrificans* was cultured by standard techniques on a nitrate-supplemented medium in order to stimulate the enzyme systems necessary for the anaerobic oxidation of sulfide.[4] The medium used thiosulfate as the primary energy source. The organism was cocultured in the

presence of heterotrophic bacteria to improve floc settling characteristics.[10] Upon harvesting, the bacterial flocs were collected and concentrated by gravity separation in a 500 gal conical bottom tank. The biomass concentrate was stored in 55 gal drums at 4°C prior to shipment to the field. Biomass yield from each 1,000 gal batch was approximately 1.41 lb dry weight.[11] This corresponded to a recoverable biomass concentration of 0.21 g/l which was much less than that anticipated on the basis of laboratory cultures. Due to time and cost constraints, only 40 lb of biomass had been cultured by the time the field pilot was initiated. This was about 10% of the targeted amount.

Actual pilot operations began on October 21, 1992. Before inoculating the pit, a complete physical and chemical survey of the pit was conducted. (Details will be given later.) Direct nutrient addition to the discharge was initiated approximately 36 hours before inoculation. The nutrient was added as a 40 wt% ammonium nitrate concentrate supplemented with 0.16 lb/gal (0.019 kg/l) P_2O_5. Both chemicals were obtained from a commercial source (Jirdon Agri Chem, Torrance, Wyoming) and were made-up in the field produced water. Initial nutrient rates were based upon the calculated nitrate requirement (3.29 mg NO_3^-/mg S^{2-}) to achieve complete sulfide oxidation under anaerobic conditions for an average daily influx of sulfide.[9] At this rate biomass requirements for nutrient nitrogen (0.084 mg NH_3/mg S^{2-}) and phosphorous (0.013 mg PO_4^{3-}/mg S^{2-}) were easily attained.[4]

To accommodate the lack of biomass inoculum, a method was devised to reduce the influx of sulfide to Pit 1 during startup. Namely, the discharge flowrate was lowered from 5,000 to 1,000 bbl/D, and the water was stripped with air upstream of Pit 1 to reduce the sulfide concentration from 100 mg/l to nearly 10 mg/l. In so doing, the potential to overload the small inoculum and thereby exceed its toxicity limit to sulfide was significantly diminished. The pit was inoculated on October 24 by pouring the biomass concentrate directly into the pit near the discharge inlet. The presence of adequate nutrients at the point of inoculation was insured by the direct addition of fertilizers to the inoculation area.

Operation

The pilot was operated continuously from October 21, 1992, to April 10, 1993. The first three days post inoculation served as an acclimation period for the bacteria. The sulfide flux to the pit was kept low by air stripping during this period. On Day 9 (elapsed time from 10/21) the water discharge rate was returned to its daily average of 5,000 bbl. As a consequence, the sulfide influx to the pit increased although stripping continued. From Day 9 to Day 14 air was gradually diverted from the stripper to the pit aeration system. By Day 14, 100% of the blower capacity (950 CFM @ 10 psig) had been diverted to the pit. At this point the sulfide

flux to the pit was approximately 92 kg/D. Flow and air rates remained constant thereafter, unless otherwise noted.

On Day 17 nutrient influx to the pit was reduced from its startup level to a level based upon twice the calculated ammonia demand. This was done to determine if nitrate was required in lieu of oxygen for anaerobic oxidation of sulfide. Further reductions in the nutrient rate were attempted throughout the program to assess the minimum nutrient requirement.

Several instances of operational upsets were of note. On three separate occasions (Days 43, 100, and 135) full discharge events occurred. These events were initiated by unforeseen injection plant failures of variable duration. The longest was on Day 135 and lasted for nearly two days. During these periods no attempt was made to adjust nutrient delivery rates in accordance with the flowrate. Hence, sulfide fluxes to the pit exceed anticipated nutrient requirements. In a related instance (Day 34), water flow to the pit fell significantly below the daily average due to a controller failure. Because this event occurred while ambient temperatures were significantly below freezing, pit temperatures briefly dropped below stated optimums for bacterial activity. On numerous other occasions, pump, aerator, or pipe ruptures interrupted air or nutrient service to the pit. Typically, these events were of short duration and negligible effect.

Monitoring

During the first weeks of startup and operation, an intensive monitoring program to assess pilot performance was undertaken. Key variables included aqueous phase sulfide, nitrate, phosphate, ammonia, sulfate, and dissolved oxygen concentrations. Flowrate, pH, and temperature were also monitored. Sampling of the discharge system routinely occurred at three locations: (1) upstream of Pit 1, (2) from Pit 1 at the influent position, and (3) the effluent from Pit 1. Periodically, additional samples were obtained elsewhere within the pit and the discharge system. Nutrient delivery rates were estimated by gauging the nutrient holding tank daily.

Most water analyses were conducted by spectral methods with a Hach DR/2000 spectrophotometer. These included Nessler ammonia, nitrate by cadmium reduction, sulfate by barium precipitation, and reactive phosphate. All reagents were obtained ready-to-use from Hach Chemical Company (Loveland, Colorado) with a stated accuracy supplied by the manufacturer. Sulfide ion was determined by Sensidyne Gastec sulfide ion analyzer tubes. The stated accuracy was ±2 ppm over a 0 to 100 ppm range. The pH and temperature were evaluated with a Beckman Φ11 portable pH meter. A two point calibration procedure was performed routinely. Dissolved oxygen was measured with Chemetric (Calverton, Virginia)

test kits for low (0–1 ppm) and high (0–10 ppm) ranges. All tests were conducted in the field within minutes to hours of sampling.

In one instance, several ions were analyzed independently by ion chromatography after sample preservation by acidification with sulfuric acid. With the exception of nitrate, ion chromatography confirmed spectrophotometric determinations. For nitrate a matrix interference with sulfide ion was identified. Hence, nitrate concentrations could not be determined unambiguously.

Ambient air quality above the pit surface was monitored periodically with an Industrial Scientific HMX 271 personal air monitor. The detection limit of the instrument is less than 1 ppm. The stated accuracy is ±2 ppm. The instrument was calibrated daily with a known H_2S/N_2 gas mixture standard.

Water flow rates were estimated by timing the water discharge into a container and averaging several determinations. The method proved effective for rates below 10,000 bbl/D. For rates above this value, estimates were calculated on the basis of the difference between daily injection plant volumes and monthly total water production averages. Estimates based on this method are subject to significant error.

Water samples for bacterial enumerations were obtained at preinoculation (Day 0), post inoculation (Day 4), Day 17, 48, and 50. Enumerations were based on the most probable number (MPN) method using thiosulfate as the sole energy source (12). Hence, MPN determinations were specific to sulfur-oxidizing bacteria.

Daily monitoring occurred from Day 0 to Day 35 and from Day 49 to Day 56. Otherwise, pilot data were collected by field personnel as time allowed. Only routine analyses (sulfide, nutrient rate, and flowrate) were attempted at these times, although periodic ammonia, pH, and temperature data were also collected. Results of the monitoring program are reported below.

Results and Discussion

Prior to inoculation and nutrient addition, a background survey of Pit 1 and the associated discharge system was conducted to determine sulfide losses under prepilot conditions. In addition to temperature and pH, specific analytes included sulfide, dissolved oxygen, ammonia, and nitrate. At the time of the survey the air stripper was operating and the flowrate to the discharge system was 3,500 bbl/D. Pit 1 was partially aerated. The results of the survey are reported in Table 2; locations are referenced to Figure 1.

On the basis of survey data, it was reasonably established that prior to inoculation no naturally significant reduction of sulfide occurred within the pit at residence times on the order of days. Specifically, upstream of the air stripper water conditions were 100 mg/l, sulfide, 107°F, and pH 7.3. After the stripper, water entered Pit 1 at 28 mg/l sulfide, 90°F, and pH 7.8. Pit 1 effluent was 28 mg/l, 81°F, and pH 8.4. As the data indicate, a significant loss of sulfide was associated with stripping but little, if any, with passive volatilization and oxidation within Pit 1. The latter was in spite of a calculated five-day hydraulic residence time. The absence of sulfide losses within the pit were potentially attributed to (1) a hydraulic short-circuit which significantly reduced fluid residence times, or (2) a shift to higher pH values and concomitantly a propensity for dissolved hydrogen sulfide to remain in solution as bisulfide ion. The temperature survey at the pit periphery (see Table 2) did indicate a hydraulic short-circuit since cold spots or stagnant regions were not identified. The pH data, however, did confirm a shift to higher pH values and presumably higher sulfide solubilities. Higher pH values were likely a consequence of contact between the discharge water and the alkaline soils of the pit.

Initial dissolved oxygen (DO) levels within the pit were uniformly less than 1 mg/l with minimal aeration. Ammonia and nitrate concentrations were also significant prior to nutrient addition. Hence, the need for aeration and nutrient addition was confirmed.

Figure 3 shows sulfide mass flowrate to Pit 1 over the duration of the pilot. Sulfide concentrations at the point of discharge to the pit (within the aerated zone) and in the effluent are reported versus elapsed operating time in Figure 4. As was mentioned previously, sulfide influx to the pit was limited by air stripping prior to and immediately after (Day 4 –Day 16) inoculation with *T. denitrificans*. During this acclimation period the sulfide influx to the pit was gradually increased from 0 to the typical daily average of 80–100 kg/D. The corresponding sulfide concentrations over this time span never exceeded 5 mg/l at the inlet. Moreover, no sulfide was detected in Pit 1 effluents. Hence, it appeared that the bacteria were capable of oxidizing average sulfide loads to the pit within a matter of days post inoculation and the cessation of stripping.

After acclimation, pit effluent sulfide concentrations remained zero but for three upset events. On a cumulative basis this performance represented a treatment efficiency approaching 99% of the total sulfide discharged to the surface system. (In the absence of upsets this treatment efficiency approached 100%.) Under normal operating conditions, the oxidation of sulfide to sulfate appeared complete. For instance, during one three-day period the average concentration of sulfide entering the pit was 110 mg/l, while the net sulfate concentration exiting the pit was 340 mg/l. This value agrees well with the expected mass ratio of sulfate to sulfide of 3 to 1, if oxidation is complete.

With respect to fugitive emissions, no H_2S could be detected immediately above the pit surface under normal operations. This was a consequence of the ability of the bacteria to instantaneously oxidize sulfides as they entered the pit; evidence of which was demonstrated by near inlet samples whose sulfide content rarely exceeded 5 mg/l. In terms of treatment efficiency, this performance represented a minimum of 95% sulfide removal within the first few feet of the aerated zone of the treatment system.

Significant discharges of sulfide from the pit occurred as upset events on Days 34, 46, 110, and 136. They could be attributed to two causes. The first (Day 34) was unique in that low flowrates to the pit and low ambient temperatures (<0°F) combined to cause water temperatures within the pit to drop below 60°F. At this temperature biological sulfide oxidation was significantly inhibited to the extent that an upset was observed. The upset was quickly remedied, however, by raising the flowrate to the pit to maintain water temperatures closer to the optimum of 85°F. The second cause for pilot upsets was largely uncontrollable. On Days 46, 110, and 136 sulfide influx to the pit significantly exceeded the daily average, i.e., >500 vs. 90 kg/D. These occurrences were caused by injection plant failures which necessitated full discharge to the pit (see Fig. 5). Each was characterized by a large sulfide flux, rapid temperature increase, and inadequate nutrient supply imposed by the unanticipated large hydraulic throughput. The pilot response to these failures was encouraging in that approximately 30% of the total sulfide was at least partially oxidized. Partial oxidation was observed as an accumulation of elemental sulfur on the banks of the pit and the absence of sulfate ion in pit effluents (see Fig. 6). In terms of sulfide removal, recovery from these events was evidenced in a matter of hours of the flow returning to normal. However, as Figure 6 indicates, complete stoichiometry oxidation of sulfide to sulfate (mass of sulfate = 3 × mass sulfide) required several days for recovery. The observation that *T. denitrificans* resorted to a partial oxidation of sulfide under stress was in keeping with laboratory observations of similar events.[4,7] Moreover, the production of elemental sulfur served as a convenient visual indicator of a stressed treatment system.

Circumstantial evidence suggested that the ability of the pilot to completely oxidize sulfide to sulfate became limited at a sulfide flux near 200 kg/D (10,000 bbl/D). This was lower than that anticipated based on the scaled lab reactor results (60,000 bbl/D).[7] At this time it is not understood whether this limitation was imposed by a lack of biomass, an insufficient residence time for reaction, an oxygen deficit, or a nutrient deficiency. Unfortunately, the ability to test these relationships in the field was severely hampered by difficulties in measuring and regulating these variables under adverse conditions. Attempts to do so were largely confounded by operational difficulties related to equipment and control failures in cold weather.

Because the addition of nitrate as an alternate electron acceptor represented the largest chemical demand for the pilot, the requirement for nitrate was assessed within days of inoculation. This was accomplished by gradually reducing the nutrient rate to the pit over a period of several days to a lower limit based upon the calculated ammonia requirement (0.084 kg NH_3/kg S^{2-}). As Figure 7 shows, by Day 14 the nitrate influx to the pit dropped below the calculated nitrate demand (3.29 kg NO_3-/kg S^{2-} times the sulfate mass flowrate in kg/D) and, thereafter, always remained below it. At no time could a pilot upset be attributed to this condition. Therefore, it was presumed that the biological oxidation of sulfide occurred in large measure aerobically. In support of this contention, dissolved oxygen levels in the aerated portion of the pit were on average about 1.5 mg/l. Although low, these levels were deemed sufficiently high to support aerobic oxidation. This conclusion was based upon the fact that oxidation of sulfide by denitrifying mechanisms is not instituted in *T. denitrificans* until O_2 levels fall below 1 mg/l. Finally, within our ability to measure, no significant differences in nitrate concentrations between the entrance and exit of Pit 1 could be determined over the lifetime of the pilot; again supporting the contention that sulfide oxidation proceeded in large part aerobically.

Figure 8 reports ammonia demand and influx for the pilot. Although nutrient delivery was erratic, ammonia influx to the pilot remained for the most part above the calculated demand. In those instances where demand exceeded influx, the cause could be either attributed to a nutrient pump failure or a full discharge event. In the former cases (Days 64 and 82), little if any effect on pilot performance could be identified. In the latter cases no attempt was made to meet nutrient demands for high discharge rates. Overall, it would appear that the calculated ammonia demand was sufficient to sustain the bacterial population in the pit with an adequate margin of safety. This contention was supported by the fact that the measured ammonia concentrations at both the pit inlet and exit always agreed within a few ppm of the estimated added concentration based on nutrient delivery rates (see Fig. 9). This strongly suggested that ammonia was always present in excess, and very little ammonia was actually consumed within the pilot area. A similar statement could be made in regard to phosphate, although measured and estimated phosphate concentrations were much lower (2–5 mg/l). Further optimization of ammonia and phosphate levels was not attempted due to operational difficulties.

The pH by location is depicted in Figure 10. Generally, the pH of the influent to Pit 1 ranged from 7.0 to 8.0 in the absence of air stripping. After microbial treatment, however, the pH of the discharge water was more alkaline (8.0–8.6) with no discernible difference from entrance to exit. The relatively low pH of the upstream fluids was consistent with the amount of dissolved H_2S in the water. Although complete oxidation of sulfide was indicated, the excess acidity associated with sulfuric acid production was apparently removed by contact with the alkaline soils

of the pit. At no time did the pH within the treatment system appear to affect sulfide oxidation by *T. denitrificans*.

Under normal flow conditions the pit temperature (see Fig. 11) averaged about 75°F with a maximum day-to-day fluctuation of about 10°F. Fluctuations were attributed to changes in flowrate or heat input to the pit and variable ambient temperatures. (Over the piloting period ambient temperatures ranged from -10° to 75°F). During full discharge events, large temperature increases were realized within the pit in a matter of hours. On one such occasion the observed change was 29°F. Fortunately, such temperature swings had no discernible lasting effect on pilot performance.

Bacterial enumerations by the MPN method are reported in Table 3. These enumerations were specific to bacteria capable of utilizing reduced sulfur as their sole energy source. Enumerations were conducted before pit inoculation, after 15 minutes, after two days, and on Days 17, 48, and 50. All water samples for bacterial counts were obtained adjacent to the discharge point to Pit 1. The background bacterial count was 4.5×10^3/ml, most of which were identified as aerobic organisms incapable of utilizing nitrate for anaerobic sulfide oxidation. Immediately after inoculation the bacterial count increased to 4.5×10^7/ml. These bacteria were capable of using nitrate and thus indicated that viable *T. denitrificans* had been introduced to the pit. Subsequent bacterial enumerations were on the order of 10^7/ml, and the bacteria were always capable of nitrate utilization. This suggested that a steady state population of *T. denitrificans* had been established. Whether indigenous organisms were significantly represented in this population is unknown.

On the basis of the laboratory scale reactor, a population count between 10^8 and 10^9/ml had been anticipated.[9] This is one to two orders of magnitude more than the actually observed stable biomass concentration. A lower than expected pilot biomass concentration offers one possible explanation for the reduced upper limit for effective sulfide treatment cited above. Causes for the biomass deficiency are not understood. Remarkably, however, they do not appear to be linked to flow conditions within the discharge system since the observed population number appeared to be very resilient to full discharge events. The Day 48 and 50 enumerations were conducted one and three days after such an event and showed no indication of biomass washout. Resistance to washout may indicate that most biomass is attached to pit surfaces and relatively little exists suspended in the water phase. Mass transfer limitations associated with settled biomass may also account for the observed lower limit for treatment effectiveness.

Finally, the primary expenses associated with pilot operations were nutrient costs and energy costs for aeration. With respect to nutrient utilization, a minimum demand based upon ammonia requirements appears to offer a reasonable lower limit. Using ammonium nitrate as the ammonia source at a cost of $.10/lb, the estimated chemical treatment cost for the LACT 10 discharge is $.0014/bbl. The rated horsepower of the blower at 1,640 rpm and 10 psig backpressure is 58. The calculated energy cost per day at $.03/kW-hr is $31.10. For an average daily flow of 5,000 bbl/D, the energy cost per treated barrel is $.0062. The total treatment cost is thus approximately $00.76/bbl or about $38/D. Contrary to operating expenses, maintenance costs were rather high. This was attributed to repeated materials failures during cold weather and in the presence of corrosive nutrient solution.

Conclusions

This work demonstrates the successful application of a bioaugmentation technology for the treatment of hydrogen-sulfide-laden produced water discharges to control fugitive air emissions from said discharges. Specifically, the following was demonstrated:

1. *Thiobacillus denitrificans* was successfully introduced into the existing discharge system of the LACT 10 unit of the Salt Creek Field, Wyoming.

2. Sulfide removal efficiencies approaching 99% could be attained for routine discharges of 5,000 bbl/D containing 100 mg/l sulfide.

3. Aerobic sulfide oxidation to sulfate was almost instantaneous and therefore no odorous emissions of H_2S could be detected emanating from the discharge under normal conditions.

4. As designed and operated, the system was capable of processing about 10,000 bbl/D or 160 kg S/D.

5. Pilot recovery from transient full discharge upset events (98,000 bbl/D) was on the order of hours. During such events, partial oxidation of sulfide to elemental sulfur was indicated.

6. Minimum nutrient requirements were based upon bacterial reduced nitrogen (NH_3) needs. No clear requirement for nitrate as an alternate electron acceptor for anaerobic oxidation of sulfide was demonstrated.

7. A steady state population of *T. denitrificans* was established at about 10^7/ml without provisions for biomass recycling. This population was stable even in the event of full hydraulic discharges.

8. Estimate treatment costs for normal discharges were less than 1 cent per barrel.

Acknowledgments

The authors gratefully acknowledge the support of Amoco Production Company and ABB Environmental Services (Portland, ME).

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Table 1 Composition of Salt Creek Discharge Brine

Component	g/l
$CaCl_2 \cdot 2H_2O$	1.33
$MgSO_4 \cdot 7H_2O$	1.32
$MgCl_2 \cdot 6H_2O$	0.23
KCl	0.018
$Na_2CO_3 \cdot H_2O$	0.086
$NaHCO_3$	1.15
NaCl	1.82
Total Dissolved Solids (TDS)	4.81
pH = 8.5	

Table 2 Pit 1 Background Survey*

Location	Temperature, °F	pH	Sulfide, mg/l
Upstream, 1	107.0	7.3	100
Inlet, 2	88.6	7.8	28
SW, 3	83.8	8.4	28
W, 4	86.2	8.3	28
NW, 5	86.4	8.2	25
Exit, 6	81.0	8.4	28
NE, 7	84.4	8.4	28
E, 8	86.8	8.4	28
SE, 9	89.0	8.5	28

* Referenced to Figure 1 and compass direction

Table 3 Bacterial Enumerations of Sulfide Oxidizers by MPN Method

Day	Bacteria/ml	NO_3^- Utilization
3	1.5×10^7	Yes
17	9.5×10^7	Yes
48	7.5×10^7	Yes
50	2.5×10^7	Yes

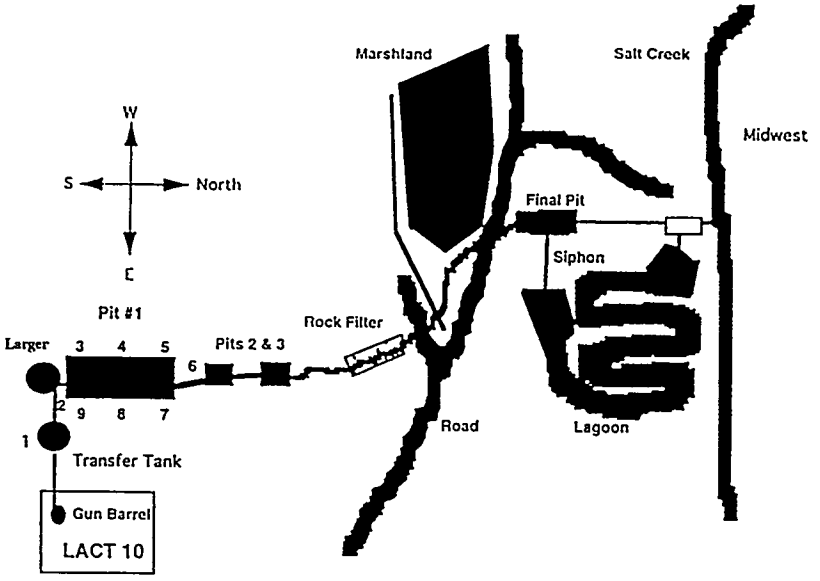

Figure 1 Amoco LACT 10 Unit, Salt Creek Field

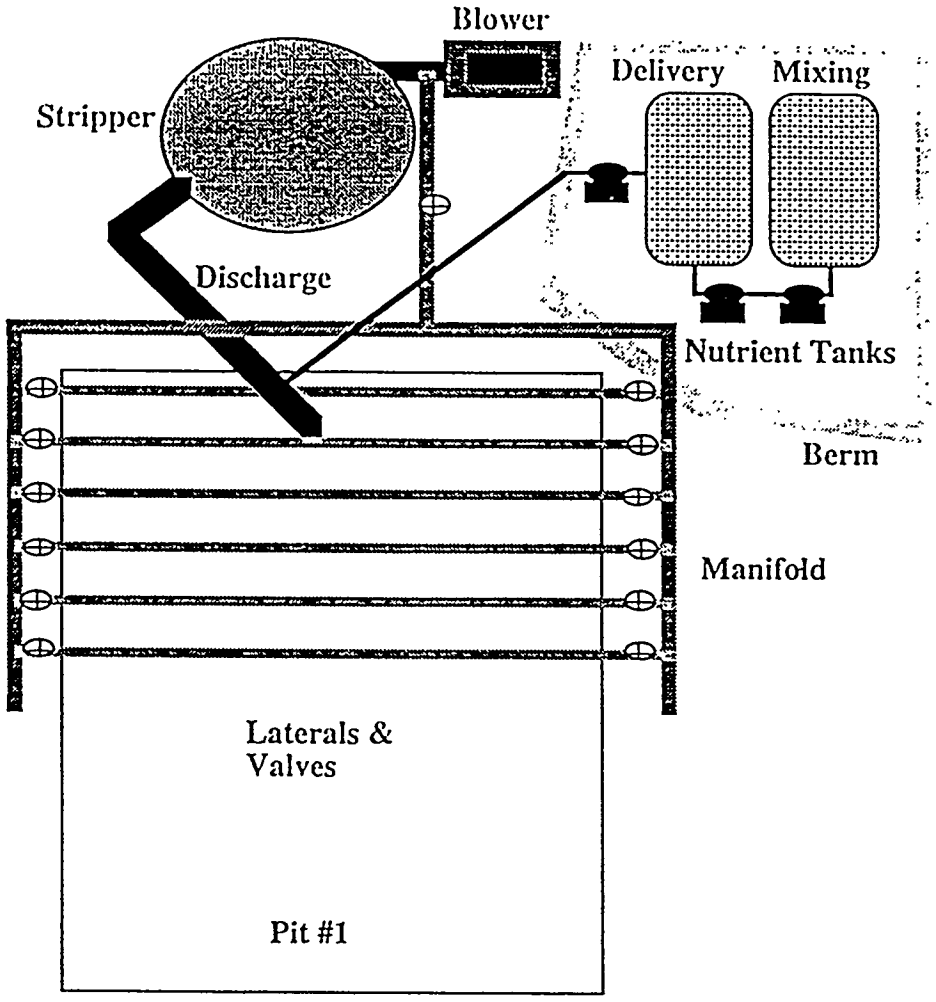

Figure 2 Facilities Modifications to the LACT 10 Unit

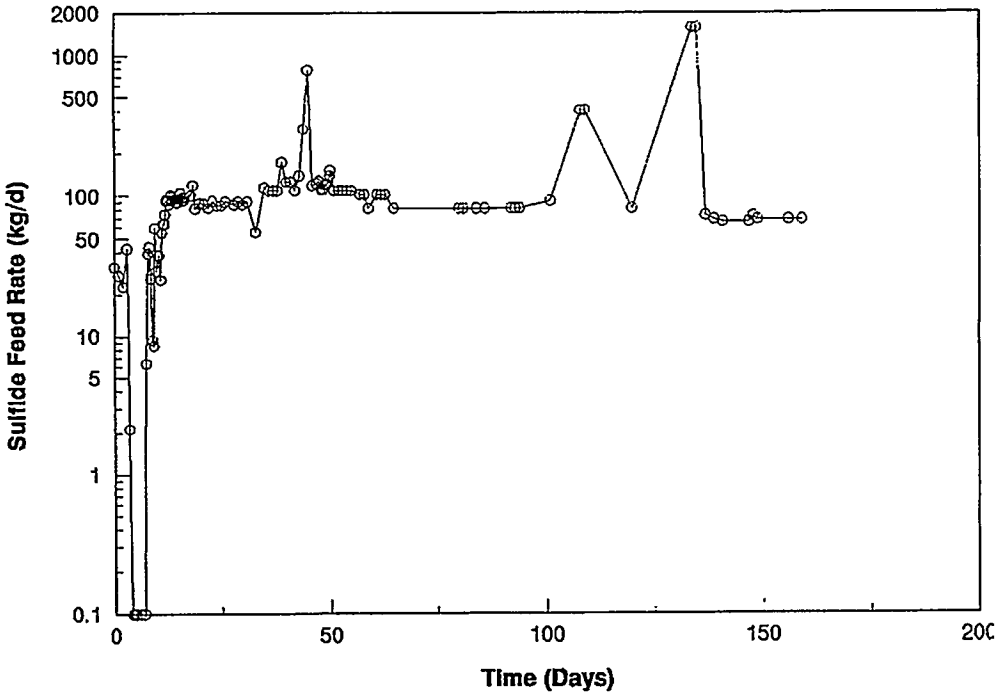

Figure 3 Sulfide Mass Feedrate (kg/D) to Pit 1.

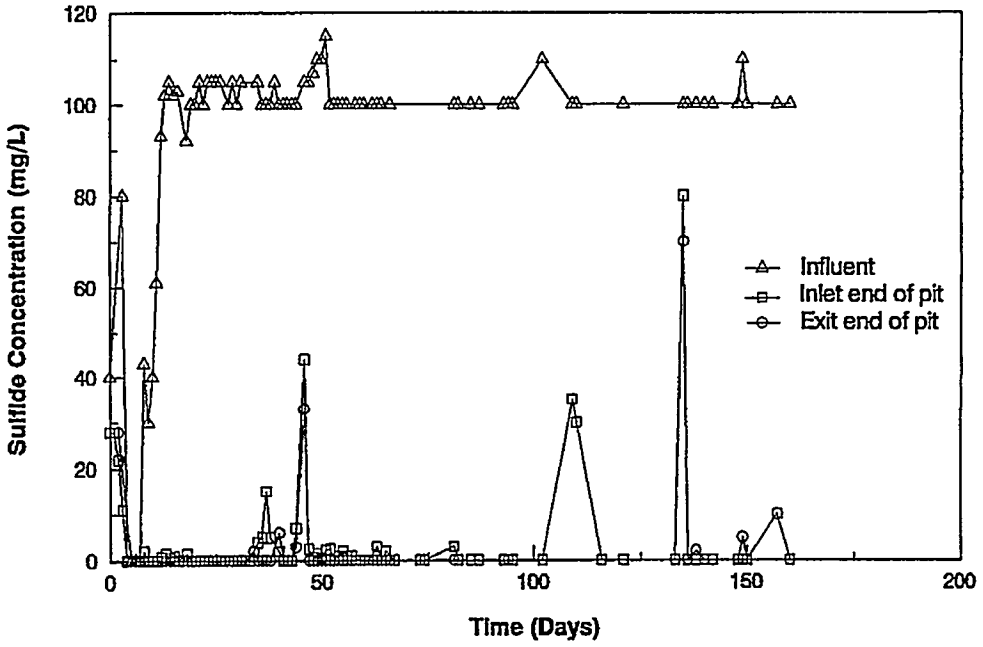

Figure 4 Sulfide Concentration in the Influent to Pit 1 and in the Inlet and Effluent Ends of Pit 1

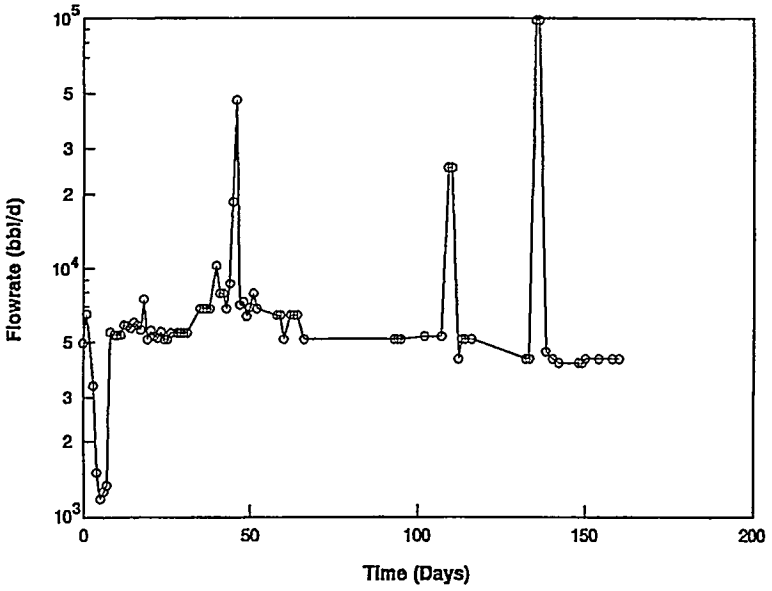

Figure 5 Hydraulic Flowrate to Pit 1

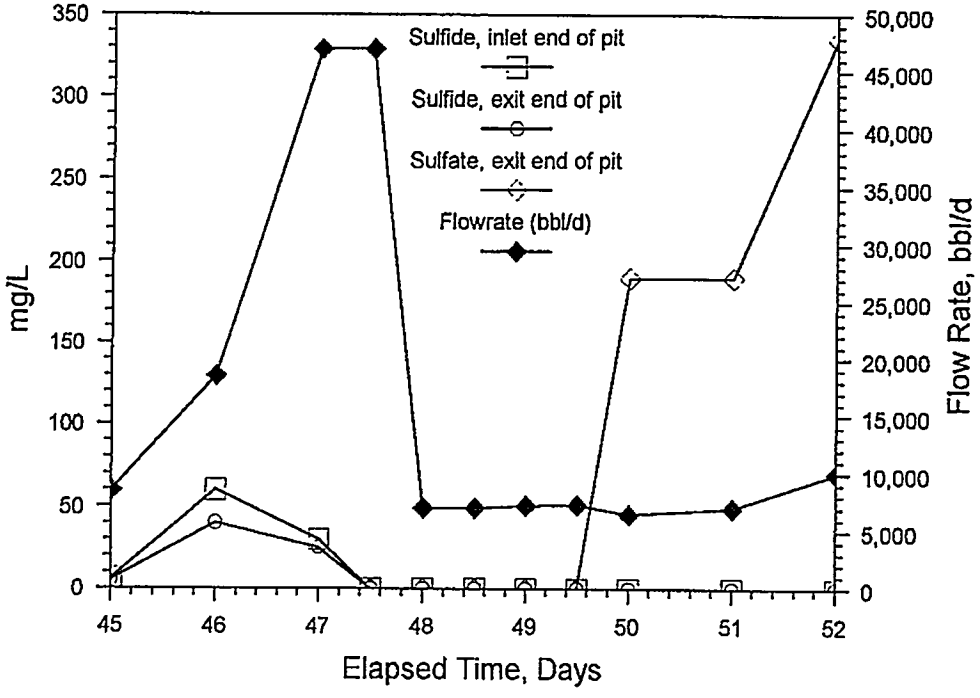

Figure 6 Sulfate Production Following a Hydraulic Upset

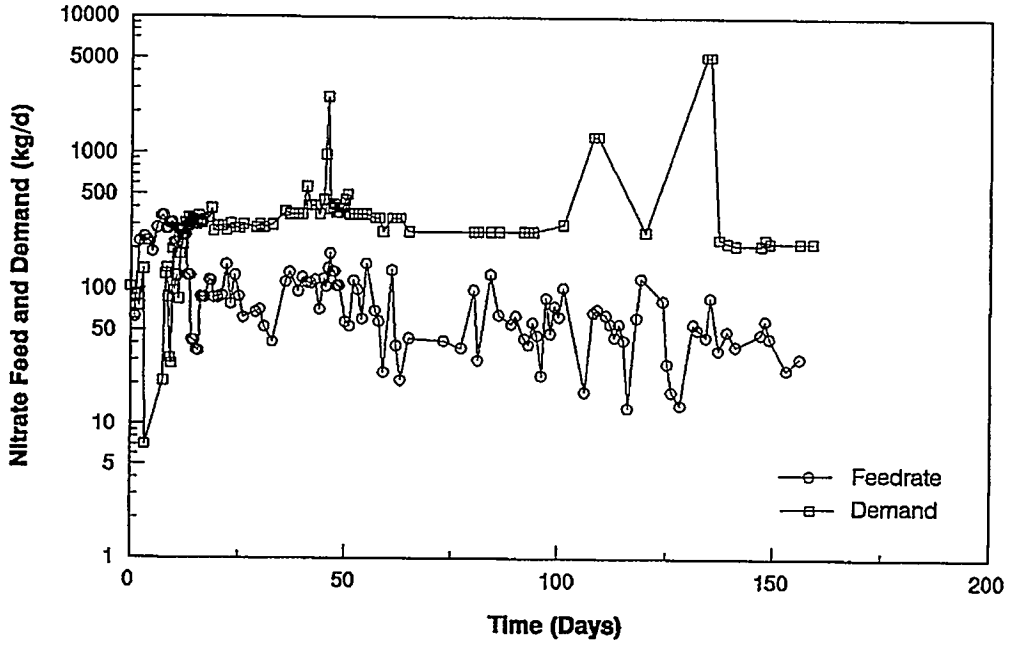

Figure 7 Nitrate Demand for Purely Anoxic Sulfide Oxidation and the Nitrate Mass Feedrate (kg/D) to Pit 1

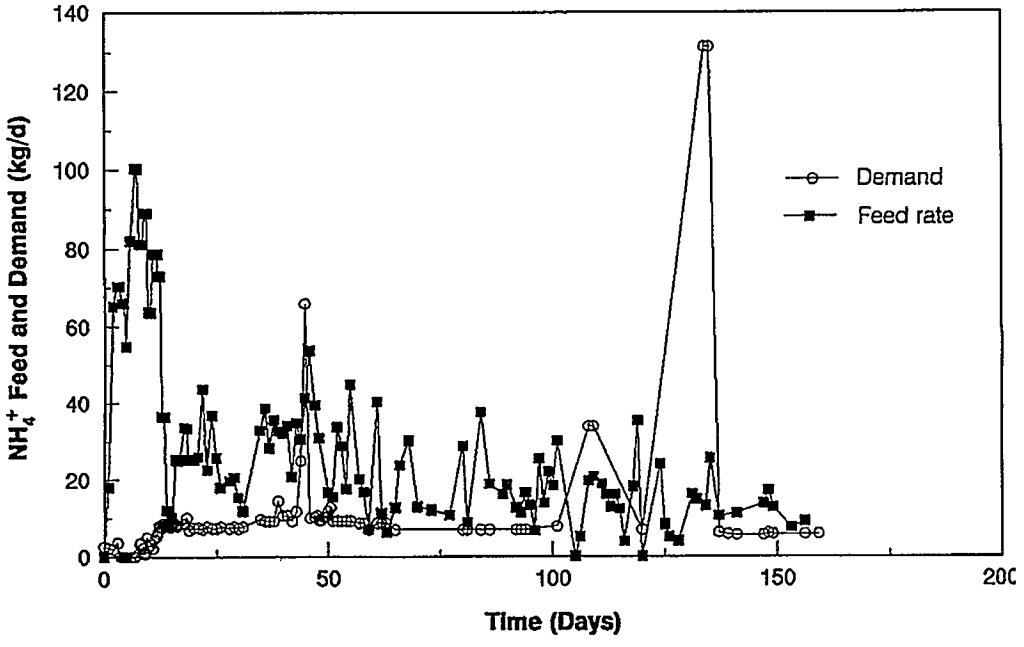

Figure 8 Demand for Ammonium Ion Required to Support Sulfide Oxidation and Ammonium Ion Mass Feed Rate (kg/D) to Pit 1

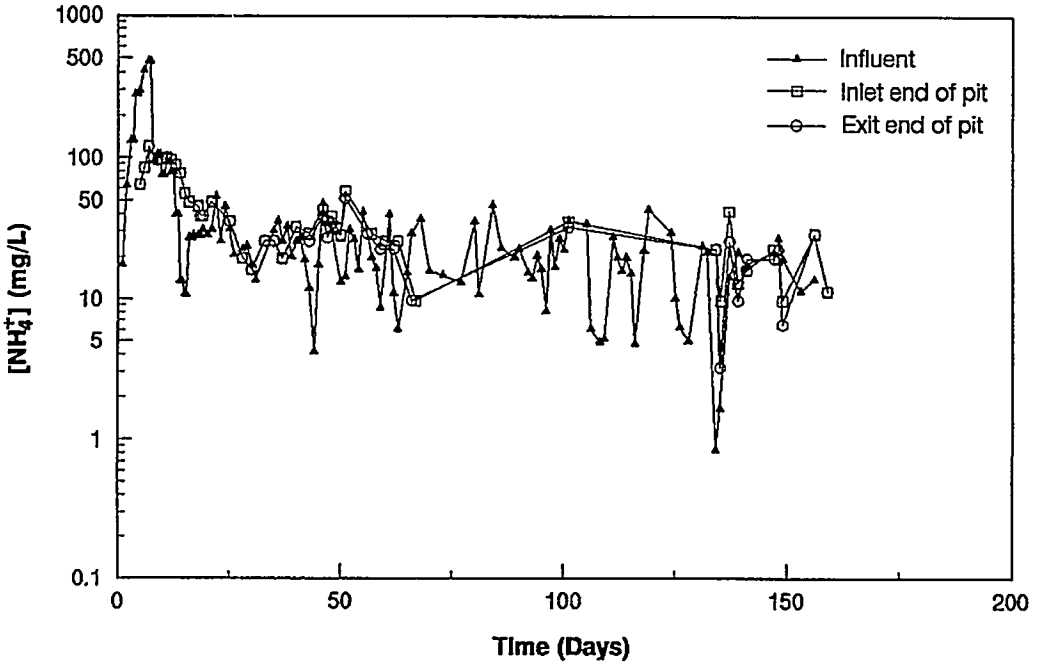

Figure 9 Ammonium Ion Concentration in the Influent to Pit 1 and at the Inlet and Effluent Ends of Pit 1

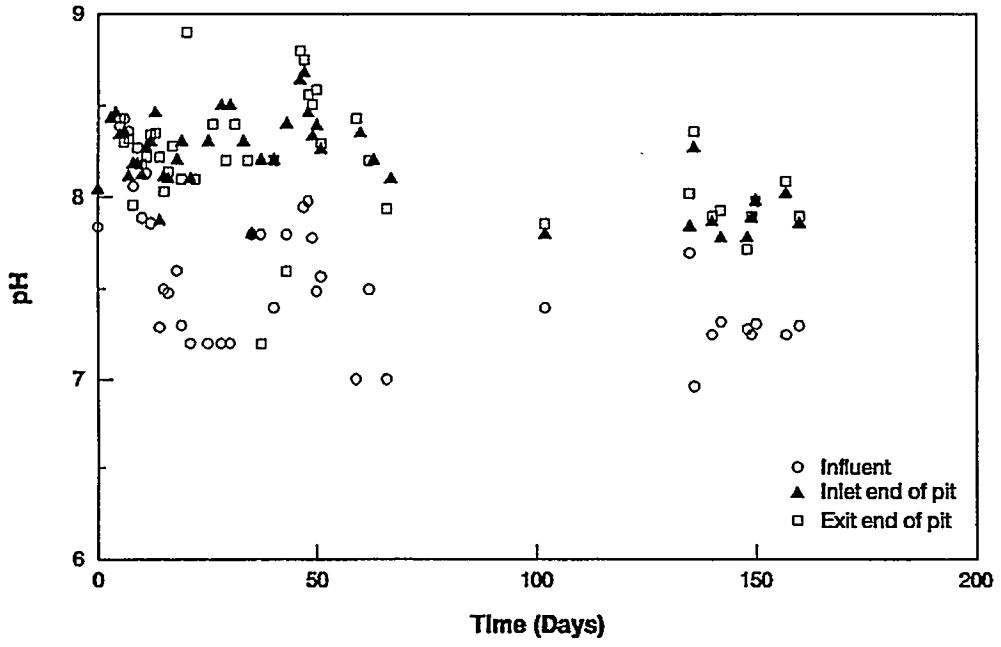

Figure 10 pH of the Influent to Pit 1 and at the Inlet and Effluent ends of Pit 1

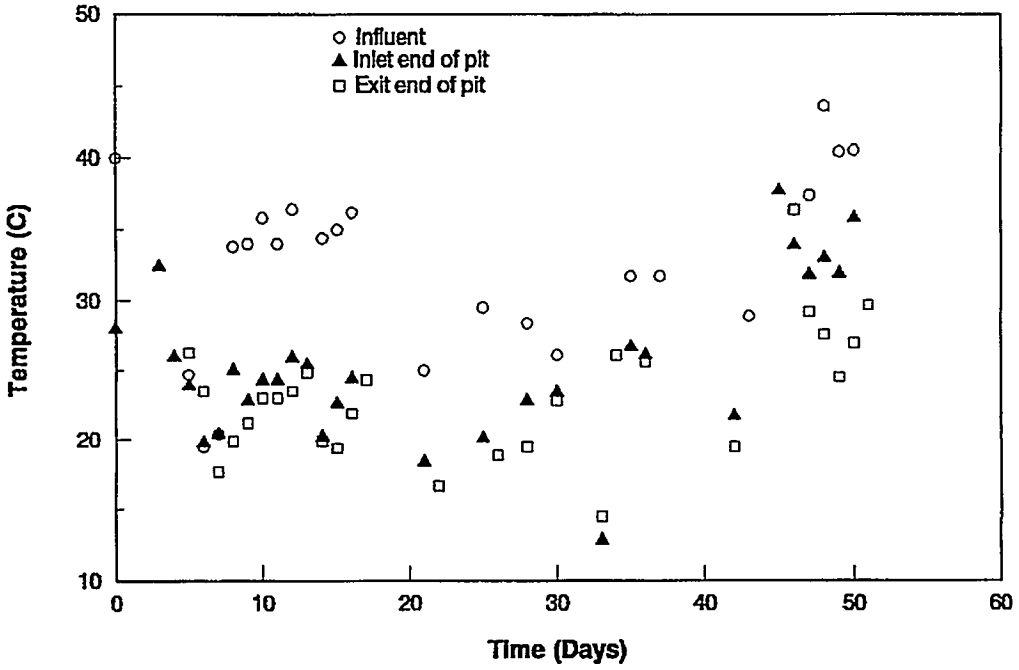

Figure 11 Temperature of the Influent to Pit 1 and at the Inlet and Effluent Ends of Pit 1

A Novel Enzyme-Based Acidizing System: Matrix Acidizing and Drilling Fluid Damage Removal

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Abstract

A novel acidizing process is used to increase the permeability of carbonate rock cores in the laboratory and to remove drilling fluid damage from cores and wafers. Field results show the benefits of the technology as applied both to injector and producer wells.

Introduction

Three years ago, a bacterial method was described for the in-situ acidizing of carbonate reservoirs.[1] A major advantage was perceived to be the slow production of acidity paralleling the growth of the injected microbes, thus allowing the action of the acid to be more widely distributed than by its direct injection into the reservoir in a highly active form.

Pediococcus, a small (1-μm diameter) spherical lactobacillus, was chosen in order to facilitate penetration through the pores of the reservoir matrix. The strain fermented molasses well and the lactic acid generated dissolved calcium carbonate in the reservoir to yield soluble calcium lactate, much of which was ultimately expelled from the reservoir when production was reinstated.

Field experience, however, revealed a number of serious limitations. Small though the bacteria were, they clearly failed to penetrate deeply enough into the very tight matrices which were the prime candidates for acidizing treatment. Furthermore, *Pediococcus* is typical of the lactobacilli in showing fastidious nutritional requirements; a considerable quantity of expensive yeast extract had to be included in the injection fluid, a cost burden which detracted seriously from the economic attractiveness of the procedure. It also became clear that perhaps as much as one-third of the injected bacterial growth medium was not rapidly produced when production resumed. Left downhole, there was in some reservoirs a risk of stimulating the growth and activity of sulfate-reducing bacteria (SRBs).

All these problems were overcome by the controlled generation of acid downhole in a noncellular system. This was achieved using the enzymatic hydrolysis of an ester; the injection fluid would be a clear liquid, initially at neutral pH and devoid of particulate matter. Acid would be generated essentially linearly with time, at a rate governed by the concentration of enzyme. Both the absence of fermentable substrates and nutritional supplements, and the fairly high concentrations of alcohol resulting from hydrolysis of the ester in the injection fluid, would restrain bacterial growth and so minimize SRB action.

The acetate is the most soluble calcium salt of the lower organic acids. Furthermore, acetate esters are readily available industrially. Enzymes able to catalyze the hydrolysis of organic acetates at temperatures up to about 80°C have been developed. The only other feedstock is water at roughly neutral pH and of suitable quality for injection.

Acetic and formic acids are normally the organic acids of choice for oil field use. While generally regarded as retarded compared with hydrochloric or hydrofluoric

acids, they do nevertheless react relatively quickly with carbonate rock or scale, and placement can be problematic. In the enzymatic hydrolysis method, the rate and quantity of acid produced are governed by the formulation of the injection fluid; thus, the acidizing fluid can be placed before most of the acid is produced, resulting in high zonal coverage and allowing true matrix acidizing and efficient drilling fluid damage removal. The process can be used for dissolving carbonate rock, scales, or carbonate fines in drilling muds, removing drilling fluid damage and other formation damage, and breaking crosslinked gels.

Basis of the System

In the Arcasolve™ system, esters are hydrolyzed enzymatically at a rate controlled by the concentration of enzyme:

e.g., methyl acetate + water $\longrightarrow$ methanol + acetic acid enzyme

Many formulations are possible, the main factors to be taken into account when planning for a particular situation being:

- Type and amount of acid required
- Acceptable shut-in period (downtime)
- Solubility in water of the ester and of the calcium salt
- Flash point of the bulk feedstock
- Possible toxicity and other hazards

Methods

Corefloods

The effects of Arcasolve treatments on the permeability of rock cores were measured with an EPS Coresystem CFA 100 Coreflood apparatus (Edinburgh Petroleum Services, Scotland). The electrically heated pressure chamber of the rig permitted core tests to be carried out over the temperature range from ambient to 100°C. Tests were generally operated at a confining pressure of 1,500 psi combined with a backpressure of 800 psi.

Core plugs were cleaned with chloroform/methanol and their helium porosity and air permeability measured. They were saturated with 3% KCl under vacuum for 16 hours, flooded with 8–10 PV of 3% KCl solution, and their permeabilities to 3% KCl were determined using Darcy's equation. Each core was then filled with the Arcasolve treatment fluid under test and incubated at the chosen temperature for the desired length of time before flushing with 3 PV of fresh 3% KCl. The permeability of each core was again measured with 3% KCl.

Field Treatments

Treatment Design. The objective of the treatments was a 20% rise in the production or injection rate by increasing the formation matrix permeability. Reservoir modeling predicted a requirement for Arcasolve stimulation in a zone of 3 m radius around the injector.

Treatment Execution. In a typical treatment, Arcasolve formulation A (designed for matrix acidizing) was pumped as three sequential 100-barrel slugs. Each was followed by a 24-hour shut-in to allow in-situ acetic acid generation and dissolution of carbonate rock. The injected fluid also contained 0.2% SS100 silt suspender. Following each shut-in period, 100 barrels were produced from the well before the injection of more Arcasolve fluid.

Drilling Mud Damage Removal

Compatibility of Arcasolve with Muds. Tests were carried out on six commercial mud types: Cat-1, Saturated Salt, KCl/Polymer, KCl/PHPA, KCl/Glycol, and Petrofree. The compatibility of the muds with Arcasolve was assessed by measuring mud viscosity changes and soluble calcium levels; calcium release indicated that active enzyme was releasing acid.

Core Plug Experiments. The effectiveness of Arcasolve at removing drilling mud damage resulting from mud filter cakes was investigated with core plugs. A core in a small confining sleeve was inserted into a pressure leak-off cell in a coreflood apparatus and its initial permeability measured. The core was then treated as follows: mud damage was applied as a mud squeeze at 300 psi for 30 minutes; the permeability of the damaged core was measured whenever possible; the core received an Arcasolve squeeze at 600 psi; the core was incubated in the Arcasolve fluid to remove mud damage and the regain permeability measured.

Large Scale Damage Removal Tests. In practice, treatments for repairing drilling damage in horizontal wells need to be effective over long formation

intervals. The potential for Arcasolve to achieve this was assessed by use of a linear conductivity flow cell[2] which enabled experiments to be conducted on much larger areas of rock core than is possible with a conventional rock core plug apparatus. Two rock wafers were mounted in the same cell, one above and one below. Mud flowed over the surface with a pressure differential pushing mud onto the surface, thereby causing damage.

Tests used KCl/Polymer and CAT-1 muds to damage Indiana limestone wafers, the resulting damage being rectified a suitable Arcasolve formulation. Permeabilities were measured before and after treatment.

Comparison of Zonal Coverage of Arcasolve and HCl

The tests evaluated the ability of the Arcasolve treatment to improve zonal coverage when treating drilling damage. Two Indiana limestone cores encased in Hassler sleeve linear flow cells were linked in series.[2] Mud damage was applied to the faces of the cores and then treated with either 15% HCl + 0.4% corrosion inhibitor or with an Arcasolve formulation suitable for the removal of drilling fluid damage. The path of the acidizing fluid led sequentially through the mud damage and matrix of the first core and then through the mud damage and matrix of the second core. Full details of the experiments and the results have been published elsewhere.[2]

Results

Core Experiments

The effects of a series of sequential Arcasolve treatments on the permeability of a dolomite rock core are shown in Figures 1–3. In each case the Arcasolve treatment is expressed as acetic acid equivalent.

After measurements of their permeabilities, cores were flooded with suitable Arcasolve formulations, shut in for up to 25 hours and their permeabilities to 3% KCl measured again. Each core in Table 1 received three sequential Arcasolve treatments; the number of treatments for the cores Table 2 are given at the bottom of that table.

The increase in permeability of the core plugs in response to Arcasolve indicates that both formations responded well to Arcasolve treatments. The deployment of three sequential Arcasolve floods led to approximately twofold to threefold increases in permeability.

Use of Arcasolve for Drilling Fluid Damage Removal. All the water-based muds were shown to be compatible with Arcasolve, the concentration of soluble calcium in each case increasing in the fluid.[2] The fact of calcium dissolution confirmed acetic acid generation and hence enzyme activity in the presence of drilling muds. Calcium dissolution was also observed in the presence of the oil-based Petrofree mud, but this did not correlate with an increase in permeability.[2] There was an initial increase in the viscosity of this mud followed by a reduction in mud viscosity during incubation. The reasons for this behavior are not understood

Following the Arcasolve/mud compatibility tests, four drilling muds were selected for examination in the core plug experiments: CAT-1, Saturated Salt, KCl/Polymer (all water-based), and Petrofree Mud (oil-based). Twelve cores were used to run three replicates of each of the muds. The concentration of soluble calcium in the Arcasolve fluid before and after incubation was also measured as an index of carbonate dissolution. The data indicated effective damage removal of water-based drilling muds from rock core plugs (see Table 3). The mechanism of damage removal appears to have been a combination of matrix acidizing (cationic polymer mud-damaged cores) and direct attack on the mud (saturated salt and KCl/polymer-treated cores). For all cores treated with water-based muds, etching of the core face was visible.

Two mud damage tests with water-based drilling muds (see Table 4) demonstrated the effectiveness of Arcasolve for removing drilling mud damage. The test with CAT-1 mud showed severe damage occurring in both the top and bottom wafers (88% and 96%, respectively). There was some variation in the initial permeabilities of the cores (upper core 43 md, lower core 125 md), but the damaged permeabilities were very close (5.0 md and 5.6 md, respectively). The greater initial permeability of the bottom core might have been the cause of the greater damage resulting from deeper mud penetration. When the cores were removed from the cell, the filter cake appeared evenly distributed over both core faces.

Tests with the KCl/Polymer also indicated severe mud damage to the lower core but an apparent increase in permeability for the upper. This might have resulted from a partial blockage of the system during the initial permeability measurement, leading to aberrant results in the subsequent determinations. When the cores were later removed from the rig, it was clear that the filter cake was thicker on the lower core.

Arcasolve removed a high proportion of the mud damage caused by both CAT-1 and KCl/Polymer muds (see Table 4). The restored permeabilities of the cores were high and, with some minor variation, visual examination showed even distribution of the filter cake on both upper and lower cores.

Comparison of Zonal Coverage of Arcasolve and HCl. Breakthrough with HCl occurred almost immediately the acid contacted the first core, with minimal effect on the second (see Table 5). By contrast, permeability data for the cores treated with Arcasolve clearly showed that the Arcasolve fluid penetrated through mud damage on both core faces and hence into the matrix of the second, thereby providing significant permeability increases in both cores.

Field Results

The Arcasolve process was used successfully in the field for matrix acidizing of injector and producer wells in West Texas. Details of the formations are given in Table 6, while Table 7 summarizes the observations in several treatments. Production and injection data from the treated wells are shown in Figures 4–6.

Significant increases in oil production or water injectivity were routinely observed. Some of the treated wells appeared to show the effects of near wellbore damage and clearly benefited from the Arcasolve treatment when appropriately formulated: it is our experience that formulation A, normally used for matrix acidizing, is also capable of removing near wellbore damage although formulation B is preferred for damage removal applications.

Discussion

Arcasolve can increase the permeability of rock cores, and the system has been used successfully in the field for matrix acidizing injector and producer wells to increase their injectivity or production rate. In-situ production of acetic acid allows true matrix stimulation of the formation, with even placement of acid throughout the treatment radius. In several cases, responses exceeded that expected from modeling. In carbonate rock cores, Arcasolve has also been shown to remove damage caused by water-based drilling muds, restoring permeabilities to close to their original values.

The Arcasolve system allows users to control the rate and quantity of acid generated, offering major benefits of greater zonal coverage when trying to place fluid over long intervals as, for example, in directional drilled wells. The importance of optimizing production to obtain maximum return on investment is recognized. The consequence of effective skin reduction on the net present value of wells has been clearly demonstrated by Daneshy.[3] Beyond the promise of more effective damage removal, the new process offers the potential for achieving simultaneous drilling damage removal and near wellbore stimulation in a single treatment. This would maximize the net present value of the well.

The use of Arcasolve avoids the need to pull corrosion-sensitive equipment or use corrosion inhibitors, as is common in conventional procedures. The injection and produced fluids present very low hazard; they are based on readily biodegradable components which minimize the disposal problems with the spent fluids. The ability to place relatively low concentrations of acid evenly over a large radius can permit the treatment of poorly consolidated formations where wellbore stability problems preclude the use of established HCl-based methods.

The Arcasolve™ system is currently being offered commercially by Cleansorb Limited, Yateley, U.K. (Tel: +44-1252-860641). The procedure is protected by patent applications.

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Acknowledgments

We acknowledge financial assistance by the U.K. Offshore Supplies Office to Archæus Technology Group Ltd, where the system was originally developed. Stimlab (U.K.) carried out the linear flow conductivity tests under subcontract. We also thank Richard Hodgson for carrying out most of the laboratory work.

Table 1 Summary of Coreflood Results: Formation 1

Core Plug	1A	1B	1C
Porosity (%)	14.5	18.2	17.8
Depth of origin (m)	1,469	1,468	1,472
Core length (cm)	6.0	6.0	6.0
Core diameter (cm)	2.52	2.52	2.52
Core cross-section area (cm^2)	5.0	5.0	5.0
Core weight (g)	75.4	62.0	—
Pore volume (cm^3)	4.3	4.7	5.6
Air permeability (md)	0.53	4.8	26
Permeability to 3% KCl (md)	0.36	3.2	34
Permeability to 3% KCl after Arcasolve treatment (md)	0.94	6.7	98
Permeability increase factor	× 2.6	× 2.1	× 2.9

Table 2 Summary of Coreflood Results: Formation 2

Core Plug	2A	2B	2C	2D
Porosity (%)	12.6	12.8	12.5	21.9
Depth of origin (m)	1,486	1,490	1,482	1,487
Core length (cm)	5.0	5.0	5.0	5.0
Core diameter (cm)	2.52	2.52	2.52	2.52
Core cross-section area (cm^2)	5.0	5.0	5.0	5.0
Core weight (g)	62.8	62.6	62.1	55.1
Air permeability (md)	1.0	0.95	0.84	9.0
Permeability to 3% KCl (md)	0.69	0.52	0.21	9.1
Permeability to 3% KCl after Arcasolve treatment (md)	3.0	2.5	0.41	18
Permeability increase factor	x 4.3	x 4.8	x 2.0	x 2.0
Number of sequential treatments*	6	3	4	4

*Optimized with respect to Arcasolve formulation

Table 3 Effects of Arcasolve Treatment on Mud Damage in Core Plugs

Mud Type	Core No.	Initial	Permeability Damaged (md)	After Treatment
Cationic polymer	1	0.55	0.03	0.39
Cationic polymer	2	0.66	0.04	0.44
Cationic polymer	6	0.74	0.05	0.25
Saturated salt	8	0.64	0	0.23
Saturated salt	22	0.68	0	0.79
Saturated salt	23	0.73	0	0.83
KCl polymer	10	0.39	0	0.47
KCl polymer	13	0.46	0	0.49
KCl polymer	14	0.50	0.01	0.49
Oil-based	17	0.49	0.02	0.05
Oil-based	19	0.69	0.02	0.04
Oil-based	21	0.54	0.03	0.04

Table 4 Mud Damage Removal Using Arcasolve

Mud Type	Initial K (md)	Final K (md)	% Damage	% Damage Removal
Controls (No Arcasolve)				
Cationic-1 Polymer				
Upper core	43	5	88	
Lower core	145	6	96	
KCl Polymer				
Upper core	10	33	–	
Lower core	154	20	87	

Mud Type	Initial K (md)	Regained K (md)	% Damage	% Damage Removal
With Arcasolve Treatment				
Cationic-1 Polymer				
Upper core	24	20		83
Lower core	29	23		79
KCl Polymer				
Upper core	51	47		94
Lower core	77	35		46

Table 5 Comparison Arcasolve and Hydrochloric Acid

Core	Initial K (md)	Recovered K (md)
Arcasolve Treatment		
A	6.9	25
B	4.0	69
Hydrochloric Acid Treatment		
A	4.7	840
B	3.4	4.8

Table 6 Details of Wells Used for Arcasolve Treatments to Improve Injectivity and Productivity

Formation 1	Formation 2
West Texas, San Andres Dolomite	West Texas, San Andres Dolomite
Bottom hole temperature 40°C (104°F)	Bottom hole temperature 40°C (104°F)
Pay zone 4,804-4,846 ft (1,441–1,454 m)	Pay zone 4,863–4,885 ft (1,458-1,464 m)
Average pay thickness 30 ft (9 m)	Not available
Average porosity 10%	Not available

Table 7 Summary of Effects of Arcasolve A Treatment on Four Wells

		Production/Injectivity (bbl/D)		
Formation	Well Type	Before Treatment	After Treatment	Notes
1	Producer	18	27	+30% increase in total fluids
2	Water injector	0 at 1,800 psi	80 at 1,800 psi	Probable formation damage correction
3	Water injector	44 at 1,840 psi	55 at 1,840 psi	
4	Water injector	60	76	

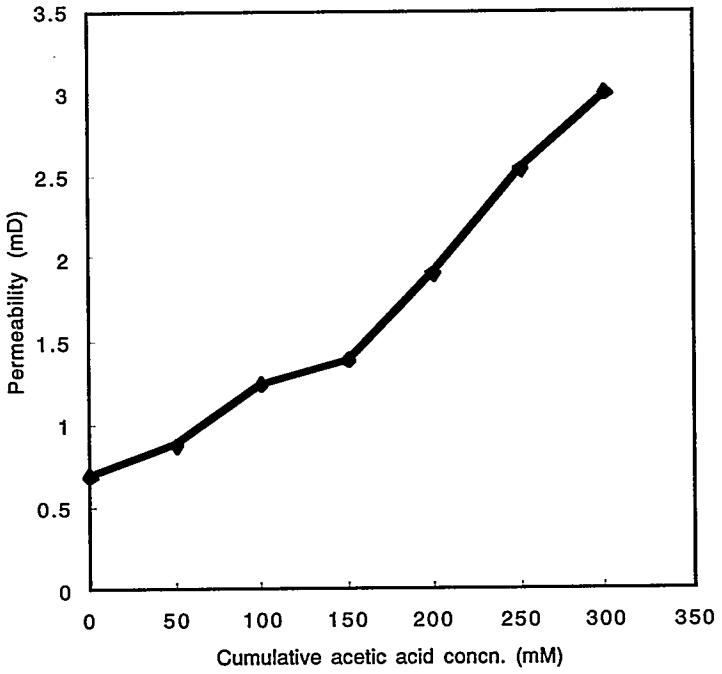

Figure 1 Effect of Arcasolve on Core Permeability (1)

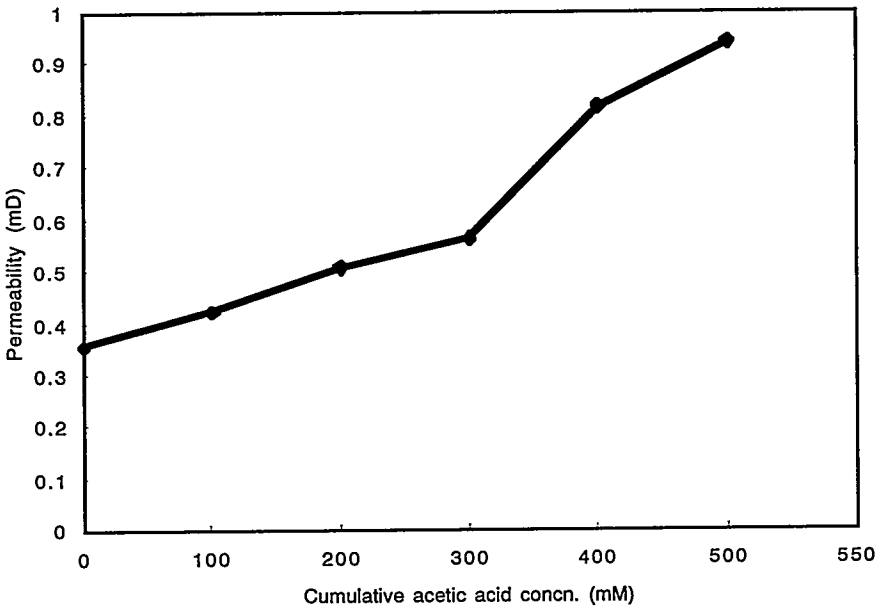

Figure 2 Effect of Arcasolve on Core Permeability (2)

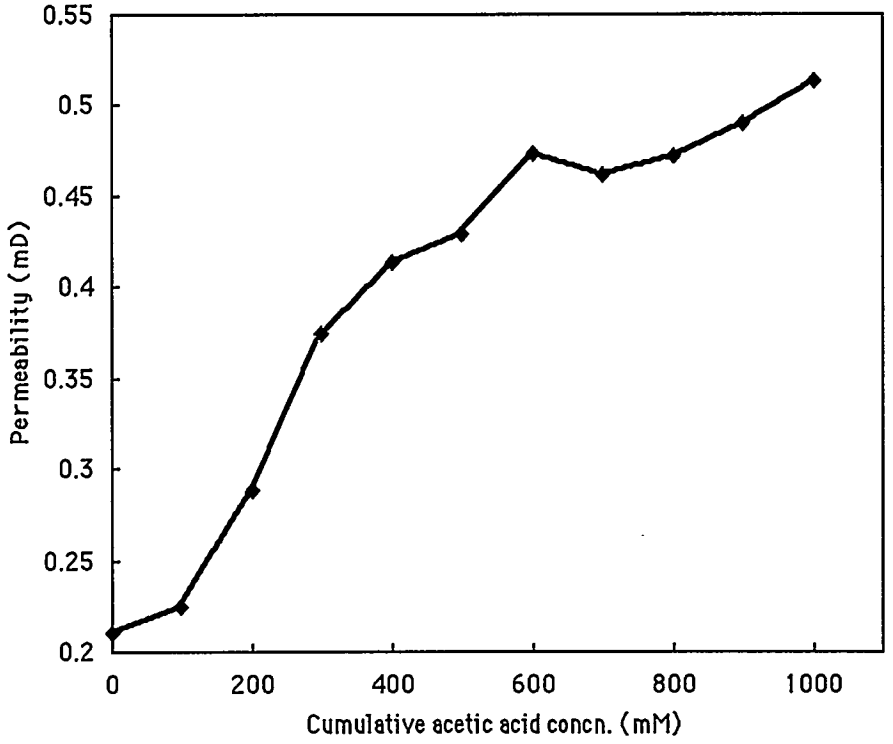

Figure 3 Effect of Arcasolve on Core Permeability (3)

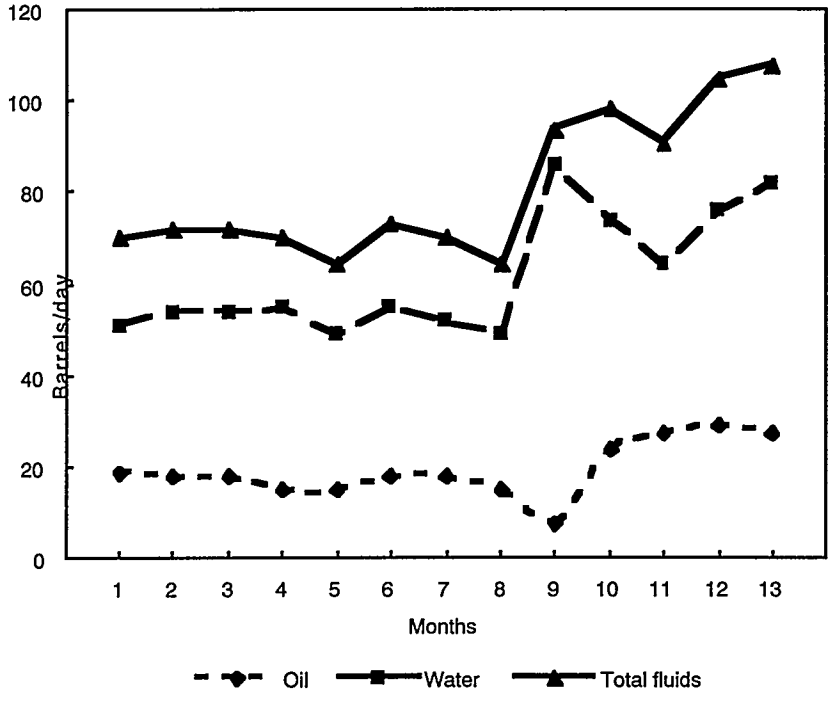

Figure 4 Results of Arcasolve Treatment of a Producer Well

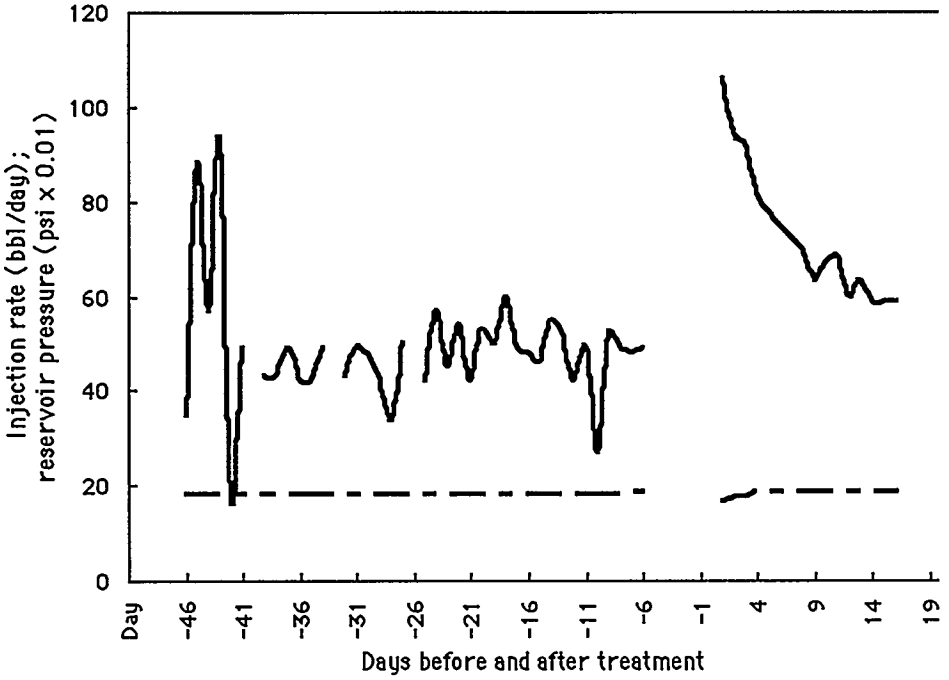

Figure 5 Water Injection Rate and Reservoir Pressure before and after Arcasolve Treatment (Well No. 1)

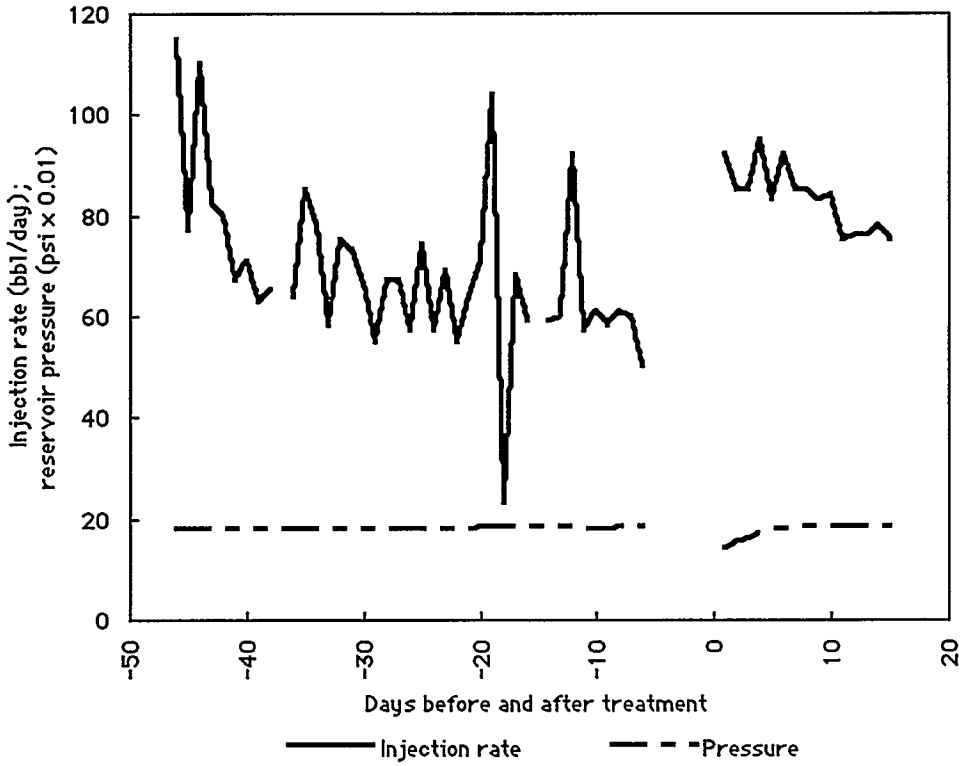

Figure 6 Water Injection Rate and Reservoir Pressure before and after Arcasolve Treatment (Well No. 2)

POSTER SESSION

Strength and Stability of Microbial Plugs in Porous Media

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Abstract

Mobility reduction induced by the growth and metabolism of bacteria in high-permeability layers of heterogeneous reservoirs is an economically attractive technique to improve sweep efficiency. This paper describes an experimental study conducted in sandpacks using an injected bacterium to investigate the strength and stability of microbial plugs in porous media.

Successful convective transport of bacteria is important for achieving sufficient initial bacteria distribution. The chemotactic and diffusive fluxes are probably not significant even under static conditions. Mobility reduction depends upon the initial cell concentrations and increase in cell mass. For single or multiple static or dynamic growth techniques, permeability reduction was approximately 70% of the original permeability. The stability of these microbial plugs to increases in pressure gradient and changes in cell physiology in a nutrient-depleted environment needs to be improved.

Introduction

The objectives of this work were to investigate the transport and growth of bacteria in porous media and to determine the magnitude and stability of mobility reduction from microbial plugs. The microbial plug refers to the system of cell mass and biopolymer produced during growth and metabolism. A schematic of bacteria and sand particles showing all the retention and permeability reduction mechanisms is shown in Figure 1. Three growth techniques were tested for improving the extent of mobility reduction. Stabilities of microbial plugs to increases in flow velocities/pressure gradients and changes in cell physiological conditions under nutrient-depleted environments were evaluated.

Low sweep efficiency is a major problem in oil recovery using displacement processes in heterogeneous reservoirs. Mobility reduction from the growth and metabolism of bacteria in high-permeability layers or channels of heterogeneous reservoirs has been a subject of research for several decades. This cost-effective technique is also referred to as microbial selective plugging.

The two basic mechanisms are permeability reduction of the rock and viscosity enhancement of the aqueous phase.[1] The three main issues are (1) distribution of indigenous/injected bacteria, (2) distribution of nutrients, and (3) growth and metabolism of bacteria in anaerobic, competitive, and mineralogically heterogeneous environment of porous media containing multiple fluid phases. The effectiveness of this process as a profile modification technique depends upon the strength and stability of bacterial plugs and the layer permeability contrasts (e.g., 1:10, 1:100, etc.) of the reservoir under consideration.

A brief discussion of the three issues is given below.

Bacteria Distribution. Either indigenous or injected bacteria can be used in the microbial plugging processes. With indigenous bacteria, transport problems are avoided, but species effectiveness becomes the central issue.

With injected bacteria, the species effectiveness is predetermined, but transport and competitive growth capacity are the main issues. Several techniques, including formation of submicron spores, have been developed for improving bacteria transport through consolidated and clay-containing porous media.[2,3] The injected bacteria must outgrow diverse indigenous bacteria, otherwise the latter will prevail with unpredictable and frequently undesirable effects.[4,5] The competition between indigenous and injected bacteria has not been studied extensively.[6,7]

Effective disinfecting process may be necessary for eliminating undesired bacterial populations. Jenneman et al.[8] reported that even after cores were steamed for 2 weeks, autoclaved for 12 hr (121°C, 15 psig), and dried at 121°C, viable indigenous populations, including *Actinomycetes* and genra such as *Pseudomonas* and *Bacillus*, remained at a concentration of 10^3 to 10^4 cells/ml. The use of a disinfectant solution (chlorine dioxide solution) reduced the counts of these populations for 24 to 48 hr. Upon the availability of desired nutrients and favorable growth conditions, these populations reestablished.

Nutrient Distribution. Design of, injection strategy for, and transport of nutrients have been investigated by several researchers. From a tracer test, Sarkar[9] found that glucose, the primary carbon source used for the present study, is not adsorbed by the rock. Even after a prolonged contact of 10 days, no significant adsorption of glucose on the sand grains could be detected. Jenneman et al.[10] showed that carbon (glucose) and nitrogen (ammonium sulfate) sources can be transported through Berea sandstone cores without much retention. Phosphates (potassium phosphate) can be transported with some retention, but Bacto-peptone, which is a protein, is retained significantly. The ratio of effluent to injected protein concentration was 0.4 at the end of 16 PV of injection.

Growth. The growth and metabolism of bacteria in petroleum reservoirs is far more complicated than fermentation of bacteria in shake-flasks. Jenneman[11] reviewed the results on effects of pore size on the growth and metabolism of bacteria. Small pore sizes and diffusion-limited mass transfer increase the lag phase and decrease the growth rate, final yield of cells, and cell size. The results were cell specific. Diffusion-limited growth has been demonstrated in many ecologically and industrially important systems, including biofilms,[12] microbial pellets,[12] and solid substrate colonization.[13] Karel et al.[14] immobilized cells uniformly within a porous polymeric matrix and then suspended it in nutrients. After a short period of time, all the viable cells were found located at the surface of the matrix in close contact with the nutrient solution. Typically, the viable cell layer was less than 200 μm thick. The cells in the interior were either dead or metabolizing extremely slowly because of mass transfer limitations.

Results from simple growth experiments conducted in shake-flasks and test tubes indicated that the presence of a solid phase and an increase in the salinity of the growth medium reduce the rate of microbial growth.[9] A higher initial glucose concentration increases the amount of residual glucose. In other words, the extent of microbial growth in porous media was limited.

Mobility Reduction. A field trial using indigenous bacteria at the North Burbank unit (Oklahoma) indicated that effective permeability decreased by 33%, but the plug was unstable following several weeks of brine injection.[15]

Results from laboratory experiments using an injected bacterium Salton-1 (closely resembles *Bacillus licheniformis*) under single-phase conditions indicated that the microorganisms in spore forms propagate easily through cores with more than 300 md permeability.[16] After bacterial growth, permeability of the second and third/last sections decreased by 90%.

Mobility reduction has been reported to be as high as 95% (of initial permeability), although in most cases it was approximately 65%. Complete sets of results, including effluent bacteria concentration histories, permeability profiles, and nutrient consumptions are usually not available for analyzing the processes in detail. In many cases, only overall (for the whole length) mobility reductions were measured. Those results were definitely influenced by facial plugging and do not reveal information about the downstream sections.

Jack et al.[17] observed more than 95% reduction in overall (for the whole length) mobility from the growth of *Leuconostoc mesenteroides* NRRL B523 in porous media consisting of fused glass beads (permeability 6.5 darcys and porosity 36%). The organism is known to produce insoluble dextran while growing anaerobically in the presence of sucrose. Silver et al.[3] conducted plugging experiments in Berea sandstone cores and slim tubes packed with crushed sandstone. The overall mobility reduction ranged from 65% to 95% depending upon the initial permeability, amount of bacteria injected, and nutrients used. The maximum mobility reduction was observed in the slim tube experiment in which the initial permeability was 6.7 darcys.

MacLeod et al.[2] found that the cell concentration profiles after acetic acid stimulation were similar to those observed after cell injection. The cell number increases from growth probably matched the cell number decreases from death; however, permeability profiles changed. A fluorescent DNA assay was used for measuring cell density along the length of the core, and starved cells of smaller size were used for improving bacteria transport.

Knapp et al.[18] used two layers of Berea sandstone slabs (105 md and 225 md) placed in capillary contact. To stimulate the growth of indigenous microorganisms, treatments consisting of injection of nutrients and incubation for 24 hr were performed. On the basis of injectivity increase of the low-permeability layer from 15% to 60%, the authors concluded that the growth occurred preferentially in the high-permeability layers.

Torbati et al.[19] evaluated pore size distributions for Berea sandstone cores plugged with products from the growth of indigenous bacteria and compared the results with those found from unplugged cores. The distributions for unplugged cores had several modes and contained pores up to about 58 μm in size. The distributions for

the plugged cores had a single mode, and the mean shifted toward smaller pore sizes. The largest pore size was about 37 μm.

Experimental Conditions

Microbe. The selection of *Bacillus licheniformis* JF-2 (American Type Culture Collection #39307) as the model microorganism followed from its selection as the model microorganism for microbial EOR processes based on its ability to grow well inside reservoirs and produce one of the most active biosurfactants known.[10,20,21] JF-2 is a facultative, motile, and gram positive bacterium. The average dimensions of rodlike JF-2 cells are 4 μm long × 1.5 μm diameter, as determined by Coulter counter measurements.

Nutrient. Cultures were grown aerobically at 42°C for 15 hr in Erlenmeyer flasks. A growth medium supplemented with glucose (usually 1%) and $NaNO_3$ (1%) for anaerobic conditions was used as the nutrient. The growth medium contained (w/v): 0.1% $(NH4)_2SO_4$, 0.025% $MgSO_4$, 1% (v/v) trace metal solution, and 0.5% NaCl in 100 mM phosphate buffer (pH 7.0).[10,21] The trace metal solution contained (w/v): 0.1% EDTA, 0.3% $MnSO_4$, 0.01% $FeSO_4$, 0.01% $CoCl_2$, 0.01% $ZnSO_4$, 0.001% $CuSO_4$, 0.001% $AlK(SO_4)_2$, and 0.001% Na_2MoO_2.

Fluids. A brine containing 0.5% NaCl was used for saturating sandpacks and conducting waterfloods. Mineral oil with viscosity of 10 cp at 24°C was used in the two-phase experiments.

Setup. A schematic of the experimental setup is shown in Figure 2. A detailed description of the experimental apparatus and of the sandpack construction has been provided elsewhere.[9,22] Sandpacks 8 in. long and 1 in. in diameter were used as the model porous medium. The sandpacks used in this study had an average permeability of 2.8 ± 0.2 darcys with grain sizes between 80 and 325 mesh (45 μm to 177 μm). The mean pore size was approximately 27 ± 5 μm as determined by capillary pressure curve measurements.[9]

Three intermediate pressure taps were used to divide the sandpack into four sections (numbered from the upstream end of the porous medium) with the following lengths : section 1, 1 in.; section 2, 2 in.; section 3, 4 in.; and section 4, 1 in. The pressure taps were connected to differential transducers arranged in series for different pressure ranges (0 to 0.5 psi and 0 to 5 psi). An electrical heating tape was placed around the sandpack holder and connected to a temperature controller to maintain the surface temperature constant at 42°C. Glass wool was used to insulate the apparatus. HPLC (high-performance liquid chromatography) pumps were used

for injecting fluids/suspensions into sandpacks by displacing them in accumulators with mineral oil.

Procedures. At the beginning of each experiment, the sandpack was first vacuum saturated with the brine, and initial permeabilities for all four sections were measured. For two-phase experiments, a residual oil saturation was established by conducting an oilflood followed by a waterflood. The sandpacks were disinfected by flowing a mixture of deionized water and ethanol (70% v/v) for at least 8 hr followed by extensive washing by more than 10 PV of sterilized water to remove any residual ethanol. After sterilization, any subsequent flood was conducted with fluids sterilized by autoclaving.

Following those initial steps, five types of operations were carried out: bacteria flood, nutrient flood, static growth, dynamic growth, and static death.

Bacteria flood is the injection of a bacterial suspension of desired concentration, prepared by diluting the culture with the growth medium, into the sandpack. For bacteria flood with high velocities, glucose was added to the bacterial suspension itself because the change in injected bacteria concentration over the injection period was not significant. For some experiments, a dispersant [0.5% (w/v) of sodium pyrophosphate] was added to the bacterial suspension for improving the bacteria transport.[22] During a nutrient flood, the growth medium containing glucose was injected into the sandpack.

After injection of bacteria and nutrient, the inlet and outlet valves of the apparatus were closed. The injected bacteria were allowed to grow inside the sandpack at 42°C for a specified incubation period. This operation is defined as the static growth. Single static growth implies one cycle of growth and multiple static growth implies two cycles of growth. After the first cycle, a nutrient flood was conducted to provide fresh nutrient to the in-situ bacteria population to increase the extent of growth. After static growths, waterfloods were conducted to measure permeabilities. For the dynamic growth, the nutrient flood at the end of a bacteria flood was continued at a slow rate for several pore volumes.

At the end of a static growth and a subsequent waterflood, the inlet and outlet valves were closed for a specified time period to determine the long–term effect of the changes in cell physiological conditions under nutrient-depleted conditions on the stability of bacterial plugging. This operation is defined as static death. To test hydrodynamic stability of bacterial plugs, waterfloods were conducted at different linear fluid velocities (pressure gradients) at the end of the static growth and static death.

Measurements. Bacteria concentrations (injected or effluent) were estimated from the number of colonies (cfu/ml) that arose after plating diluted samples on agar plates (containing the growth media and 1% glucose) and incubating them at 42°C for two days. Glucose concentrations (injected or effluent) were measured using a glucose analyzer (Model YSI-23A). Pressure drops were continuously monitored for evaluating permeabilities.

Results And Discussion

Six experiments conducted for the study can be categorized in three groups as follows.

Single Static Growth (Group I)

Single Phase, With Dispersant (Experiment 1)

Single Phase, No Dispersant (Experiment 2)

Two Phase, No Dispersant (Experiment 3)

Multiple Static Growth (Group II)

Single Phase, No Dispersant (Experiment 4)

Two Phase, No Dispersant (Experiment 5)

Dynamic Growth (Group III)

Single Phase, With Dispersant (Experiment 6)

The descriptive name for each experiment indicates whether the oil phase was present and whether the dispersant was added to the bacterial suspension. The results for these experiments after bacteria flood, nutrient flood, waterflood, static growth, and static death operations are given in Table 1. The complete results are given elsewhere.[9]

The results on effluent bacteria concentration, buildup pressure, effluent glucose concentration, and permeability ratio profiles were considered together to explain microbial growth and plugging mechanisms. The effluent glucose concentrations were representative of the in-situ concentration profiles. However, the effluent concentrations after 0.65 PV of injection were affected by dispersion of the displacing brine, which contained no glucose. Thus, the glucose concentrations for the upstream one–third of the length of the sandpack could not be obtained. Results on permeability ratios for the first section are relevant for the bacteria transport.

However, permeability ratios for the downstream sections are relevant for predicting mobility reduction in regions away from the wellbore of a reservoir.

Single Static Growth

The three experiments conducted in this group are defined as With Dispersant (Expt. No. 1), No Dispersant (Expt. No. 2), and Two Phase (Expt. No. 3). For these experiments, the nutrient was added to the bacterial suspensions, and bacteria floods were conducted at the same high flow velocity of 100 ft/D. The effluent bacteria concentration ratio (based on the injected bacteria concentration) histories are shown in Figure 3. The pressure buildup histories during the static growth are shown in Figure 4. The effluent glucose concentration ratio (based on the injected glucose concentration) histories are shown in Figure 5. The permeability ratio profiles measured at the end of the bacteria flood, static growth, and static death are shown in Figures 6, 7, and 8.

Initial Bacteria Distribution. The maximum bacteria effluent concentration ratios were 0.95, 0.5, and 0.2 for the With Dispersant, No Dispersant, and Two Phase experiments, respectively. The total number of bacteria injected were 3.2×10^{10} cfu, 0.66×10^{10} cfu, and 3.5×10^{10} cfu; and the total sandpack-retained bacteria fractions (at the end of 5 PV) were 0.37, 0.80, and 0.96, respectively.

Permeability ratio profiles at the end of bacteria flood (see Figs. 6, 7, and 8) indicated that bacteria retentions were higher in the upstream section of the medium. Very little permeability reduction occurred in the downstream sections, even when high bacteria concentrations of 10^7 cfu/ml flowed through the sandpack in the With Dispersant case. The presence of a residual oleic phase increased bacteria retention but decreased the extent of permeability reduction. The bacteria transport results are discussed in more details by Sarkar et al.[22] Initial bacteria concentrations in the downstream sections were presumably higher for the With Dispersant experiment but lower for the No Dispersant and Two Phase experiments.

Pressure Buildup. The maximum buildup pressure for the No Dispersant and Two Phase experiments were similar (4.5 psi), but was twice as large for the With Dispersant experiment. For all three cases, more than 80% of the pressure buildup occurred during the first four days of growth. For the With Dispersant experiment, the waterflood at the end of growth was conducted with a backpressure of 20 psi. No gas was detected when the backpressure was released at the end of the waterflood after static death. The small amount of gas that was produced during the growth process remained dissolved in the aqueous phase and was carried out during the subsequent waterflood.

Glucose Consumption. Glucose consumption was uniform for the With Dispersant experiment, but was higher in the upstream section and lower in the downstream sections for the other two cases. For the Two Phase experiment, the waterflood conducted at the end of the static growth was performed after reversing the sandpack (i.e., the downstream end became the upstream end) to find out glucose consumption at the original upstream end. The nonuniformity occurred because the initial bacteria concentration in the downstream sections were lower. The average glucose consumption was 75% for the With Dispersant case and 55% for the No Dispersant and Two Phase experiments.

For the With Dispersant experiment, the bacteria retention was minimal but the pressure buildup and the average glucose consumption were maximal. This occurred because bacteria concentrations were high throughout the length of the medium. For the other two cases, glucose consumptions were lower in the downstream sections because the growth in the downstream sections was limited by the low initial bacteria concentration.

By using the dispersant, the bacterial transport can be facilitated to achieve uniform growth throughout the length of the medium. The chemotactic and diffusive fluxes were not very effective in creating uniform growth.

Mobility Reduction. For the With Dispersant experiment, the permeability profile after the static growth became almost uniform at a value of 0.3 (see Fig. 6). After static death (3 days), the permeability ratios in the downstream sections increased to the level that was present at the end of the bacteria flood. The permeability ratio profiles after static growth and static death were determined only at high velocities of 100 ft/D. The bacteria transport and growth were very effective in this experiment. Even in this case, the microbial plug was not stable.

For the No Dispersant experiment, the permeability ratios were evaluated at 2 and 100 ft/D after the static growth (see Fig. 7). The 2 ft/D profile was nonuniform—varying from 0.01 in the upstream end to 0.23 in the third section. With an increase in the velocity from 2 ft/D to 100 ft/D, the ratios increased to 0.14 in the upstream end and to 0.38 in the third section. Similarly, the permeability ratios were evaluated at 10 and 100 ft/D after the static death. With an increase in the velocity from 10 ft/D to 100 ft/D, the ratios increased especially in the third section to 0.8. An increase in the velocity destabilized the plugs in both the cases, particularly at the end of static death.

For the Two Phase experiment, permeability ratios after the static growth were higher compared to those for the other two cases. (Permeability ratios for the last section of the sandpack could not be obtained.) The effluent bacteria concentrations and glucose consumptions were similar to those for the No Dispersant case.

However, the permeability ratios decreased by only 10% to 20% (of initial permeability) compared to the values after bacteria flood. The reason for the slight increase in permeability ratios at the first section is not understood.

Multiple Static Growth

The two experiments conducted under this group are defined as Single Phase (Expt. No. 4) and Two Phase (Expt. No. 5). For both of these experiments, the bacteria flood was conducted at 100 ft/D, the injected bacteria concentration was 10^6 cfu/ml, and the maximum effluent bacteria concentration ratio was 0.02. The static growth periods were 7 days, except for the for the Two Phase second growth period of 5 days.

For the Single Phase experiment, the average nutrient consumption was 35% for both the growth periods, and the pressure buildups were 4 psi and 3 psi for the first and second static growths, respectively. The permeability ratio profiles at the end of bacteria flood, first static growth, and second static growth are shown in Figure 9. The permeability ratios after the first static growth ranged from 0.35 in the upstream section to 0.55 in the downstream section. After second static growth, permeability ratios decreased by another 10% only.

For the Two Phase experiment, nutrient consumptions, in comparison with the single phase results, were similar but pressure buildups were less— about 2 psi for the first and second static growth periods. The permeability ratio profiles at the end of bacteria flood, first static growth, and second static growth are shown in Figure 10. The permeability ratios after the first static growth ranged from 0.3 in the upstream section to 0.85 in the downstream section. After the second static growth, permeability ratios in the fourth section decreased to 0.65. After static death, permeability ratios evaluated at a low velocity of 10 ft/D did not change much. However, when the velocity was increased to 100 ft/D, permeability ratios in the fourth section increased to a high value of 0.9.

Knapp et al.[18] found that for experiments using indigenous bacteria, several treatments (static growth) were necessary probably because the initial concentrations of indigenous bacteria were much less.

Dynamic Growth

For the single experiment (Expt. No. 6) conducted in this group, the bacteria flood was conducted at a flow velocity of 25 ft/D. The injected bacteria concentration was 1.6×10^8 cfu/ml and the maximum effluent concentration ratio was 0.60. Figure 11

shows the effluent glucose concentration ratio history for the two nutrient floods conducted successively at very low velocities of 0.5 and 0.25 ft/D after the bacteria flood. The glucose concentration ratio became steady at 0.75 during the 0.5 ft/D flood and at 0.65 during the 0.25 ft/D flood. The dynamic growth was conducted for 7 days (first and second nutrient floods).

The glucose consumption was 35% (at 0.25 ft/D) compared to 80% observed for the With Dispersant experiment (Expt. No. 1) of Single Static Growth. This is not surprising in the sense that the residence time allowed was 2.7 days (0.25 ft/D velocity and 8 in. long sandpack), whereas the active growth period was 4 days (see With Dispersant curve in Figure 4). However, the effluent concentrations became steady after 1.0 PV of injection, indicating the development of a steady bacterial populations after the short residence time.

After dynamic growth, a static growth was allowed for four days. Figure 11 also shows the glucose concentration history during the waterflood conducted after the static growth. Glucose concentrations in the downstream sections did not decrease during the static growth. This is another indication that the residence time was sufficient and was not a factor in limiting growth/glucose consumption. The growth itself was limited.

The permeability ratio profiles after the bacteria flood and static growth are shown in Figure 12. The permeability ratios after static growth ranged from 0.55 in the first section to 0.15 in the fourth section. The reasons for not achieving a lower permeability ratio in the first section are not properly understood. One explanation is that during the nutrient floods the suspended bacteria were gradually pushed to the pore walls and were less effective in reducing permeability. Growth in that section should be more vigorous because of higher initial bacteria concentration and the availability of fresh nutrient.

Thus, even in a system where fresh nutrient is continuously injected and growth inhibitors are flushed out, the growth in a porous media is limited. A significant increase in cell mass could not be achieved to increase the extent of permeability reduction.

Implications for Field Applications

Results from the three growth techniques—Single Static Growth, Multiple Static Growth, and Dynamic Growth—indicate that the magnitude of permeability reduction from growth of cells in porous media is limited to 70% of the original permeability. With this degree of permeability reduction, the profile modification technique may be effective in reservoirs with low-permeability contrasts (2 or 3).

Production of biopolymers along with increase in cell numbers, as observed for other species,[17] may increase the degree of permeability reduction.

The instability of microbial plugs can limit the applicability of this technique. The velocity effects appear to be more influential than the physiology effects. In field applications, where the flow is radial, the flow velocities in the near wellbore region are higher, but in the deep reservoir region are much lower (1 to 2 ft/D). Hence the issue of stability to velocity effects are less important. Moreover, instability to velocity effects may be helpful because mobility reduction is undesired in the near wellbore area. The time period for conducting displacement processes in the field may reach several months or years. The physiology effects over a much longer static death period need to be evaluated.

The length of the porous media, which was 8 in. for the experimental setup, may be more than 500 ft in field applications. Consequently, the residence time for nutrients will be much longer, even if nutrient injections are conducted at high rates and the injected nutrients enter only into the high-permeability layers. With sufficient concentrations of carbon source being added, nutrients that will reach the downstream sections may contain enough carbon source but higher concentrations of growth inhibitors unless their transport is retarded by porous media through physical or chemical interactions.

Conclusions and Recommendations

The following conclusions can be made from the results of this study.

(1) Sufficient initial bacteria concentration is needed for effective bacteria growth and mobility reduction in a porous media.

(2) The chemotactic and diffusive fluxes are not significant even in a static condition and do not affect the overall growth.

(3) The magnitude of permeability reduction due to an increase in cell mass in a porous medium was limited to approximately 70% of the initial permeability.

(4) The stability of bacterial plugs to increases in pressure gradients or flow velocities and changes in cell physiology in a nutrient-depleted environment need to be improved. The cell mass alone is not sufficient for a significant and stable mobility reduction.

(5) Systems of suitable gelling agents and effective biomass-producing bacteria should be developed for significant and stable mobility reduction.

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Table 1 Results for Single Static, Multiple Static, and Dynamic Growth Group Experiments after Bacteria Flood, Nutrient Flood, Waterflood, Static Growth, and Static Death

Expt. No.	Velocity ft/D	Bacteria flood k_2/k_{12} (k_{12}) fraction (darcy)	Bacteria flood Ce/Ci (Ci) fraction (cfu/ml)	Static growth IT days	Static growth BP psi	Static growth NC %	Waterflood Velocity ft/D	Waterflood k_2/k_{12} fraction	Static Death days	Waterflood Velocity ft/D	Waterflood k_2/k_{12} fraction	Nutrient flood Velocity ft/D	Nutrient flood k_2/k_{12} fraction
I. Single Static Growth													
1. Single Phase, with Dispersant													
	100	0.86 (3.14)	1.0 (1.2×10^8)	11	9	80	100	0.31	3	100	0.82		
2. Single Phase, No Dispersant													
	100	0.79 (3.51)	0.48 (2.6×10^7)	5	4	50	2	0.12	4	10	0.34		
							100	0.23					
3. Two Phase, No Dispersant													
	100	0.48 (2.27)	0.20 (7.2×10^7)	6	4.5	60	100	0.28	-	-	-		
II. Multiple Static Growth													
4. Single Phase, No Dispersant													
	100	0.82 (3.27)	0.02 (1.4×10^6)	7	4.2	35	100	0.42	-	-	-	100	0.36
	(2nd static growth)			7	2.6	35	100	0.35	6	10	0.67		
5. Two Phase, No Dispersant													
	100	0.83 (1.64)	0.02 (1.1×10^6)	7	2	40	100	0.46	-	-	-	100	0.44
	(2nd static growth)			5	1.4	30	100	0.49	7	100	0.49		
III. Dynamic Growth													
6. Single Phase, With Dispersant													
	25	1.00 (2.30)	0.6 (1.6×10^8)	-	-	-	-	-	-	-	-	0.5	0.25
	(static growth at the end of dynamic growth)			4	0	0	25	0.52	-	-	-		

K = permeability, darcy; k_i = initial permeability, darcy; C_e = effluent bacteria concentration, cfu/ml; C_i = injected bacteria concentration, cfu/ml; IT = incubation time; BP = buildup pressure, psi; NC = nutrient consumption, % of injected concentration; subscript 2 = second section of sandpack

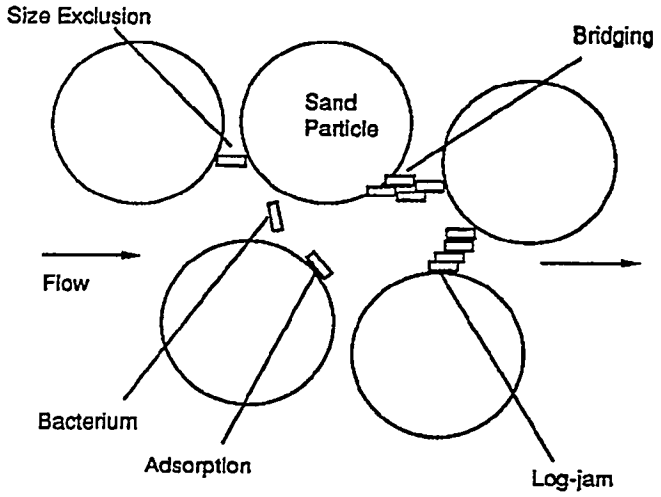

Figure 1 Schematic of Bacteria and Sand Particles Showing All the Retention and Permeability Reduction Mechanisms

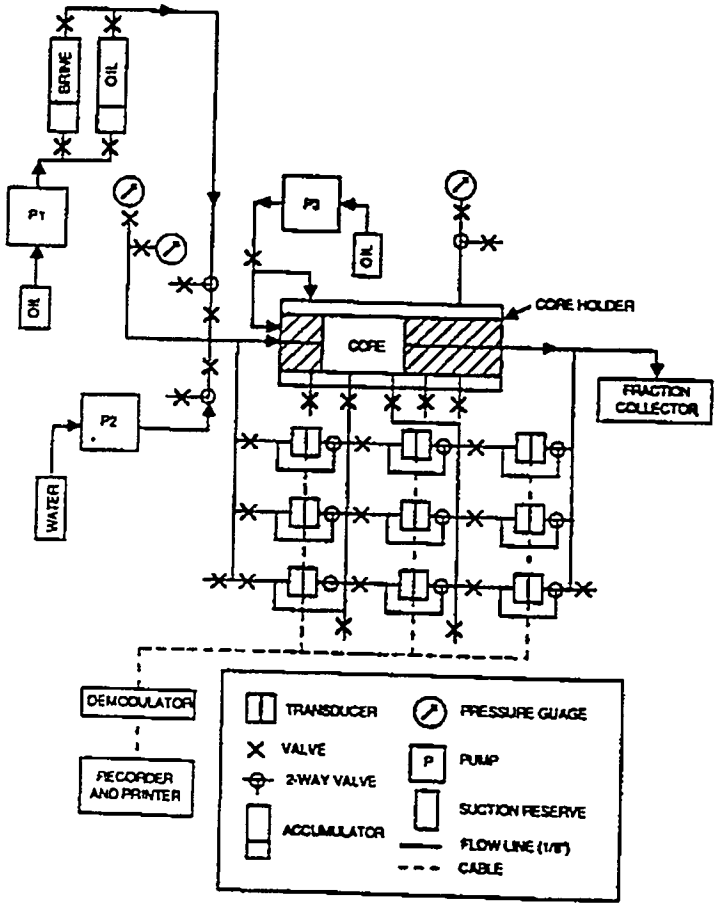

Figure 2 Schematic Diagram of Experimental Apparatus

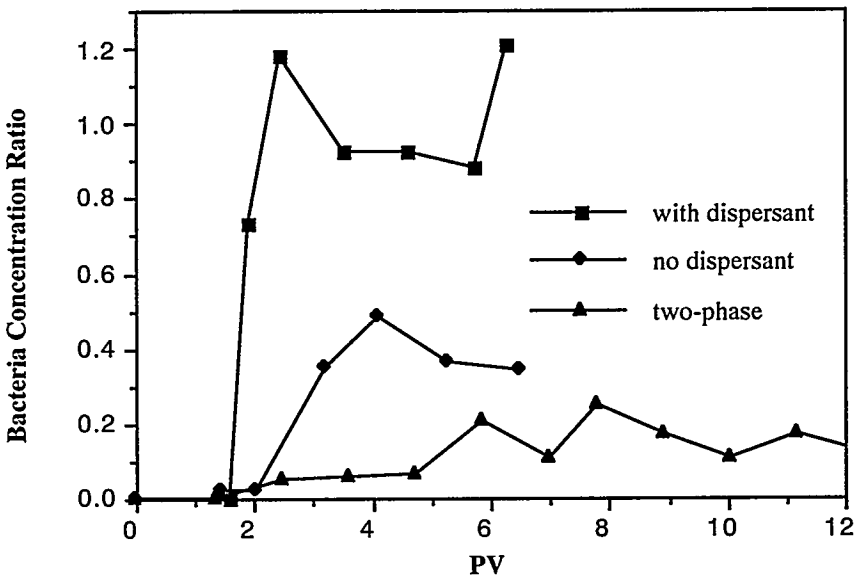

Figure 3 **Effluent Bacteria Concentration Ratio Histories of the with Dispersant, No Dispersant, and Two-Phase Experiments of the Single Static Growth Group**

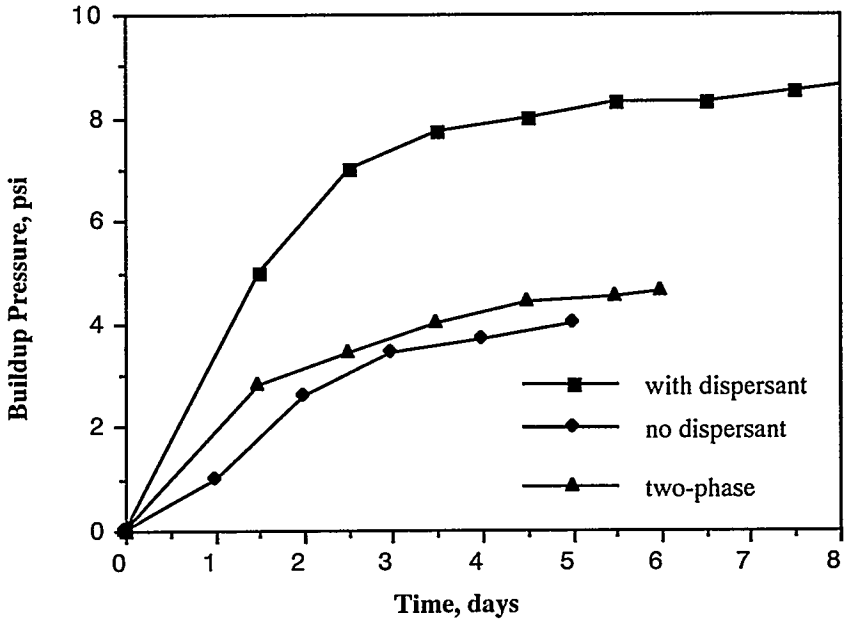

Figure 4 **Pressure Buildup during the Static Growth for the with Dispersant, No Dispersant, and Two-Phase Experiments of the Single Static Growth Group**

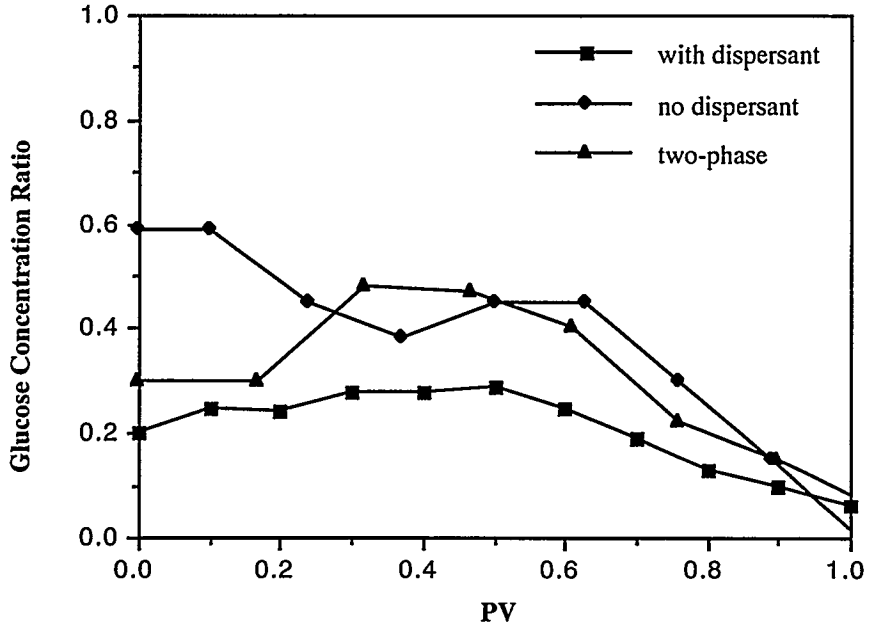

Figure 5 **Effluent Glucose Concentration Ratio Histories for the with Dispersant, No Dispersant, and Two-Phase Experiments of the Single Static Growth Group**

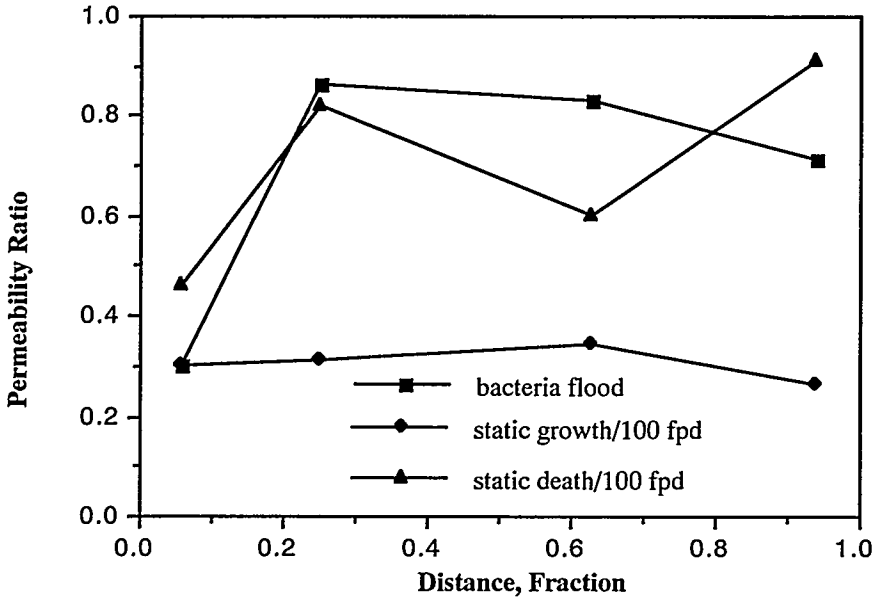

Figure 6 **Permeability Ratio Profiles for the with Dispersant Experiment of the Single Static Growth Group at the End of Bacteria Flood, Static Growth, and Static Death Operations**

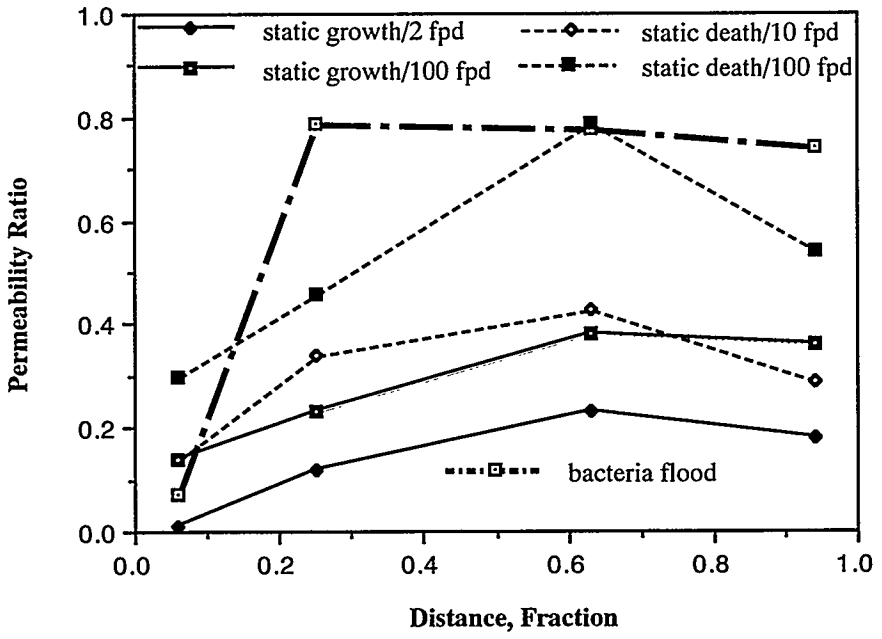

Figure 7 **Permeability Ratio Profiles for the No Dispersant Experiment of the Single Static Growth Group at the End of Bacteria Flood, Static Growth (2 and 10 ft/D), and Static Death (10 and 100 ft/D)**

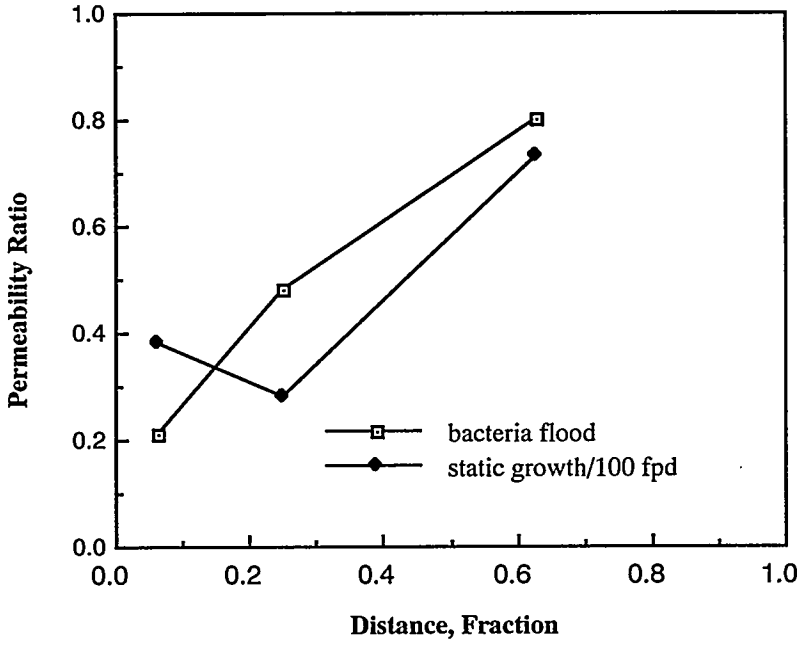

Figure 8 **Permeability Ratio Profiles for the Two-Phase Experiment of the Single Static Growth Group at the End of Bacteria Flood and Static Growth**

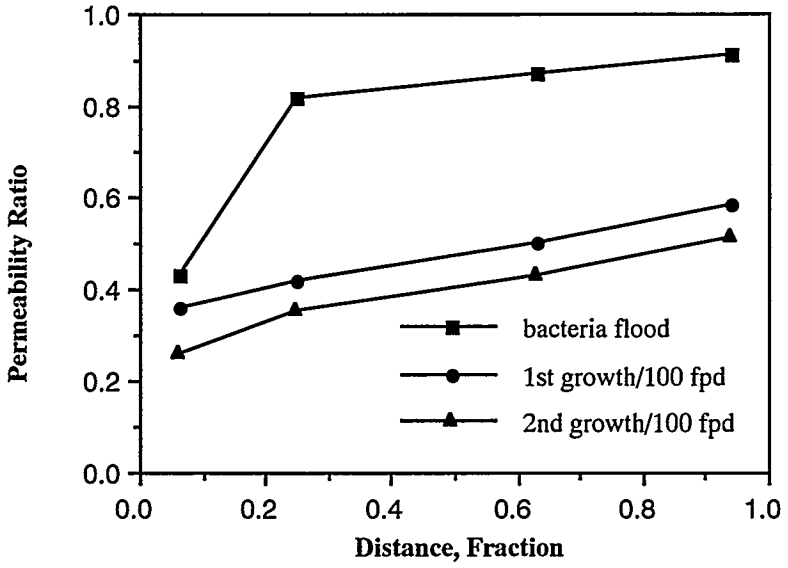

Figure 9 **Permeability Ratio Profiles at the End of Bacteria Flood, First Static Growth, and Second Static Growth for the Single-Phase Experiment of the Multiple Static Growth Group**

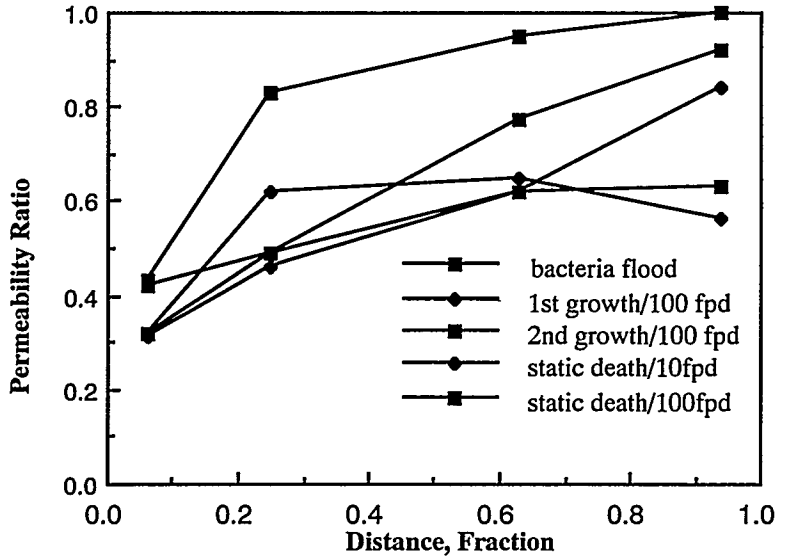

Figure 10 **Permeability Ratio Profiles at the End of Bacteria Flood, Static Growth, and Static Death for the Two-Phase Experiment of the Multiple Static Growth Group**

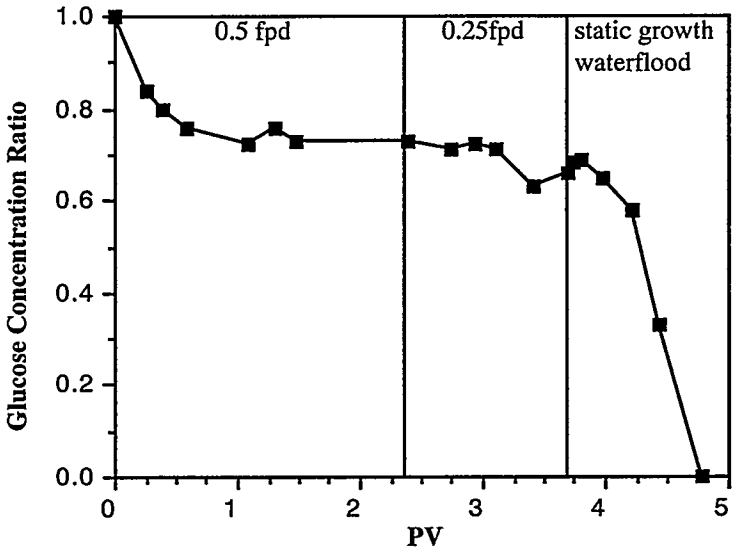

Figure 11 Effluent Glucose Concentration Ratio History for the Single-Phase Experiment of the Dynamic Growth Group during Nutrient Floods at Different Velocities

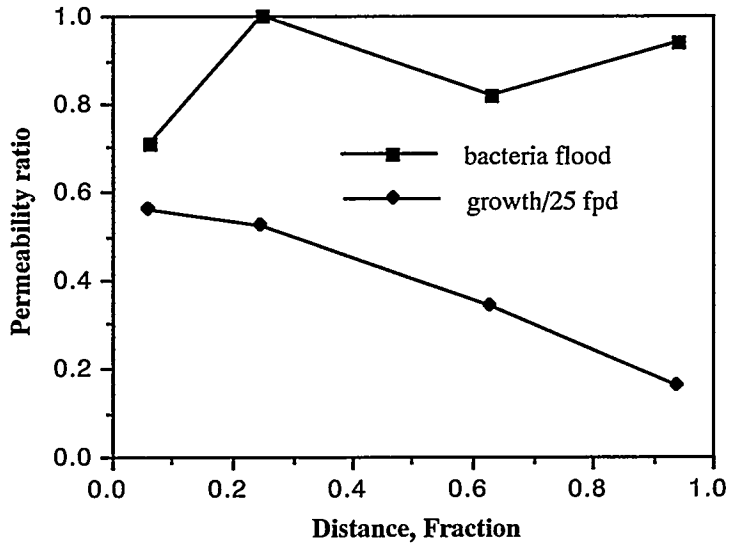

Figure 12 Permeability Ratio Profiles at the End of Bacteria Flood and Static Growth for the Single-Phase Experiment of the Dynamic Growth Group

Bioconversion of Heavy Crude Oils: A Basis for New Technology

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Abstract

Systematic studies of chemical mechanisms by which selected microorganisms react with crude oils have led to the identification of biochemical markers characteristic of the interactions of microbes with oils. These biomarkers belong to several groups of natural products ranging from saturate and polyaromatic hydrocarbons containing heterocyclics to organometallic compounds. The biochemical conversions of oils can be monitored by these chemical markers, which are particularly useful in the optimization of biochemical processing, cost efficiency, and engineering studies. Recent results from these studies will be discussed in terms of biochemical technology for the processing of crude oils.

Introduction

Biochemical reactions which occur during the interaction of select microorganisms and crude oils follow distinct trends which can be monitored by means of unique chemical markers.[1,2] The chemical markers that are used represent several groups of compounds ranging from those containing sulfur and nitrogen to organometallic compounds, saturates, aromatics, resins, and asphaltenes, each representing certain chemical properties of oils which are affected by the action of microorganisms. These markers resemble a group of compounds, known as "biomarkers," which are used in petroleum exploration, source rock, and reservoir correlations, as well as maturation and degradation studies.[3] The versatility and applicability of chemical markers has been discussed in detail elsewhere in this volume.[2] For this discussion, it suffices to say that bioconversion of crude oils results in a 20% to 45% reduction in sulfur content, a 15% to 45% reduction in nitrogen content, and a 16% to 99% reduction in the concentration of trace metals such as vanadium, nickel, arsenic, and others. Further, current data indicate that the biochemical action occurs in the heavy fractions of crude oils such as resins and asphaltenes and favors formation of lighter fractions. The chemical markers serve as diagnostic tools for monitoring several aspects of the biochemical conversion. These include the nature and the extent of bioconversion of the crude by microorganisms, properties essential in the cost-efficiency analyses of any processes based on microbial interactions with crude oils. This paper will briefly discuss the use of a chemical marker in the development of a heavy crude oil upgrading process.

Materials and Experimental

Chemical and biochemical methods have been discussed elsewhere[1,4] and will not be dealt with here. Assuming a 10-year life span of the plant, there are three major steps to consider in cost analysis. To obtain the total capital investment is the first step.[6] The detailed cost estimation for each unit is derived from graphs and formulas using published data.[6,7] The second step is to calculate manufacturing cost. This step combines the ECI and Ulrich methods.[6,8] The third step is to calculate the net present value and payback period which relates to the interest rates, i.e., the cost of borrowed money. For this step, the procedure described in Chapter 8 of reference 6 has been used.

Results and Discussion

This discussion will deal with the results obtained by using a biochemical process for the upgrading of low grade oil, as shown in Figure 1. In this process, a 55-

gallon bioreactor has been used. There are two major parts to this process. The first part is a biochemical batch process in which the oil and the biocatalysts are mixed by concurrent pumping through a mixer to make a water-in-oil emulsion. The process is set to run in a batch mode and a 36-hr, 50-cycle pass. The second part deals with the processing of waste products. In this particular example, the aqueous phase is separated from oil by sedimentation centrifuge, although other de-emulsification processes can be used. The aqueous phase is then further treated using several available technologies, such as co-precipitation and/or absorption of metals and by-products.

A single chemical marker, i.e., total concentration of sulfur, has been used in the economic and technical analysis of the process. For the analysis, a heavy crude with 3% sulfur oil has been used as the material.[8] In this particular case, as shown in Figure 2, the annual net profit increases with the decrease in the reaction time regardless of the amount of sulfur removed, e.g., from 33% to 45%. The analysis described in Figure 3 shows that the annual net profit increases as a function of an increase in the level of sulfur removal from the oil at different reaction times. The combined data given in the two figures clearly indicate that in order to maximize the annual net profit, it is necessary to shorten the reaction time and simultaneously remove maximum amount of sulfur from the oil.

Using this approach, a cost-efficiency analysis has been carried out yielding results which can be applied directly to the design and optimization of biochemical processes using a single chemical marker such as sulfur. Figure 4 shows the cash flow profiles for the oil upgrading process which assumes a 10-year life span for a plant that can remove 33% of sulfur from oil within 48 hours of reaction time. In this case, there is no profit regardless of interest rates which may be charged by financial houses for borrowed capital funds. With a decrease in reaction time to 42 hours and an increase in the extent of sulfur removal to 36%, a profit can be obtained. However, the invested capital represents in-house funds and does not involve any monies from outside financial groups, a scenario represented by Figure 5. In this case the payback period is 7.4 years with a net present value of $0.6 million, assuming that the plant will operate for 10 years to reach its anticipated life time.

On the other hand (see Fig. 6), if the reaction time can be reduced to 36 hours and at the same time the sulfur removal from the oil increased to 40%, then the payback period can be reduced to 2.7 years. With these reductions, the net present value after operating for 10 years becomes $3.6 million, without borrowing any moneys from financial houses (see Fig. 6). Using such cost analyses enables us to define laboratory engineering experimental protocols to identify the optimization parameters needed to reduce the reaction times and at the same time reach the maximum sulfur removal efficiency. Concurrent with this strategy, further cost-

efficiency fine tuning can be accomplished by tailoring the use of the end product such as utilities, refining, and others. This can be accomplished by modifying process variables to account for nitrogen and trace metals removal. In all cases, such R&D strategies use chemical markers in the development and application of new upstream and downstream oil processing operations.

Conclusions

Ultimate success of any applicable process depends on its technical feasibility and its cost-efficiency. Use of chemical markers to evaluate the bioconversion of crude oils by microorganisms allows to monitor major variables characteristic of microbial action on crude oils. These include changes resulting in the following:

1. Composition of organic sulfur compounds
2. Composition of nitrogen compounds
3. Composition of organometallic compounds
4. Distribution of hydrocarbons

In addition, the use of chemical markers allows one to predict the cost-efficiency of a process and simultaneously guides the R&D effort in process optimization.

Acknowledgments

This work is supported by the U.S. Department of Energy, Division of Fossil Fuels, under Contract No. AS-219-ECD, and Contract No. DE-AC02-76CH00016 with the U.S. Department of Energy. We wish to express our gratitude to Ernie Zuech of the U.S. DOE, Bartlesville office, for the supply of OSC oils and the Synfuels Research Corp. and the Santa Fe Energy Resources, Inc. of Houston for the supply of MWS oil. We also wish to acknowledge Yao Lin of Brookhaven National Laboratory for the graphic materials.

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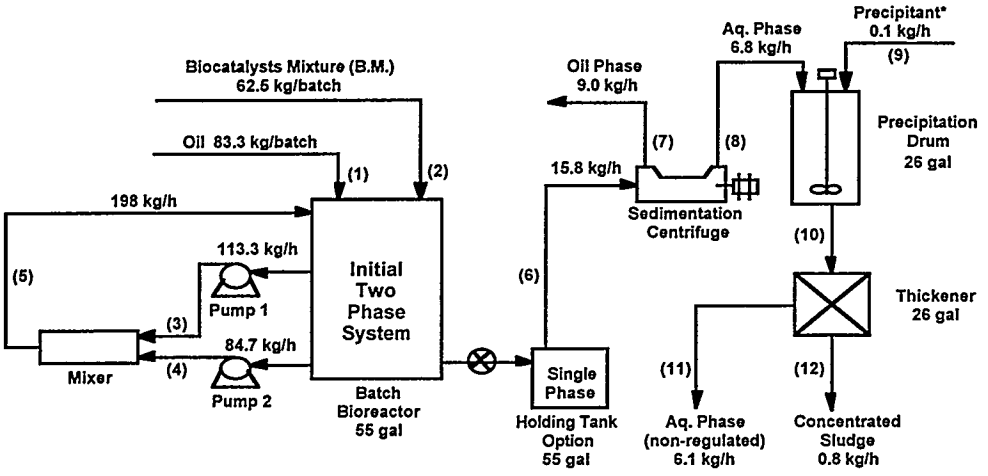

1. Biochemical batch process. (36 hour pass)

2. Product and waste processing plant. (9.2 hour)

* Based on the experience of biochemical processing of geothermal brines.

Figure 1 Biochemical Process for Upgrading of Low-Grade Oil (Batch per Pass, 55 gal Bioreactor, 1 Set)

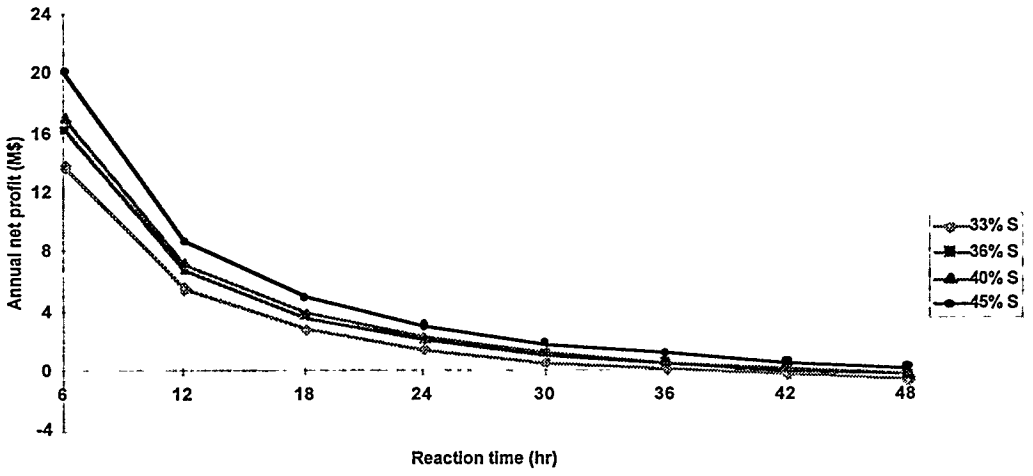

Figure 2 The Annual Net Profit at Various Reaction Times in Oil Upgrading Process with Different Sulfur Removal Levels (1,000,000 gal Batch per Pass)

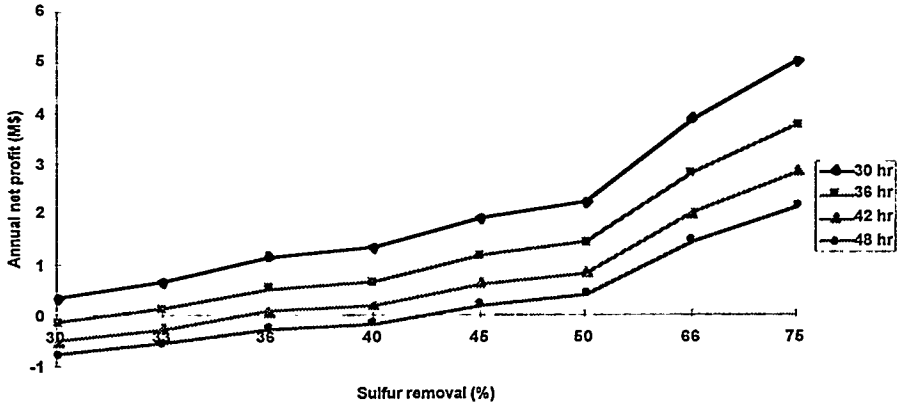

Figure 3 The Annual Net Profit for Various Sulfur Content Removal in Oil Upgrading Process at Different Reaction Times (1,000,000 gal Batch per Pass)

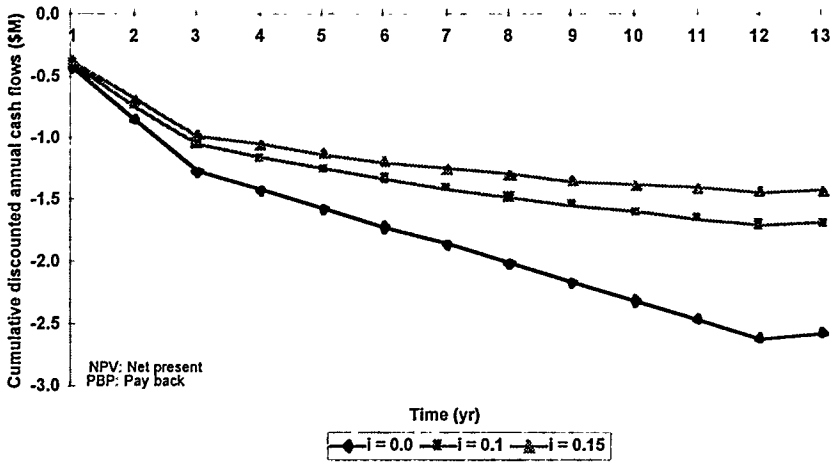

Figure 4 Cash Flow Profiles for Oil Upgrading (48 hr Batch, 33% S Reduction)

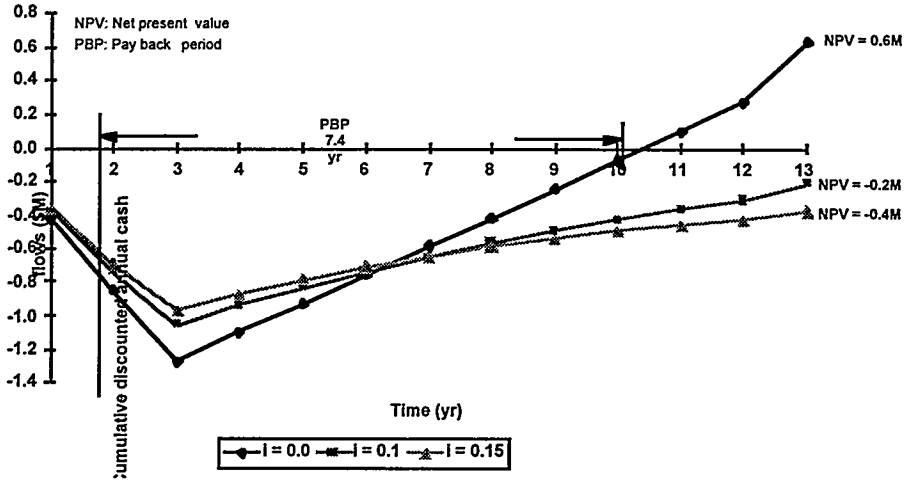

Figure 5 Cash Flow Profiles for Oil Upgrading (42 hr Batch, 36% S Reduction)

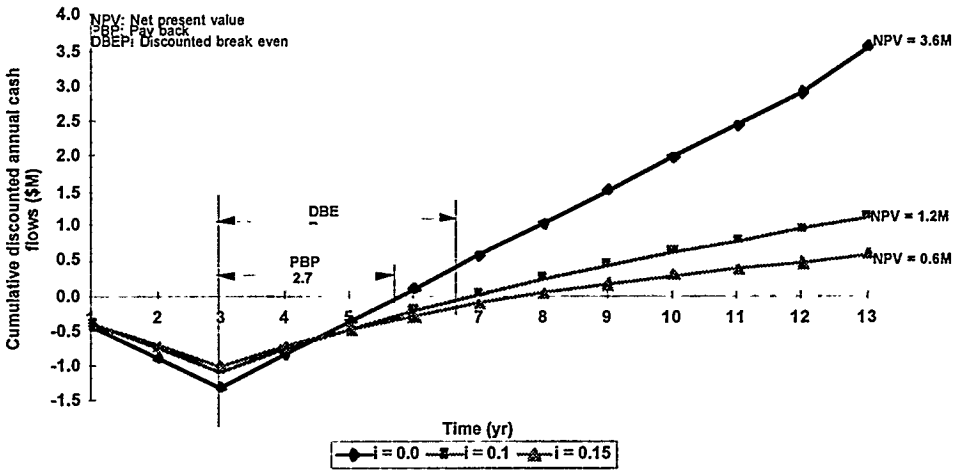

Figure 6 Cash Flow Profiles for Oil Upgrading (36 hr Batch, 40% S Reduction)

Microbial Enhancement of Non-Darcy Flow: Theoretical Consideration

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Abstract

In the near well-bore region and perforations, petroleum fluids usually flow at high velocities and may exhibit non-Darcy-flow behavior. Microorganisms can increase permeability and porosity by removing paraffin or asphaltene accumulations. They can also reduce interfacial tension by producing biosurfactants. These changes can significantly affect non-Darcy flow behavior.

Theoretical analysis shows that microbial activities can enhance production by decreasing the turbulence pressure drop and in some cases increasing the drag force exerted to the oil phase. This implies that the effects of microbial activities on non-Darcy flow are important and should be considered in the evaluation of microbial well stimulation and enhanced oil recovery.

Introduction

The alteration of Darcy flow characteristics of fluids in porous media, by various microbial mechanisms in order to enhance oil production, has been widely studied.[1–4] Microorganisms can decrease the viscosity of petroleum fluids by breaking large molecules into smaller ones. They can also increase the absolute permeability by removing formation damage caused by paraffin or asphaltene accumulation and change the relative permeability curves by reducing interfacial tensions. Therefore, the mobility of petroleum fluid is increased. So far, however, how these mechanisms influence non-Darcy flow has not been examined. Flow at relatively high velocities in porous media no longer obeys Darcy's Law and can only be accurately described by a non-Darcy flow model. This non-Darcy flow behavior happens in the near well-bore region and perforations, usually associated with high gas production. Obviously, the understanding of how microbial activities affect non-Darcy flow is important to the accurate evaluation of well stimulation or enhanced oil recovery by microbial activities.

There have been many investigations of non-Darcy flow in porous media.[1] Tutu et al.[5] presented a model that includes not only the inertial effect but also the momentum transfer between two phases which is related by the interfacial behavior of fluids. Microorganisms can produce bioproducts by various metabolic activities and change interfacial properties of petroleum fluids; thus, altering non-Darcy flow behavior. Based on the model of Tutu et al., this paper discusses how microorganisms affect non-Darcy flow by changing the properties of porous media and petroleum fluids, and offers a better understanding of the mechanisms of microbial well stimulation.

Theoretical Analysis

As we know, Darcy's equation only applies to laminar flow in porous media. At high flow rates, the flow becomes turbulent, extra energy is consumed by the turbulence, and flow can not be described by Darcy's Law. Just as in the case of single-phase flow in porous media, a two-phase flow system will experience pressure drop changes continuously from laminar flow to turbulent flow, or from Darcy-flow to non-Darcy-flow. Forchheimer[1] added a turbulent pressure-drop term or inertial term to the Darcy flow equation. For two-phase flow, Tutu et al.[5] further

proposed a momentum equation that includes an interfacial term without the inertial term. This term is caused by drag force at the interface between the two phases. The non-Darcy flow momentum equation can be written as:

$$-\frac{\partial p1}{\partial x} + \rho_1 \mathrm{Sin}\theta = \frac{\mu_1}{k k_{r1}} u_1 + \frac{\rho_1 \beta}{k_{r1}} u_1^2 + \frac{F_{12}}{S_1}, \tag{1}$$

$$-\frac{\partial p2}{\partial x} + \rho_2 \mathrm{Sin}\theta = \frac{\mu_2}{k k_{r2}} u_2 + \frac{\rho_2 \beta}{k_{r2}} u_2^2 + \frac{F_{21}}{S_2}, \tag{2}$$

where k is rock permeability, ϕ is rock porosity, ρ_1, ρ_2 are densities of phases 1 and 2, S_1, S_2 are saturations of phases 1 and 2, u_1, u_2 are Darcy velocities of phases 1 and 2, β is called non-Darcy flow coefficient and is a function of permeability and porosity. The relation used by Schulenberg and Muller[6] is

$$\beta = \frac{3.21 \times 10^4}{k^{1.25} \phi^{0.75}}. \tag{3}$$

The constant and the exponents in this equation were determined by experimentation; different values are given by other authors. F_{21} or F_{12} are called drag force and determined by dimensional analysis, and

$$F_{21} = -F_{12} = \frac{k \beta \rho_1 g}{\sigma} (\rho_1 - \rho_2)\left(\frac{u_1}{s_1} - \frac{u_2}{s_2}\right) W(s_l), \tag{4}$$

where σ is interfacial tension between two phases, $W(s_1)$ is an empirical dimensionless function of saturation obtained from experiments.

The form is

$$W(s_1) = W^0 s_l^m (1 - s_l) \tag{5}$$

Schulenberg and Muller[5] suggested W^0=350 and m=7.

There are three terms in the momentum equation; we refer to them as Darcy, inertial, and drag force terms respectively. The inertial term always gives extra pressure drop and reduces flow rates. But the drag force term can either reduce flow rate or increase flow rate, depending the relative velocities of the two phases. For wells with high gas production, because the gas velocity is higher than oil velocity, the drag force always favors the oil production. In wells with high water

cut, because the water flows faster than oil, the drag force also always favors the oil production.

The non-Darcy flow equation implies that non-Darcy flow coefficient is inversely proportional to permeability and porosity. Bacteria can increase permeability and porosity by removing paraffin or asphaltene accumulation in the near-wellbore region, thereby reducing turbulent pressure drop and improving oil production. The equation also shows that drag force is inversely proportional to interfacial tension between phases. The drag force is caused by the molecular momentum transfer between two phases at the interface. Reducing the interfacial tension enables the molecular momentum to be transferred more easily because the energy barrier is brought down. Bacteria can produce biosurfactants by metabolic activities that reduce interfacial tension, increasing the drag force exerted to the oil phase by gas or water. This can improve production, even without an increase in permeability or porosity.

Quantitative comparisons of the effects of various properties of porous media and fluids on non-Darcy flow were performed using the model above. In order to know the relation between fractional flow and saturation, as needed in the calculation, Corey's relative permeability model was used as follows:

$$k_{r1} = k^0_{r1} \left(\frac{S_1 - S_{1I}}{1 - S_{1I} - S_{2I}} \right)^{e1}, \tag{6}$$

$$k_{r2} = k^0_{r2} \left(\frac{S_2 - S_{2I}}{1 - S_{1I} - S_{2I}} \right)^{e2}, \tag{7}$$

where k^0_{r1} and k^0_{r2} are the end relative permeabilities of phases 1 and 2, S_{1I} and S_{2I} are residual saturation of phases 1 and 2, respectively.

Biosurfactants can reduce interfacial tension between phases, thus increasing the endpoint relative permeability, k^0_{r1} and k^0_{r2}, and decreasing the residual saturation, S_{1I} and S_{2I}. If the interfacial tension were equal to zero, e_1 and e_2 would be 1.0.

If only one phase is considered, quantitative calculations can be done using the above momentum equation. Although the calculations are based on empirical relations, the results can give insight into what extent bacteria can alter the non-Darcy flow behavior. The parameters used in our calculations are summarized in Table 1. The drag force F_{12} is assumed to have negative values at fixed saturation, so that it increases liquid flow rates.

Figure 1 shows the contributions of Darcy, inertial, and drag force terms to pressure gradient. It is noticed that at low velocity, flow obeys Darcy's Law. While at high velocity, the inertial term is large and flow deviates from Darcy's Law. In this case, the interfacial tension is 30 dynes/cm which is a normal value for oil and gas in reservoirs, and the drag force term is negligible.

Keeping other parameters constant, we substituted different values of permeability and porosity into the equations and completed a similar calculation to that in Figure 1. In Figure 2 the inertial term is greatly reduced by increasing permeability and porosity, and the deviation from Darcy-flow is subdued.

Figures 3 and 4 illustrate the effect of interfacial tension on flow in porous media. At a high interfacial tension (see Fig. 3), the drag force term can be neglected and flow deviates significantly from Darcy-flow. At very low interfacial tension (see Fig. 4), the drag force is so large that it cancels the effect of the inertial term and flow obeys Darcy law at higher flow rates. In other words, at high flow rates the energy consumed by turbulence is compensated by the momentum transferred from the faster-flowing phase in the porous media.

Figure 5 shows the results calculated with the increased relative permeability. It shows that the inertial term can be reduced by increasing relative permeability. This reduces the deviation from Darcy's Law (compare with Figure 1).

As the above calculations demonstrate, the effects of microbial activities on non-Darcy flow cannot be neglected in the accurate evaluation of microbial well stimulation and enhanced oil recovery.

Conclusions

- The effects of microbial activities on non-Darcy flow are important factors for the accurate evaluation of microbial well stimulation and enhanced oil recovery.

- Microorganism activities decrease the inertial pressure gradient by removing paraffin or asphaltene accumulation, improving production.

- Microorganism activities may increase the drag force exerted to the oil phase by producing biosurfactants that decrease interfacial tension, improving production.

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Table 1 Parameters in Calculations

Figure Number	Permeability (darcy)	Porosity (%)	Relative Permeability	Interfacial Tension (d/cm)
1	0.9	0.25	0.15	30
2	1.87	0.40	0.15	30
3	1.3	0.25	0.15	30
4	1.3	0.25	0.15	0.3
5	1.3	0.25	0.30	30

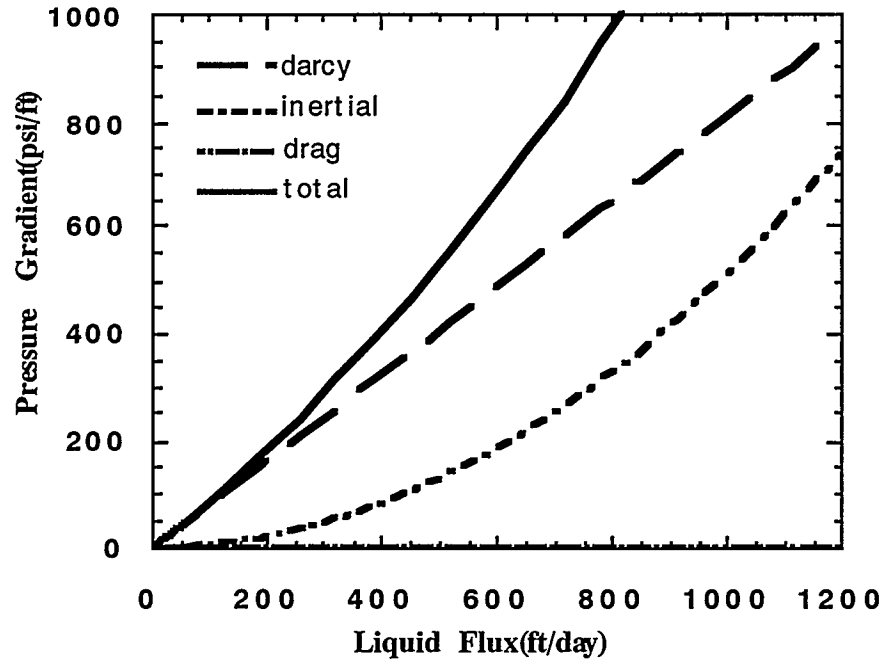

Figure 1 Contributions of the Darcy, Inertial, and Drag Force to Pressure Gradients As a Function of Liquid Flux at Lower Permeability and Porosity

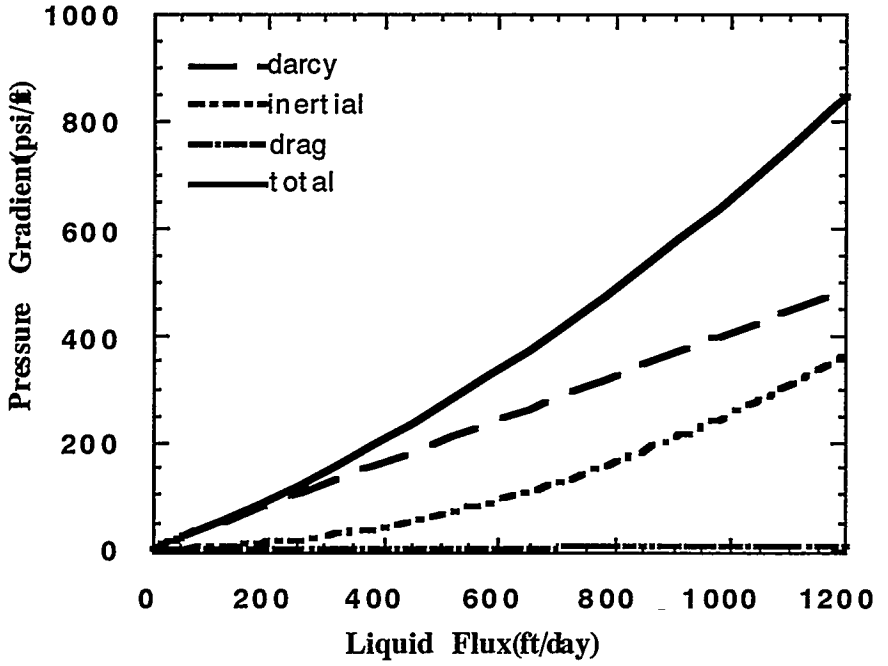

Figure 2 Contributions of Darcy, Inertial, and Drag Force to Pressure Gradients As a Function of Liquid Flux at Higher Permeability and Porosity

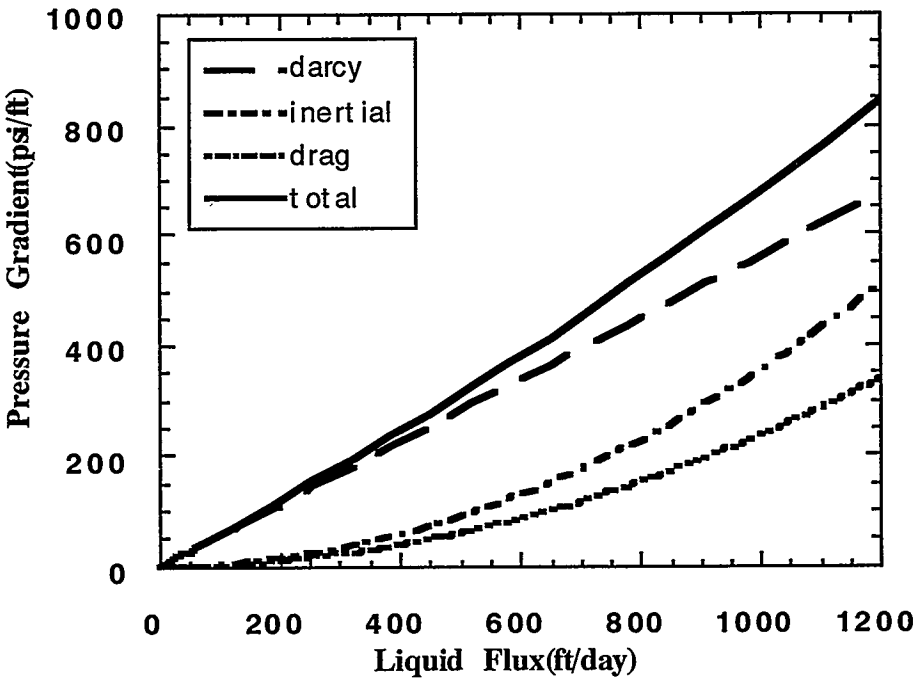

Figure 3 Contributions of Darcy, Inertial, and Drag Force to Pressure Gradients As a Function of Liquid Flux at a High Interfacial Tension

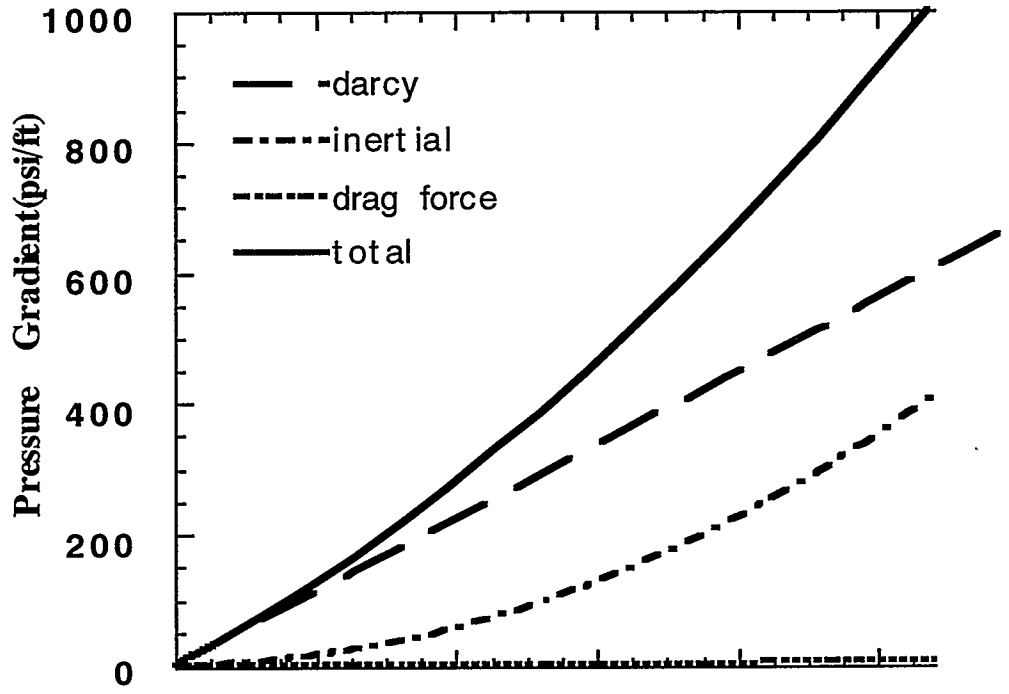

Figure 4 Contributions of Darcy, Inertial, and Drag Force to Pressure Gradients As a Function of Liquid Flux at Small Interfacial Tension

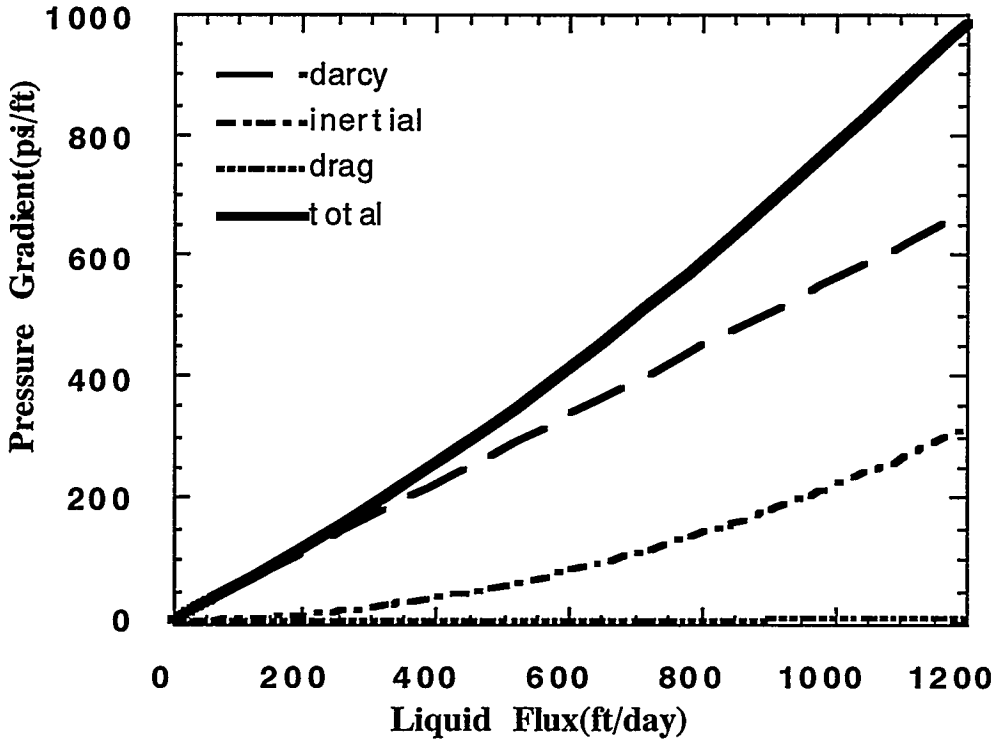

Figure 5 **Contributions of Darcy, Inertial, and Drag Force to Pressure Gradients As a Function of Liquid Flux for Increased Relative Permeability**

Anaerobic Thermophilic Bacteria Isolated from a Venezuelan Oil Field and Its Potential Use in Microbial Improved Oil Recovery

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Abstract

The objective of this work is to determine the ability of indigenous bacteria from a Venezuelan oil field to grow under reservoir conditions inside a porous media, and to produce metabolites capable of recovering residual crude oil. For this purpose, samples of formation waters from a central-eastern Venezuelan oil reservoir were enriched with different carbon sources and a mineral basal media. Formation water was used as a source of trace metals. The enrichments obtained were incubated at reservoir temperature (71°C), reservoir pressure (1,200 psi), and under anaerobic conditions for both outside and inside porous media (Berea core). Growth and metabolic activity was followed outside porous media by measuring absorbance at 660 nm, increases in pressure, and decreases in pH. Inside porous media bacterial activity was determined by visual examination of the produced waters (gas bubbles and bacterial cells).

All the carbohydrates tested outside porous media showed good growth at reservoir conditions. The pH was lowered, gases such as CO_2 and CH_4 were identified by GC. Surface tension was lowered in some enrichments by 30% when compared to controls.

Growth was decreased inside porous media, but gases were produced and helped displace oil. In addition, 10% residual oil was recovered from the Berea core.

Mathematical modeling was applied to the laboratory coreflood experiment to evaluate the reproducibility of the results obtained.

Introduction

Most Venezuelan crude oil reservoirs are over 4,000 ft deep, thus most reservoirs are thermophilic (over 60°C), and have high pressure values (1,200–1,600 psi). Therefore, the study of indigenous microorganisms from these reservoirs have very interesting results.

For MIOR the use of these microorganisms constitute a challenge, since they not only have to survive at such harsh conditions, but they also must produce useful metabolites to recover residual crude oil.

There are examples of successful MIOR treatments in deep, hot reservoirs.[1,2,3] But in those cases allocthonous bacteria were injected, making the process more expensive since the microorganisms have to be produced ex situ.

The objective of this study is to evaluate the ability of the indigenous microorganisms to produce metabolites capable of enhancing oil production at reservoir conditions utilizing an inexpensive carbon source. For this purpose a coreflood experiment was carried out. This experiment was then mathematical modeled as a chemical equation in order to understand the mechanisms utilized by bacteria to liberate residual oil.

Study Area

The study area was centered in the central-eastern part of Venezuela, El Tigre, in the state of Anzoátegui. The reservoirs studied belong to Corpoven S.A. (Guara field, San Tomé). Two different reservoir formation water were analyzed: GG-2 (East Guara field) and GG-63 (West Guara field).

The wells sampled from reservoir GG-2 were: GG-95, GG-143, and GG-69. From reservoir GG-63 only one well was sampled: GG-159. Both reservoirs have a depth average around 4,500 ft, a pressure of 1,600 psi, and a temperature of 71°C.

Methods and Materials

Media and Conditions of Cultivation

A mineral salt medium was used which consisted in the formation water of reservoir GG-2 and 2% Tanner's Mineral Medium.[4] The carbohydrates utilized were: glucose (1%), sucrose (1%), starch (0.5%), molasses (2%), and root beer (2%). All media were prepared under anaerobic conditions (80% N_2 : 20% CO_2) with cysteine sulfide as the reduction agent. The incubation temperature was 70°C at atmospheric conditions.

For the incubation at reservoir pressure (1,200 psi) the medium used consisted of molasses (2%) as carbon source with 0.1% diammonium phosphate as nitrogen and phosphate source. This same medium was used for the core displacement test.

Analytical Methods

Growth was monitored by absorbance at 600 nm using a Hewlett Packard spectrophotometer. Metabolic activity was followed by decreases in pH, pressure increases, and biogas analysis with a Carle 500 Gas Chromatograph with thermal conductivity detector. Surface tension was evaluated at 60°C using a ring type digital tensiometer.

Coreflood Experiments

For this experiment a Berea core was used. The core was 7 cm long, 4.3 cm in diameter, and had 31.1 ml of porous volume, an absolute permeability of 660 md, 21.8 ml of OOIP, and an initial oil saturation of 69.6 (%PV).

Figure 1 shows the equipment used for the core displacement experiment. The core was brought to residual oil conditions before injecting the indigenous microorganisms isolated from reservoir GG-2, a pressure of 1,200 psi, temperature of 70°C, and saturation with molasses.

Wettability Studies

Changes in the wettability of the Berea core after the treatment with microorganisms were evaluated with the Amott-Harvey technique.[5] This study was performed with

clean Berea sandstone, Berea with residual oil plus molasses, and Berea with residual oil plus molasses and microorganisms.

Mathematical Modeling / Simulation

The simulator Stars was used to performed the mathematical modeling of the coreflood experiment. Stars is a multiphase thermal simulator implemented for EOR purposes such as vapor and additives injection, dry and wet combustion, and for different chemical processes. It can be used at laboratory and field levels.

Simulation Data

The growth and metabolism of bacteria were described as a chemical reaction based on a generalized stoichiometric equation[6] presented below:

$$C_{12}H_{22}O_{11} + 1.2\,NH_4 + H_2O \rightarrow 6CH_{1.8}N_{0.2}O_{0.5} + C_3H_6O_3 + 0.5C_2H_4O_2 + CH_4 + CO_2 + 3H_2O$$

In this equation sucrose is utilized as carbon source simplifying molasses, ammonia as nitrogen source and water, this will yield cells, plus organic acids and gases. The reaction is taken as a first order reaction. Other requirements are: energy produced from the equation = 10^6 joules/mol, activation energy = 32,000 joules/mol, and frequency of reaction = 10^8. The last two values were taken from the literature.[7]

Another important parameter to include is the different phases of the components in the reaction. In total there are 9 components: 2 oleic components (oil and cells), 2 gaseous components (CO_2 and CH_4), and the rest were aqueous components. Cells were taken as oleic components since they were simulated as phospholipid micelles.

The petrophysical data used was the same obtained in the coreflood experiment.

Results

Growth and Metabolism at Reservoir Temperature

The enrichments showed the best growth with glucose, sucrose, and starch as carbon source having a maximum growth at the fourth day of incubation. Poor growth was observed with root beer as carbon source. With molasses and only ammonium phosphate as mineral salt (see Fig. 2), sample GG-143 (reservoir GG-2) showed good growth, as good as with sucrose and Tanner´s mineral medium.

This suggests that an inexpensive medium can be used to grow the indigenous microorganisms that belong to well GG-143.

Figure 2 also shows a rapid decrease in bacterial cells after the maximum growth is achieved, not having a stationary phase. This might be happening because a decrease in pH from 7.8 (GG-2 formation water pH) down to 5.3 for glucose and 5.0 for sucrose. With molasses, the change in pH was not so drastic, and the decrease in growth was also not so drastic.

The volume of gas produced during 10 days of incubation can be observed in Figure 3. All enrichments produced a greater volume of gas than did controls (55.5 ml). The greatest volumes were obtained with glucose, sucrose, and molasses, producing cumulative pressures up to 140 psi (GG-143 with sucrose). In all enrichments the gases detected were CO_2, CH_4, and H_2.

Surface tension was not lowered by all enrichments. Only with sample GG-143 reductions around 30% compared to controls (70 mN/m) were obtained with sucrose (50 mN/m) and starch (45 mN/m) as a carbon source.

Growth and Metabolism at High Pressure and Temperature

Sample GG-143 was used for this purpose. Figure 4 shows a decrease in absorbance of 60% compared to growth at atmospheric pressure. The growth rate was increased as seen by the slope of the curve in the exponential phase. Gas production was not so severely affected, pressure was increased from 1,200 psi up to 1,800 psi, this is equivalent to 120 ml in just two days.

Coreflood Experiments

Results are shown in Table 1. Changes in permeability were also recorded. Figure 5 shows how the relative permeability of both oil and water decreases after the microbial treatment. This suggest that the rock surface becomes less wet to both liquids. The results obtained from the wettability experiments show the same pattern. The rock changes from highly water wet to neutral wettability (see Table 2).

Mathematical Modeling

Figure 6 shows the results obtained from the simulator, which are in concordance with the results observed in the laboratory during the coreflood experiment. During

the time of water (IA) and molasses (IS) injection, there was no production of oil or gas. When the injection of the inoculum started, there was a small increase in gas production. During the incubation period (PI), no production was observed since the core was shut-in. After 15 days of incubation (368 hr) the core was open and there was a brisk increase in oil and gas production. Approximately one milliliter of oil was produced, the same amount obtained at the laboratory.

Pressure increase during the incubation period can be observed in Figure 7. The simulator predicted that the pressure is increased by almost 100% during the incubation period. In the laboratory coreflood experiment the increase in pressure was not registered, but during static experiments at temperature and reservoir pressure, the pressure increased from 1,200 psi to 1,800 psi.

Figure 8 shows how oil saturation changes inside the core during the incubation period. It can be seen that the oil is pushed to the production end. During the time of injection there is no redistribution of the oil saturation, this suggests that the metabolism of the microorganisms is affecting that redistribution of the oil saturation during the incubation period (PI).

When the cells were utilized in the simulator as an aqueous component, very little oil was produced, but as an oleic component, the same quantity of oil was produced as in the coreflood experiment (data not shown).

Discussion

With the coreflood experiment it was demonstrated that the indigenous bacteria were able to recover a 10% additional oil at reservoir conditions, utilizing a very inexpensive type of nutrient. The mathematical simulation of this experiment gave the same results as that obtained in the laboratory. Figure 7 suggests that the biogases produced during the incubation period made the pressure increase almost by double, and observing Figure 8, during the same incubation time, there was a rearrangement of the oil saturation, creating a bank of oil at the production end. It seems that gases were helping the entrapped oil to be mobilized to the production end.

Also the different responses obtained when the cells were utilized as an oleic or aqueous phase suggest that the lipophilicity of the cells may change the wettability of the rock, making it more neutral wet (see Table 2), which lowers the capillary tensions inside the Berea core, realizing the entrapped oil.

In summary it can be said that the indigenous microorganisms of the Guara field were able to recover additional crude oil at reservoir conditions. The

microorganisms produced enough gases to help displace oil, and they changed the wettability of the rock, enhancing the recovery of the remaining oil.

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Table 1 Coreflood Experiment	
Waterflood	
Injection velocity (ft/D)	1.2
Oil recovered after W.F. (ml)	11.1
Secondary oil recovered (% OOIP)	50.9
Residual oil (ml)	10.7
S_{or} (% PV)	34.2
Microbial Treatment	
Microbial culture injected (PV)	1
Additional oil recovered (ml)	1
Tertiary oil recovered (% OOIP)	4.6
Tertiary oil recovered (% S_{or})	9.4

Table 2 Wettability Index of the Berea Core before and after the Microbial Treatment

System	w	o	I[a]
Berea after Molasses Flood	0.71	0.05	0.66
Berea after Microbial Flood	0.23	0.16	0.07

[a] $+0.3 \leq I \leq +1.0$ water wettability

$-0.3 \leq I \leq +0.3$ neutral wettability

$-1.0 \leq I \leq -0.3$ oil wettability

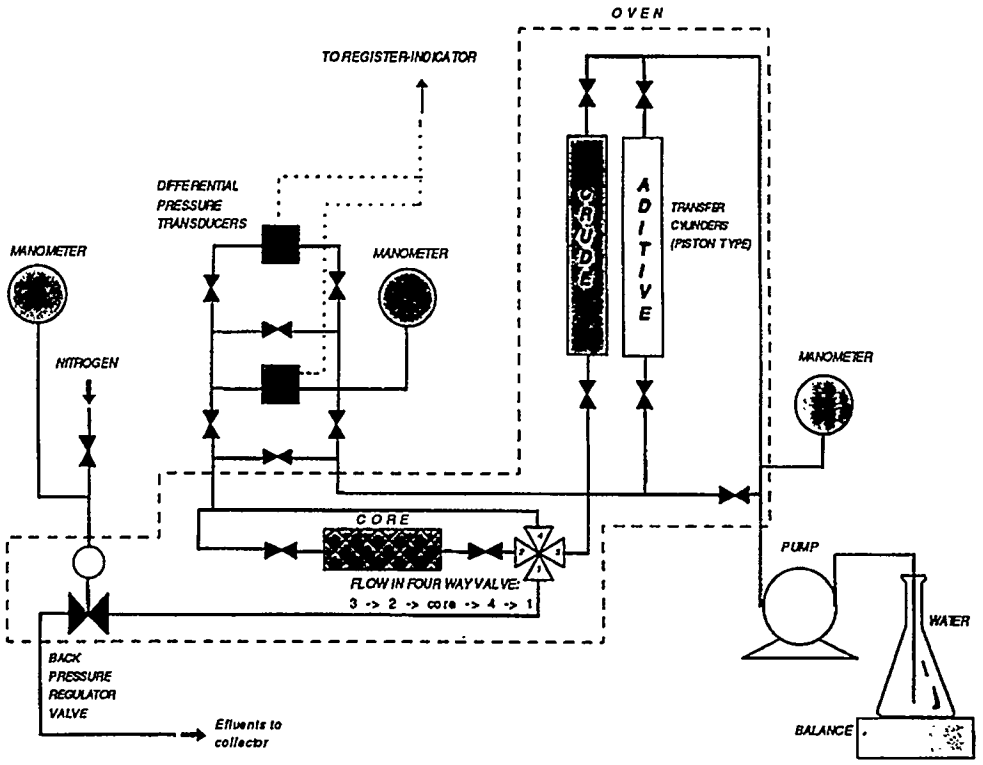

Figure 1 Coreflood Equipment

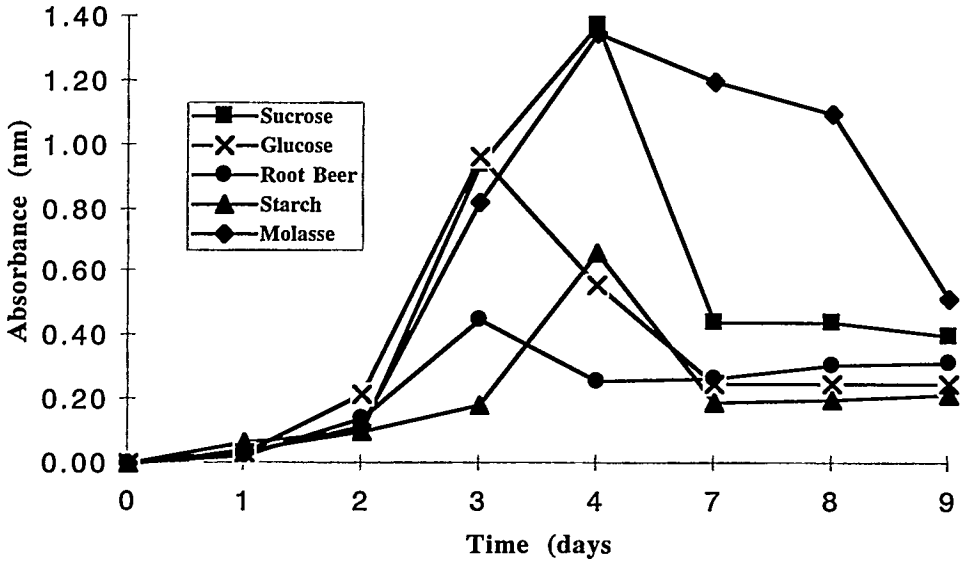

Figure 2 Growth Curves with Different Carbohydrates

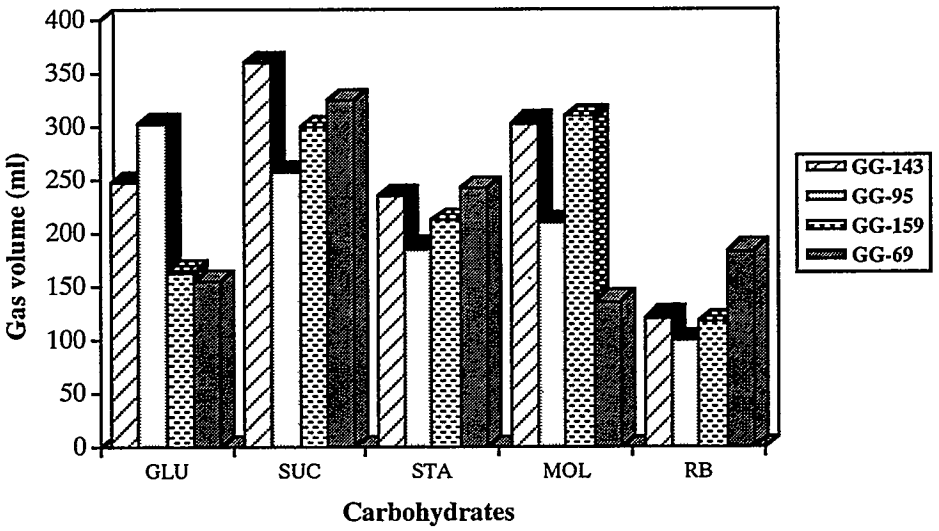

Figure 3 Volume of Gas Produced with Different Carbohydrates

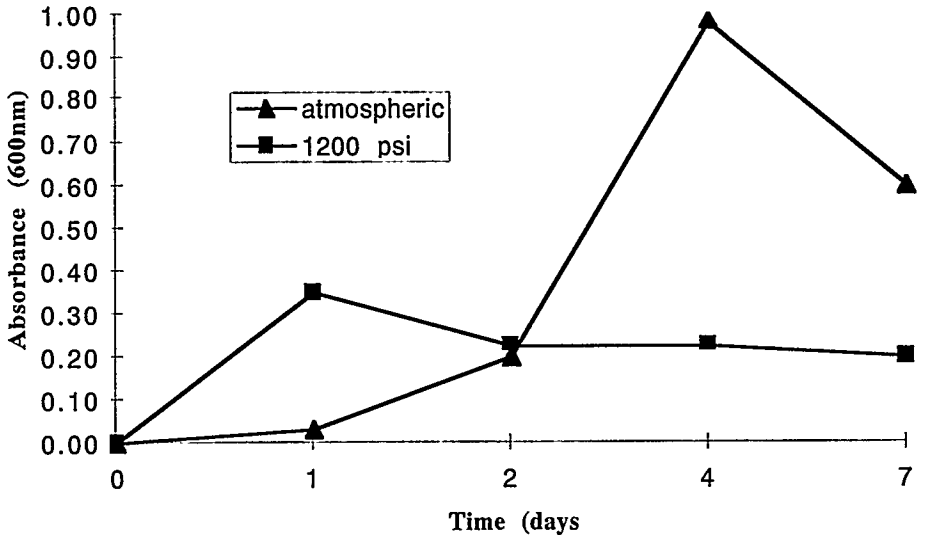

Figure 4 Growth Curves at Atmospheric and Reservoir Pressure with Molasses As Carbon Source

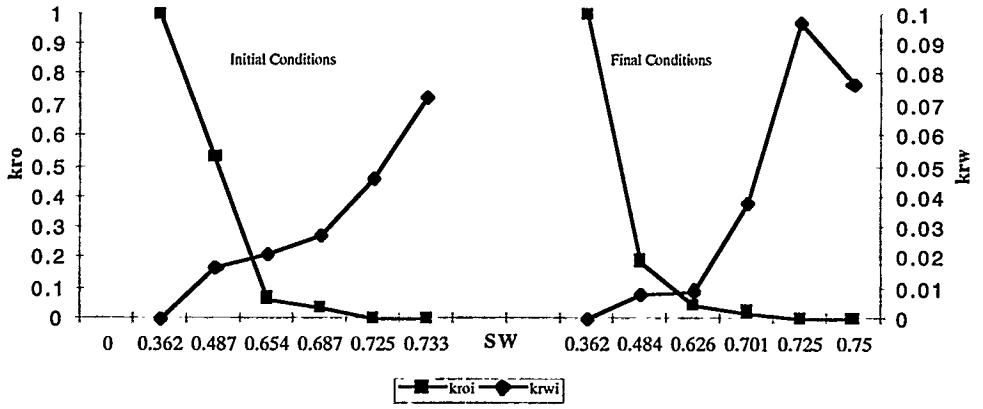

Figure 5 Relative Permeability Changes after Coreflood Experiment

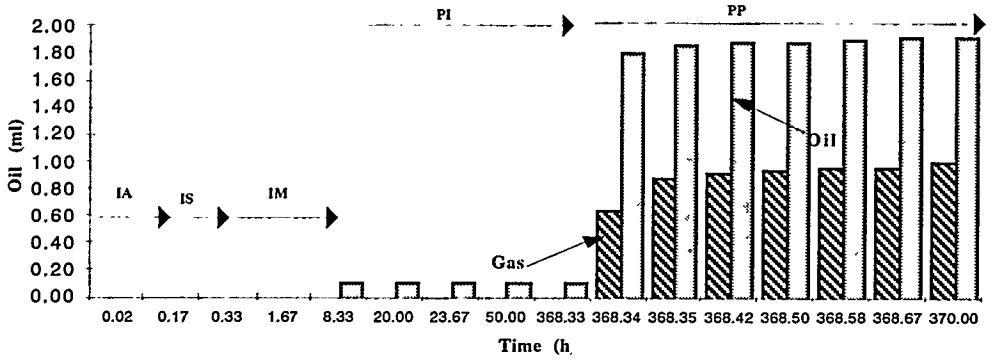

Figure 6 Gas and Oil Production during Coreflood Experiment

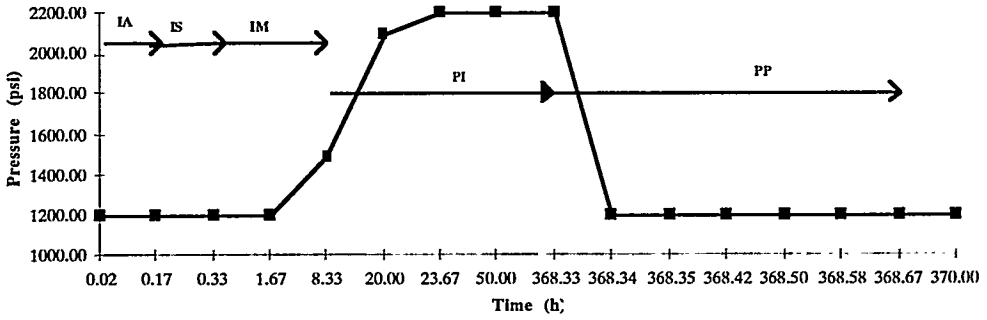

Figure 7 Pressure inside the Pore Media during the Coreflood Experiment

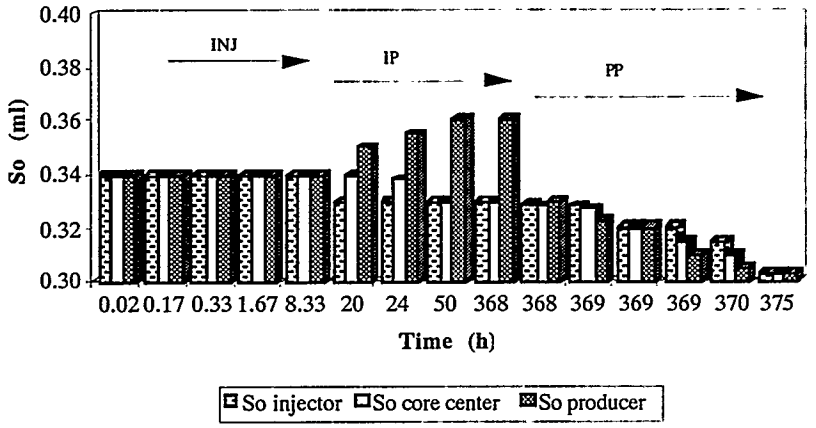

Figure 8 Oil Saturation inside the Core during the Coreflood Experiment

SOURING: CONTROL AND TREATMENT

Microbial Control of Hydrogen Sulfide Production

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Abstract

A sulfide-resistant strain of *Thiobacillus denitrificans*, strain F, prevented the accumulation of sulfide by *Desulfovibrio desulfuricans* when both organisms were grown in liquid medium. The wild-type strain of *T. denitrificans* did not prevent the accumulation of sulfide produced by *D. desulfuricans*. Strain F also prevented the accumulation of sulfide by a mixed population of sulfate-reducing bacteria enriched from an oil field brine. Fermentation balances showed that strain F stoichiometrically oxidized the sulfide produced by *D. desulfuricans* and the oil field brine enrichment to sulfate.

The ability of a strain F to control sulfide production in an experimental system of cores and formation water from the Redfield, Iowa, natural gas storage facility was also investigated. A stable, sulfide-producing biofilm was established in two separate core systems, one of which was inoculated with strain F while the other core system (control) was treated in an identical manner, but was not inoculated with strain F. When formation water with 10 mM acetate and 5 mM nitrate was injected into both core systems, the effluent sulfide concentrations in the control core system ranged from 200 to 460 μM. In the test core system inoculated with strain F, the effluent sulfide concentrations were lower, ranging from 70 to 110 μM. In order to determine whether strain F could control sulfide production under optimal conditions for sulfate-reducing bacteria, the electron donor was changed to lactate and inorganic nutrients (nitrogen and phosphate sources) were added to the formation water. When nutrient-supplemented formation water with 3.1 mM lactate and 10 mM nitrate was used, the effluent sulfide concentrations of the control core system initially increased to about 3,800 μM, and then decreased to about 1,100 μM after 5 weeks. However, in the test core system inoculated with strain F, the effluent sulfide concentrations were much lower, 160 to 330 μM.

Introduction

Hydrogen sulfide is a toxic and corrosive gas that greatly increases the cost of recovery of oil and natural gas. An increase in hydrogen sulfide concentrations is often noted after a petroleum reservoir has been waterflooded to improve oil recovery.[1,2] A major mechanism for hydrogen sulfide production in petroleum/gas reservoirs below 80°C is believed to be microbial metabolism.[1] Since many petroleum reservoirs have environmental conditions favorable for microbial growth,[3] and much evidence exits to support the conclusion that these reservoirs contain active microbial populations,[4,5] this suggests that souring may be caused by the inadvertent introduction of some limiting nutrient such as a suitable organic electron donor, or a source of nitrogen or phosphorous during waterflood operations.[6,7] Because of their diverse metabolic properties and widespread occurrence, sulfate-reducing bacteria (SRB) were thought to be the only agents responsible for microbially induced souring. However, sulfate reducers are not the only organisms found in oil/gas reservoirs that produce sulfide.[2] In fact, the most commonly detected sulfide-producing bacteria, such as *Shewanella putrefaciens*, do not use sulfate as an electron acceptor, but use other sulfur oxyanions. Thus, methods to detect or control souring based solely on methods to detect or control sulfate-reducing bacteria may not be effective in actual field situations.

The detrimental activities of sulfide-producing bacteria can be controlled by the effective use of biocides. The use of biocides is most successful in controlling unwanted activities in surface facilities. However, controlling these activities in the reservoir through the use of biocides is often difficult and expensive. Our approach is to manipulate the ecology of the system so that the terminal electron-accepting process is changed from the reduction of sulfur oxyanions to nitrate reduction.[8,9] Thus, even if sulfide producers are present in the reservoir, the accumulation of the unwanted product of their metabolism, sulfide, is prevented. This can be done by adding nitrate and a sulfide-tolerant strain (strain F) of *Thiobacillus denitrificans*.

T. denitrificans is an obligate autotroph and facultative anaerobe which, under anaerobic conditions, oxidizes sulfide to sulfate by reducing nitrate to nitrogen (Equation 1).

$$5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_20 \quad (1)$$

T. denitrificans strain F is not inhibited by inorganic sulfide concentrations in excess of 1,000 μM, which inhibit the growth of wild-type strains of *T. denitrificans*.[10] Because *T. denitrificans* strain F is a chemoautotrophic bacterium, the addition of organic nutrients would not be needed which would limit the growth

of indigenous organisms present in the reservoir. The addition of nitrate inhibits sulfide production in many environments.[8] Thus, a combination of strain F and nitrate could effectively control sulfide accumulation in oil and natural gas formations.

In the study reported here, the ability of strain F to control biogenic sulfide production was investigated by culturing this strain with SRB such as *Desulfovibrio desulfuricans* in liquid cultures. We have also investigated the efficacy of nitrate and *T. denitrificans* strain F in controlling H_2S concentration in experimental systems using cores and water from an underground gas storage facility. A previous study showed that sulfide levels in formation as groundwater infiltrated the formation after the stored gas had been removed.[11]

Materials and Methods

Organisms and stock cultures

Wild-type *T. denitrificans* (ATCC 23462) was obtained from the American Type Culture Collection (Rockville, Maryland). A sulfide- and glutaraldehyde-resistant strain of *T. denitrificans* (strain F) was obtained by enrichment from cultures of the wild-type as described in detail elsewhere.[10] Stock cultures of *T. denitrificans* strains were maintained anaerobically in thiosulfate medium as previously described.[12] In this medium thiosulfate is the energy source, nitrate is the terminal electron acceptor, bicarbonate is the source of carbon, and ammonium ion is a source of reduced nitrogen. Stock cultures were transferred every 30 days and stored at 4°C until used.

Desulfovibrio desulfuricans, strain D, was obtained from Dr. M. P. Bryant, University of Illinois (Urbana, Illinois). Sulfate-reducing enrichments were obtained from (1) sewage sludge from a secondary anaerobic digester in Norman, Oklahoma, (2) two separate sites from the aquifer underlying the Norman, Oklahoma, municipal landfill, (3), the Tuscaloosa aquifer underlying the Savannah River Plant in Savannah, Georgia, and (4) an oil field brine from Wilmington Field, Long Beach, California. The latter was provided by Dr. E. Donaldson of the University of Oklahoma. The thiosulfate medium was modified to grow *D. desulfuricans* in pure culture and in coculture with *T. denitrificans* strains and for enrichments of environmental samples. The modified medium was identical to that used to grow *T. denitrificans* in pure culture except for the deletion of thiosulfate and the addition of 0.1% (w/v) Na_2SO_4, 0.05% (v/v) sodium lactate syrup (60%), and 0.1% (v/v) Balch vitamin solution.[13] When *T. denitrificans* was grown in pure culture in the modified medium, thiosulfate was added. The preparation and use of

media were according to the anaerobic methods of Hungate as modified.[14,15] The medium was bubbled for 30 min with O_2-free nitrogen and dispersed into serum tubes under this same gas phase. The tubes were sealed with number 0 black rubber stoppers crimped with aluminum seals and autoclaved (121°C for 20 min). The tubes were inoculated using sterile syringes and needles which had been flushed with O_2-free nitrogen. Late exponential phase cultures were used as the inocula. All cultures were incubated without shaking at 30°C. Cultures of both *T. denitrificans* and *D. desulfuricans* were checked for purity by microscopic analysis and by inoculation of thioglycolate medium in which none of the above strains grew.

A mixed population of SRB was also enriched from formation water (Davis #6 well, St. Peter formation) from a gas storage reservoir in Redfield, Iowa. The enrichment was produced using a medium which contained a bicarbonate buffer, mineral salts solutions, trace metals, 20 mM sodium lactate, 40 mM sodium acetate, and 20 mM sodium sulfate. The composition of the bicarbonate buffer, mineral salts solution, and the trace metals solution are given in Tanner (16). The enrichment medium was prepared anaerobically and dispensed to serum bottles in 20-ml volumes. Bottles were autoclaved for 20 min (fast exhaust) before inoculating with 20 ml of formation water. Enrichments were incubated at room temperature or 30°C. The SRB enrichment was transferred to a medium which consisted of Davis #6 formation water (see Table 1) with 5 mM sodium lactate, and could be repeatedly transferred in this medium without any other nutrient addition. The lactate supplemented formation waster was not prepared by the Hungate technique, but the headspace of the bottle was flushed with N_2. The final concentration of SRB in these enrichment cultures was about 10^7 cells per ml. The final sulfide concentration was about 5 mM. The initial sulfide concentration in the formation water was typically 1.2 to 40 μM. These enrichments were used to inoculate the core system in the extended core experiments described below.

Preliminary Core Experiment

Core System. The core systems used in these preliminary experiments were assembled by Bioindustrial Technologies, Inc. (BTI, Grafton, New York) and located in the laboratory on-site. One core system was previously used to test the effectiveness of biocide formulations in controlling sulfide levels in the cores. Following the completion of BTI studies, the core system was flushed with formation water at 75 ml/hr for 7 days before the experiments described here were initiated.

The core system contained three cylindrical cores of St. Peter sandstone of about 2.5-cm diameter and 7.6-cm length, each of which was mounted in polyvinyl chloride (PVC) tubing. The cores were connected in series to each other using

stainless steel tubing and compression fittings (see Fig. 1). The intake line of the core system had a 5-μm pore size membrane filter to remove suspended solids from the fluid before injection into the cores. A sampling port was located at the inlet side of each core and tubing was set up exiting the last core. The porosity of the St. Peter sandstone was 30%. From the porosity of the cores and the volume of the tubing, the fluid volume of the core system was estimated to be 240 ml. The flow rate of fluids through this core system was 75 ml/hr, giving a hydraulic retention time of 3.2 hr.

A second core system constructed in an identical manner as described above was only used to determine the effect of nitrate addition on sulfide production. This core system had not been treated with biocides. Because of this, the maximum amount of fluid that could be injected into this core system was lower than that of the first core system. The flow rate of the second core system was 14 ml/hr, giving a hydraulic retention time of 16.7 hr.

Growth of Cells for Core Injection. *T. denitrificans* strain F cells were grown in thiosulfate medium in 2-L cultures in a B. Braun Biostat M (12). Temperature was maintained at 30°C and pH controlled at 7.0 by the addition of 6M NaOH. The culture received a gas feed consisting of 30 ml/min of a mixture containing 5% CO_2, with the balance being N_2 to ensure that the culture did not become carbon limited. When the OD_{460} of the culture medium reached approximately 1.0 (about 10^9 cells/ml), cells were harvested by centrifugation at 5,000 × *g* and 25°C. Cells were then washed by resuspending the pellet in 15 mM phosphate buffer (pH 7.0), and centrifuging the suspension as above. Cells were shipped as a wet pellet by overnight delivery service to the test site. Sufficient medium was used to resuspend the pellet in a 5-liter beaker so that the suspension was slightly turbid. The viable cell concentration of the suspension was estimated by the end-point dilution method using the above medium with thiosulfate. Medium that was injected into the core system was not sterilized.

Core Experiments. An experiment was conducted to determine whether indigenous microbial populations capable of oxidizing sulfide and using nitrate as the electron acceptor were present in the core system. Formation water supplemented with 40 mM sodium nitrate was injected into each core system. After each 24-hr period, a sample was collected from the sample port located upstream of each core and from the tubing exiting the last core. Samples were analyzed immediately to determine the concentrations of sulfide, sulfate-reducing bacteria, acid-producing bacteria, and strain F. The remainder of each sample was frozen and then analyzed for nitrate, nitrite, sulfate, and sulfite at a later date.

Only the core system with a flow rate of 75 ml/hr was used for the remainder of the experiments described below. The *T. denitrificans* growth medium without

thiosulfate was injected into this core system for 40 hr to determine whether the addition of nutrients would stimulate the production of sulfide in the core system. Samples for chemical and microbiological analyses were taken after 20 and 40 hr of medium injection.

The core system was then inoculated with strain F to determine the effectiveness of this organism in controlling sulfide levels in a continuous flow system. A suspension of about 10^5 viable cells/ml of strain F in growth medium without thiosulfate was injected into the core system for 6 hr (about 0.5 liter). This was followed by injection of growth medium without thiosulfate for 24 hr. This inoculation procedure was repeated once.

Preliminary studies showed that the formation water contained a substance that was inhibitory to the growth of strain F. In order to acclimate strain F to the formation water, a mixture of growth medium and formation water starting with 9% (v/v) growth medium without thiosulfate and 10% (v/v) formation water with 40 mM sodium nitrate was injected into the core system. Every 12 hr, the percent of formation water with nitrate injected into the core system was increased by 10% until only formation water with nitrate was injected into the core system. When the fluid mixture injected into the core was 30%, 60%, and 80% formation water with nitrate, the core system was again treated with strain F. A cell suspension of strain F, prepared as described above, was injected into the core system for 6 hr, followed by a 6-hr treatment of the respective mixture of growth medium (without thiosulfate) and formation water with 40 mM sodium nitrate. Samples for chemical and microbiological analyses were taken every 12 hr.

During the time that the core system received only formation water with 40 mM nitrate, samples from the core system contained a compound that interfered with the detection of sulfide. This has been previously observed during nutrient-limited growth of strain F and results in incomplete oxidation of sulfide or reduction of nitrate (Sublette, K. L. unpublished data). Because of this problem, the core system was treated with growth medium without thiosulfate for 6 hr. For the remainder of the experiment, nutrient-amended formation water with nitrate was injected into the core system. The nutrient-amended formation water with nitrate contained 10 mM $NaNO_3$, and (in g/l) KH_2PO_4 (1.8), $MgSO_4 \cdot 7H_2O$ (0.4), NH_4Cl (0.5), $CaCl_2$ (0.03), $NaHCO_3$ (1.0). Samples for chemical and microbiological analyses were taken after each 24-hr period.

Extended Core Experiment

Core System. Core material from the St. Peter formation was provided by Northern Natural Gas Co., Redfield, Iowa. The core material was cut into

cylindrical cores, 5 cm in diameter by 15 cm long. The outside surface of each core was coated with epoxy, wrapped with fiberglass, and then coated with another layer of epoxy. A small portion of each end of the cores was cut off with a diamond saw to provide a smooth, flat surface.

Two core systems were constructed as described in Figure 1. All materials used for the core system construction were noncorrosive. Polypropylene was machined to form endplates for each end of the cores. The endplates and cores were encased in epoxy. Nylon or Tygon tubing was used throughout, and all fitting and valves were composed of nylon, polypropylene or Teflon. Gauges were mounted on gauge guards (Plast-O-matic Valves, Inc., series GGM) which consisted of PVC bodies and Teflon diaphragms. Sampling ports contained Teflon-lined gas chromatography septa. The formation water reservoirs were glass 2-liter bottles.

The core system had a common filter that connected the drum containing formation water to each of the 2-liter reservoirs. The 2-liter reservoirs and the drum containing formation water were flushed with carbon dioxide for 15 sec every min. Two Gilson variable-speed peristaltic pumps were used to supply formation water to the core systems.

Two 55-gal drums of formation water from the Davis #6 well at the Redfield site were delivered to our laboratory by surface freight transportation. Acetate (1.8 mM) was the only identifiable carbon and energy source for SRB in this water. The drums were stored at room temperature throughout the duration of the study.

The permeability of each core was tested prior to inoculation by connecting individual cores to a flow system that consisted of he peristaltic pump and an inlet pressure gauge. A synthetically prepared formation water with the following composition (in g/l) was used: KH_2PO_4, 1.8; $MgSO_4 \cdot 7H_2O$, 0.4; NH_4Cl, 0.5; $CaCl_2 \cdot 2H_2O$, 0.03; $NaHCO_3$, 1.0; and NaCl, 0.8. Three cores had backpressure less than 2 psig, while one core had a backpressure of 20 psig. The core with 20 psig backpressure was frozen at -20°C overnight to create minute fractures in the rock to increase permeability. After thawing and reconnecting the core to the flow system, it was determined that the backpressure was still high. The core was then flushed with 0.1 N HCl to dissolve carbonate minerals. This acid treatment reduced the backpressure to less than 2 psig.

Preparation of Thiobacillus Denitrificans Strain F for Core Inoculation. As noted earlier, *T. denitrificans* strain F had been previously shown to be inhibited by unknown components in formation water from Davis #6 well. Therefore, *T. denitrificans* strain F was adapted for growth in Davis #6 formation water prior to inoculation of the core system. *T. denitrificans* was anoxically grown in 2-liter fermenter with thiosulfate-mineral-salts medium

supplemented with 50 mM nitrate to an optical density (460 nm) of 0.8 (12). The cells were harvested by centrifugation at 5,000 × *g* at 25°C, resuspended in the same medium without thiosulfate, and returned to the fermenter. The culture was fed a gas mixture consisting of 1.0% H_2S, 5% CO_2, with the balance being N_2, at a flow rate of 30 ml/min. The culture was incubated at 28°C, and the pH was maintained at 7.0. During this time, all of the H_2S in the feed gas was removed by contact with the culture, and stoichiometric amounts of sulfate accumulated in the culture medium.

After 48 hr of operation as described above, 500 ml of the culture was removed and replaced with an equal amount of Davis #6 formation water supplemented with medium components and nitrate, but without thiosulfate. The H_2S-bearing feed gas rate was increased to 45 ml/min. Every 24 hr for the next four days, an additional 500 ml of the culture was removed and replaced with nutrient-supplemented formation water. The amount of medium removed and replaced each day was then increased to 750 ml until the volume percent of formation water in the reactor reached 93%. Throughout this acclimation period, H_2S continued to be completely removed from the feed gas, and stoichiometric amounts of sulfate accumulated in the culture medium. No elemental sulfur was detected. After a final 24-hr period of operation with H_2S gas feed and 93% formation water, cells were harvested by centrifugation at 5,000 × *g* and shipped as a wet cell pellet by overnight delivery to the University of Oklahoma where the core system was operated.

Establishing an Active SRB Population in the Core Systems. Each core system was flushed with 1 ml/min of formation water for 72 hr followed by inoculation with 200 ml of an SRB enrichment grown in formation water supplemented with 5 mM sodium lactate and 5 mM sodium sulfate. Lactate was the carbon and energy source and sulfate the terminal electron acceptor for the SRB. After 24 hr, the tubing in the core systems had a black coating indicating the production of iron sulfide. A concentrated solution of lactate and sulfate was then injected into each core through the sampling ports. After 48 hr, microscopic examination of fluid in the tubing between the cores in each system showed the presence of a mixed microbial population comprised of rod-shaped bacteria of various sizes. None of the organisms were motile. Enumeration studies showed that the liquid in the tubing of each core system contained about 10^{10} SRB per ml. The core systems were then operated at various feed rates of formation water or formation water supplemented with additional acetate (10 mM).

Effect of Nitrate on Sulfide Formation. When the test and control core systems had reached steady state with respect to sulfide formation at a feed rate of 0.1 ml/min with formation water supplemented with 10 mM acetate, the effect of nitrate on sulfide formation was investigated. Formation water supplemented with 10 mM acetate was injected into each core system for two weeks. This was

followed by a one-week period with formation water with 10 mM acetate and 5 mM nitrate.

Effect of T. Denitrificans Strain F on Net Sulfide Production. The cell pellet of *T. denitrificans* strain F described previously was suspended in 75 ml of anoxic formation water (boiled and allowed to cool with an 80% N_2:20% CO_2 gas phase) amended with 10 mM sodium acetate and 5 mM sodium nitrate. Twenty-five ml of this suspension was injected into the test core system via syringe into each of the three sampling ports. The proximal and distal valves of the test core system were closed after inoculation. After 48 hr the flow of formation water with acetate and nitrate was resumed at 0.1 ml/min. Microscopic examination of liquid from the sampling ports showed the presence of gram-negative rods resembling *T. denitrificans* along with other rod-shaped bacteria of various sizes. Enumeration studies of the effluent of the test core system showed that it contained about 10^3 strain F-like cells per ml after one week of operation.

After inoculation, the test core system contained to receive a feed of 0.1 ml/min formation water supplemented with 10 mM acetate and 5 mM nitrate for five weeks. The control core system received a feed of 0.1 ml/min formation water with 10 mM acetate for three weeks and then with an additional supplement of 5 mM nitrate for two weeks.

After five weeks of operation under these conditions, the amount of sulfide production in the control core system was lower than expected based on the amount of sulfate and acetate available in the influent. This suggested that sulfide production may have been nutrient limited. At this time, the feed to both core systems was changed to formation water supplemented with KH_2PO_4 (0.1 g/l), NH_4Cl (0.5 g/l), and 5 mM sodium lactate and 10 mM nitrate. After five weeks of operation with this nutrient-supplemented formation water with lactate, the nitrate concentration was increased to 20 mM until the conclusion of testing.

Analytical

Growth in liquid cultures was followed spectrophotometrically at 460 nm. Qualitative estimates of the numbers of *D. desulfuricans* and *T. denitrificans* present in the co-cultures were done microscopically.

In liquid cultures nitrate was determined colorimetrically using the Szechrome NAS reagent by Polysciences, Inc. (Warrington, Pennsylvania) (17). Lactate and acetate were determined by high pressure liquid chromatography (HPLC) using a Bio-Rad, 200 × 7.88 mm Aminex HPX-87H column and a UV detector (208 nm). The mobile phase buffer solution was 0.2 N H_2SO_4, and the flow rate was 0.9 ml/min.

Sulfate was also determined by HPLC using a Wescan PRP-X100 column, a Wescan conductivity detector, and a LDC Milton pump. The mobile phase buffer solution was 2 mM hydrogen potassium phthalate at pH 4.5, and the flow rate was 2 ml/min.

In core studies sulfate, nitrate, nitrite, and thiosulfate were also measured with an HPLC equipped with a Vydac column (250 mm × 4.6 mm, phase 3021) and a conductivity detector. The mobile phase consisted of 1 mM phthalate, set to pH 4.9 with a 6% boric acid solution. The flow rate was 2 ml/min. The injection volume was 90 µl. Sulfide was measured colorimetrically by a modified version of the methylene blue sulfide assay as described previously.[18]

Acetate was also measured with a gas chromatograph equipped with a flame ionization detector and a glass column (6 ft, 1/4-in OD, 2-mm ID) containing GP 10% SP-1200/1% H_3PO_4 matrix. The oven temperature was 130°C, the injector temperature was 170°C, and the detector temperature was 175°C. The carrier gas (He) flow rate was 30 ml/min. One-ml volumes of samples and standards were acidified with 200 µl of a 25% phosphoric acid. Injection volume was 1 µl. All samples were centrifuged for 3 min at 12,000 × *g* before chromatographic analysis.

Enumeration. In core experiments sulfate-reducing bacteria (SRB), acid-producing bacteria (APB), and strain F and strain F-like organisms (denitrifying *Thiobacilli*) were enumerated by the end-point dilution method once a week. Sampling and transfer inoculations were done via syringe. SRB and acid-producing bacteria (APB) were estimated using BTI-SRB medium and BTI-APB medium (Bioindustrial Technologies, Georgetown, Texas) in the preliminary core experiments. In the extended core experiment SRB were estimated using (API)-RST medium of Tanner.[16] Positive tubes were indicated by the formation of a black precipitate. APB enumerations were done using an anoxic medium containing 0.5% purple broth base (Difco) and 1% glucose. Acid production was demonstrated by the change in the pH indicator, bromcreosol purple, from purple to yellow. Strain F was enumerated using the medium of Sublette and Sylvester.[12]

Results

Liquid Cultures

Thiobacillus denitrificans is a chemoautotrophic facultatively anaerobic bacterium which grows in a low-organic environment but is inhibited by a high concentration of certain organic molecules. As noted above, *T. denitrificans* uses sulfur

compounds such as thiosulfate, elemental sulfur, and sulfide as electron donors, and in the absence of oxygen it uses nitrate as its electron acceptor. Most sulfate-reducing bacteria use organic compounds such as lactate as the electron donor for the reduction of sulfate to sulfide and usually require B vitamins and other organic growth factors. To develop a medium that would support the growth of both organisms, modifications were made to the thiosulfate medium used for the growth of *T. denitrificans*.[12] There was little effect on the growth of *T. denitrificans* when yeast extract (0.1%), trypticase (0.1%), and sulfate (0.1%) were added to the thiosulfate medium. However, when sodium lactate was added, a longer lag time and a slower growth rate resulted. Cultures of *T. denitrificans* with 5.3 mM (0.1%) sodium lactate had lag times of six days, while cultures with lower lactate concentrations had lag times of about three days. A concentration of 2.65 mM (0.05%) sodium lactate was chosen, since this concentration was high enough to allow for easy quantitation of the lactate and its fermentation products, but low enough so the growth of *T. denitrificans* was not severely inhibited. Figure 2 shows the growth of pure cultures of *D. desulfuricans* and of *T. denitrificans* grown in medium modified with the addition of 2.65 mM sodium lactate, 0.1% (w/v) sodium sulfate, and 0.1% (v/v) Balch vitamins. Thiosulfate was added when *T. denitrificans* was grown in pure culture.

D. desulfuricans was grown alone and in coculture with the wild-type strain or the sulfide-resistant strain of *Thiobacillus* to determine how effective the two *Thiobacillus* strains were in using biologically produced sulfide. High amounts of sulfide were produced in all pure cultures of *D. desulfuricans* and in all cocultures containing the wild-type strain of *T. denitrificans* (see Table 2). However, cocultures inoculated with 0.3 ml of the wild-type of *T. denitrificans* had lower sulfide levels than cocultures inoculated with 0.1 and 0.2 ml of wild-type, indicating that large numbers of cells were needed before sulfide use was observed. When *D. desulfuricans* was grown in coculture with the sulfide-resistant strain F of *T. denitrificans*, little or no sulfide was produced. The small amount of sulfide which was produced after 14 days (4.0 mg/l) disappeared after 19 days. This amount was measured by the colorimetric assay performed and could be visualized by the disappearance of iron sulfide which accumulated in the form of a black precipitate. This black precipitate was always present when sulfide was detected and was absent in those tubes where no sulfide was found. This experiment was repeated several times with similar results. The addition of a known amount of sulfide to a sample from these cultures showed that the cocultures did not contain any substances that could have interfered with the colorimetric assay. *T. denitrificans* cultures were not tested for sulfide, since it is known that this organism does not produce sulfide.

In order to demonstrate that *T. denitrificans* was actually oxidizing sulfide to sulfate by reducing nitrate, a balance of substrates and products of metabolism was

performed on *D. desulfuricans* cultures with and without strain F. With an initial lactate concentration of 1.5 mM, one would expect 1.5 mM acetate and 0.75 mM sulfide to be produced if all lactate is utilized by *D. desulfuricans*. Values of 1.4 mM acetate (83% recovery) and 0.6 mM sulfide (83% recovery) were found, as seen in Table 3. In coculture with strain F, lactate was completely utilized with a concomitant decrease in the nitrate concentration, indicating the growth of both *D. desulfuricans* and strain F. No sulfide was detected either visually or colorimetrically. The final sulfate concentration was the same as the initial concentration indicating that *Thiobacillus* stoichiometrically oxidized the sulfide produced by *D. desulfuricans* to sulfate. A slight decrease in nitrate concentration was observed in pure cultures of *D. desulfuricans* indicating that this strain can use nitrate as has been shown for other *Desulfovibrio* strains.

In order to assess the efficiency of strain F in using sulfide generated by other bacterial populations, five separate environmental samples were collected and grown in modified medium without thiosulfate. Each sample was transferred three times in the absence of nitrate in order to select for growth of sulfate-reducing bacteria. When inoculated into modified medium with nitrate, only the enrichment from the oil field brine produced sulfide (data not shown). The addition of nitrate to the other enrichments either shifted the flow of electrons from sulfate reduction to nitrate reduction or inhibited the growth and metabolism of the SRB.[19] The results of experiments similar to those described above in which the brine served as a source of SRB are also given in Table 3. In cultures containing the organisms enriched from the oil field brine, similar results were observed, except that there was no acetate detected. Approximately 50% more sulfide was detected in cultures without strain F added than in cultures of *D. desulfuricans* alone. This suggests the presence of sulfate-reducing bacteria which use acetate, or some other acetate user which produces H_2 for sulfate-reducers capable of growing on hydrogen. When strain F was grown with the brine consortium, sulfide was not detected. Nitrate concentration decreased, and sulfate concentration was approximately the same as the starting concentration. Thus strain F was able to grow and effectively utilize all the sulfide produced by this mixed bacterial population.

Preliminary Core Experiment

The addition of nitrate alone to the formation water which was injected into the core system operated at a hydraulic retention time of 3.2 hr resulted in lower effluent sulfide levels (see Table 4). Concomitant with the reduction of sulfide was the decrease in nitrate concentrations in the core effluent, suggesting the presence of indigenous microbial populations capable of oxidizing sulfide using nitrate as the electron acceptor. However, no growth was observed in the medium used to enumerate denitrifying *Thiobacilli*, either prior to or after treatment of the core

system with nitrate alone, indicating that the core system did not contain chemoautotrophic denitrifiers similar to *Thiobacilli.* The addition of nitrate did not affect the numbers of sulfate-reducing bacteria and acid-producing bacteria. Interestingly, the sulfide levels in the influent and the effluent before the treatments began were similar. This suggested that little or no sulfide production occurred within the core system when organic nutrients were not added to the formation water to support the growth of sulfate-reducing bacteria.

In the core system operated at a hydraulic retention time of 16.7 hr, the effluent sulfide concentration was 60 μM, even though the influent sulfide concentration was the same as that injected into the other core system (170 μM). The reason for the decreased effluent sulfide concentration in this core system was not determined. After nitrate treatment, the effluent sulfide concentration of this core system decreased to 3 μM, supporting the conclusion that the addition of nitrate is useful in controlling sulfide levels.

Injection of nutrients into the core system operated at a hydraulic retention time of 3.2 hr did not stimulate sulfide production. The influent and the effluent sulfide levels were low (1.3 and 1.6 μM, respectively) when only *T. denitrificans* growth medium without thiosulfate was injected into the core system. The numbers of sulfate-reducing bacteria were not affected (data not shown). This again suggested that little or no sulfide production occurred within the core system. After inoculation with strain F and injection of the growth medium without thiosulfate for 24 hr, 10^7, 10^5, and 10 cells/ml of denitrifying *Thiobacilli* were detected at sampling locations SP-1, SP-2, and EF (see Fig. 1), respectively. The number of denitrifying *Thiobacilli* increased to 10^5 cells/ml at location EF after the subsequent treatment with cells followed by medium injection. These bacteria were presumed to be *T. denitrificans* strain F since no denitrifying *Thiobacilli* were detected prior to inoculation. Thus, strain F was maintained in the core system when growth medium without thiosulfate was used. Only low levels of sulfide (1 to 2 μM) were detected during these treatments.

Preliminary studies suggested that the formation water contained a compound inhibitory to the growth of strain F (data not shown). Therefore, the fraction of formation water injected into the core was increased in steps in order to acclimate strain F. Relatively high concentrations (10^5 to 10^7 cells/ml) of strain F were detected at locations SP-1, SP-2, and FE during the course of this treatment, suggesting that strain F was active and growing in the core system. Throughout this period, the concentration of sulfide in the effluent of the core system was 70 to 89% lower than the influent concentration. There was also a reduction in the nitrate concentrations in the core system, suggesting that these two processes were linked. The sulfate concentrations in the effluent relative to the influent concentration of the

core system increased from 2.6 to 4.2 mM after strain F inoculation, which was much higher than that expected from the oxidation of influent sulfide alone.

When the influent was shifted completely to formation water with 40 mM, the concentration of strain F in the core system decreased from 10^7 cells/ml to 10^3 cells/ml, and interferences in effluent sulfide analyses were observed. This suggested that some essential nutrient may limit the growth of strain F, which would result in incomplete oxidation of sulfide or in reduction of nitrate. In subsequent treatments, the concentration of nitrate was decreased from 40 mM to 10 mM, and nutrients were added to the formation water.

The treatment of the test core system with strain F and the subsequent injection of formation water with reduced nitrate concentrations and nutrient amendments resulted in the reestablishment of strain F in the core system (see Table 4). Concomitant with the increase in strain F was the disappearance of the interfering substance from the effluent of the core system. After the population of strain F was reestablished, an 84% to 99% decrease in sulfide concentration in the effluent compared to the influent was observed. There was a substantial reduction in the levels of nitrate and a substantial increase in the levels of sulfate in the effluent compared to the influent of the core system. However, the amount of sulfate detected in the effluent of the test core system was much higher than that expected if strain F completely oxidized only the sulfide present in the formation water.

Although these experiments demonstrated the potential of microbial sulfide control, problems with the experimental system prevented any definitive conclusions regarding the need for strain F inoculation. The small differences between the influent and effluent sulfide concentrations prior to nitrate treatment suggested that the sulfate-reducing biofilm was not very active. The difference in hydraulic retention times between the control core system and the test core system made comparisons impossible. Finally, the duration of the project was too short to determine whether strain F established itself in the rock biofilm. Therefore, an extended core experiment was conducted to determine the effectiveness of strain F versus nitrate treatment alone in controlling sulfide production by a metabolically active sulfate-reducing biofilm and to determine if inoculation and growth with strain F in the core system causes plugging and loss of injectivity.

Extended Core Experiment

Baseline Operation. When formation water without any supplementation was injected into the core systems, the highest effluent sulfide concentration, 185 μM, occurred when the flow rate was 0.1 ml/min. At this flow rate, the concentration of SRB in the effluent was 10^6 cells per ml and the concentration of APB was 10^6 to

10^7 cells per ml. The differences between the influent and effluent sulfate and acetate concentrations were 0.5 mM and 1.5 mM, respectively. Faster flow rates (0.2 to 1 ml/min) gave effluent sulfide concentrations about equal to influent sulfide concentrations (50–100 μM), indicating no net sulfide production. Thus, the system was operated at a flow rate of 0.1 ml/min for the remainder of the study. No organisms capable of growing autotrophically with thiosulfate as the electron donor and nitrate as the electron acceptor were observed in the influent or the effluent of either core system at any of the flow rates used.

Storage of the formation at room temperature resulted in a decrease in the acetate concentration from 1.8 mM to below the detection limit. When this formation water was injected in the core system, little or no sulfide production occurred; the influent and effluent sulfide concentrations were similar, about 90 to 125 μM. To stimulate sulfide production in the core systems, 10 mM sodium acetate was added to the formation water prior to injection into the core. With the addition of 10 mM acetate to the formation water, the effluent sulfide concentrations in each core system were similar, and higher than the influent sulfide concentrations (< 90 μM), ranging from 220 to 270 μM (see Table 5). Effluent sulfate concentrations ranged from 4.5 to 5.1 mM. Sulfur recoveries were good; 95% to 107% of the sulfur injected into the core systems as sulfate and sulfide was recovered in the effluents as these two compounds. A 10-fold difference between influent and effluent SRB counts was observed in each core system. Influent and effluent APB counts of both core systems varied from 10^6 to 10^7 cells per ml. On one occasion, a few organisms (10 cells/ml) capable of growing autotrophically with thiosulfate and nitrate (strain F-like organisms) were detected in the effluent of the control core system.

Effect of Nitrate on Sulfide Formation. Formation water with 10 mM acetate and 5 mM nitrate was injected into each core system. No consumption of nitrate was observed. The influent and effluent sulfide concentrations averaged 60 μM and 223 μM, respectively, comparable to the concentrations observed prior to nitrate addition (see Table 5). Levels of SRB and APB bacteria remained unchanged and no strain F-like organisms were detected. Nitrate addition also had no effect on influent and effluent sulfate levels. These results indicated that the two core systems did not contain large numbers of microorganisms capable of using nitrate.

Effect of T. Denitrificans Strain F on Net Sulfide Production. After the test core system was inoculated with *Thiobacillus denitrificans* strain F, the effluent sulfide concentration decreased from 220 μM to an average of about 100 μM and remained at these levels for several weeks (see Fig. 3). The effluent sulfide in the test core system decreased 79% compared to that observed prior to inoculation, and the effluent sulfide concentration was 85% lower than that of the control core system when both received nitrate. During the first two weeks of

injection with formation water without nitrate, the sulfide production in the control core system was about 120 to 130 μM (see Fig. 3). During the third week sulfide production increased to 440 μM. After nitrate was added to the formation water, the amount of sulfide produced by the control core system decreased to 245 μM by the fifth week. After nitrate injection, influent and effluent sulfate concentrations in the control core system averaged 4.9 mM and 4.8 mM, respectively, versus 4.9 mM and 5.1 mM, respectively, from the test core system. Sulfur recoveries from both core systems ranged from 95% to 109% during this period, indicating that all of the sulfur injected into the cores as sulfide and sulfate was recovered in the effluent as these two compounds.

Approximately 1.2 to 1.8 mM nitrate was consumed by the organisms present in the test core system. No nitrate consumption was observed in the control core system. The amount of nitrate consumption in the test core system was much larger than the expected amount of nitrate consumption (390 μM) based on the difference between the effluent sulfide concentrations of the two core systems (210 μM), and the stoichiometry given in Equation 1.

Numbers of SRB in the effluent of the control and test core systems were 10^7 and 10^8 cells/ml, respectively. About 10^7 cells/ml of APB were detected in the effluents of both core systems. The numbers of strain F-like organisms in the effluent of the test core system increased from 10^3 cells/ml immediately after inoculation to 10^4 cells/ml two weeks after inoculation. No strain F-like organisms were detected in the effluent of the control core system. These data clearly show that the inoculation of strain F was more effective than the addition of nitrate alone in controlling sulfide production.

Effect of Nutrient Addition on Sulfide Production. The maximum amount of sulfide produced by the control core system (345 μM) was much lower than that expected (5 mM) based on the amount of sulfate and acetate present. This suggested that sulfide production in the cores may have been nutrient limited. To stimulate sulfide production, the formation water with 10 mM nitrate was supplemented with inorganic nutrients, and lactate was used in place of acetate. Figure 4 shows that these changes greatly stimulated sulfide production in the control core system. Very high effluent sulfide concentrations, ranging from 3,060 to 3,780 μM, were observed. In the test core system which contained strain F, sulfide production remained low with effluent sulfide concentrations < 330 μM (see Fig. 4). This clearly demonstrates the effectiveness of strain F in controlling sulfide production. Sulfur recoveries from the test core system ranged from 99% to 109%, but sulfur recoveries from the control cores were high (123% to 168%) during the first four weeks with nutrient supplemented formation water, indicating that more sulfur compounds were produced than were injected into the system.

For two weeks after the addition of the above nutrients, additional peaks were present that interfered with the detection of nitrate by HPLC, so the amount of nitrate used during this time could not be determined. During the third week of injection with the nutrient-supplemented formation water, little or no nitrate consumption was detected in the control core system, while 4.9 mM nitrate was consumed in the test core system. The difference in the amount of sulfide produced by the control core system compared to that of the test core system during this week was 2,800 μM. Based on the stoichiometry given in Equation 1, the amount of nitrate needed to oxidize this amount of sulfide to sulfate is 4.4 mM, which is very close to the observed nitrate consumption in the test core.

The concentration of strain F-like organisms detected in the effluent of the test core system increased from 10^4 to 10^7 cells/ml during the three weeks after nutrient-supplemented formation water was used. Concentrations of SRB and APB in the effluents of the two core systems were 10^8 and 10^7 cells/ml, respectively.

After the third week of injection of nutrient-supplemented formation water, consumption of nitrate (1.3 to 2.9 mM) in the control core system was observed. Concomitant with this was a decrease in effluent sulfide concentration from about 3,800 μM to 1100 μM (see Fig. 4). This lower effluent sulfide concentration was still four times higher than the effluent sulfide concentration (250 μM) of the test core system at this time. Growth was observed in some of the thiosulfate/nitrate mineral medium dilution bottles inoculated with effluent from the control core system, indicating the presence of 10^3 to 10^5 nitrate-using bacteria per ml. Microscopic examination showed that the predominant morphology in these bottles was a motile rod distinctly different from strain F. It should be noted that the numbers of strain F-like organisms detected in effluents of the test core system were always three orders of magnitude greater than the numbers of nitrate users detected in effluents from the control core system.

Increasing the nitrate concentration in the formation water from 10 to 20 mM did not markedly affect the sulfide production in either of the two core systems. During this time the control core system produced about 1,000 μM sulfide, while the test core system produced 100 to 200 μM sulfide. Little or no nitrate was consumed in the control core system at this higher nitrate concentration. Also, the numbers of nitrate-using bacteria detected in the effluent of the control core system were very low, about 10 cells/ml. In the test core system, levels of strain F-like organisms remained high, 10^5 to 10^6 cells/ml, and the amount of nitrate consumed (5 mM) was similar to that observed when 10 mM nitrate was used. The concentration of APB remained high at about 10^7 to 10^8 cells/ml in both systems.

The addition of 40 mM nitrate to the nutrient-supplemented formation water resulted in high nitrite levels which interfered with the analysis of sulfide. No further work was done at this nitrate concentration.

Plugging. The use of nutrient-supplemented formation water increased the biomass present in the injection lines and on the tubing walls between cores in both core systems. There was an initial increase in the inlet pressure of each core in the two core systems from 1 psig to about 3 psig. However, after several weeks of injection of nutrient-supplemented formation water, the inlet pressures decreased to less than 2 psig. Increasing the flow rate 20-fold (from 0.1 to 2 ml/min) did not increase the inlet pressure of any of the cores. Thus, little or no plugging occurred during the course of this experiment, even after extended incubations (about eight months). The small amount of plugging that was observed was probably due to the development of the sulfate-reducing biofilm.

Conclusions

A sulfide-resistant strain of *Thiobacillus denitrificans* was successfully grown in coculture with the sulfate-reducing bacterium *Desulfovibrio desulfuricans* in liquid culture without the accumulation of sulfide. Microbial sulfide production in an enrichment from an oil field brine was also controlled by the presence of this sulfide-resistant strain F. The effectiveness of strain F is due to its ability to grow and utilize sulfide at levels which are inhibitory to the wild-type strain of *T. denitrificans*. There are many sulfide-oxidizing bacteria, but these bacteria are usually inhibited when H_2S concentrations reach a nuisance level. Strain F not only removed sulfide in cultures of *D. desulfuricans* with lactate as the energy source, but it also did so in the presence of a mixture of sulfate-reducing bacteria which use lactate and products of lactate metabolism, acetate, and H_2 for sulfide production.

In the preliminary core experiment, the efficacy of *Thiobacillus denitrificans* strain F in controlling sulfide production in the core system at the Redfield site was studied. However, as noted above, several factors complicated the interpretation of our data, and prevented us from obtaining data that clearly showed that inoculation of *T. denitrificans* strain F was required. The two core systems used in the extended core experiment had excellent injectivity, which allowed both systems to be operated over a range of identical hydraulic retention times, and the current core systems actively produced sulfide. When formation water supplemented with only 10 mM acetate was used, 200 to 300 μM sulfide was produced. When the formation water was supplemented with lactate, ammonium nitrogen, and a phosphorous source, large amounts of sulfide, in excess of 3,000 μM, were produced. This provided an ideal experimental system to test the effectiveness of

strain F under conditions that simulated actual field conditions as closely as possible.

The data clearly show that inoculation of *T. denitrificans* strain F was needed to effectively control sulfide production, and that strain F was effective in systems that had the potential to produce large amounts of sulfide. When formation water with acetate and nitrate was used, a 78% reduction in effluent sulfide concentration was observed in the test core system inoculated with strain F compared to the control core system. When formation water supplemented with lactate, ammonium nitrogen, phosphorous, and nitrate was used, the effluent sulfide concentration of the test core system was 92% less than that of the control core system. A reduction in effluent sulfide concentration was observed after extended operation (5 weeks) in the control core system when nutrient-supplemented formation water and 10 mM nitrate were used. However, the effluent sulfide concentrations in the control core system were still very high, 1,000 μM, indicating that the addition of nitrate alone was not very effective in controlling sulfide production. Nitrate consumption was detected in the control core effluents, indicating that some nitrate-using bacteria were present. Most likely, these bacteria were SRB capable of using either sulfate or nitrate as the electron acceptor,[20] since the predominant organisms in enumeration bottles were morphologically similar to several types of sulfate reducers and the cells were much larger than strain F. The numbers of these nitrate-using bacteria in the control core system effluents decreased to low levels after 20 mM nitrate was used.

The extended core also shows that strain F can be maintained in the system for long periods of time without reinoculation. High concentrations of strain F were detected three to four months after inoculation at the two sampling ports downstream from each core in the test core system. Growth of strain F in the core system did not lead to additional plugging of the core. The small increase in the inlet pressure that occurred after nutrient-supplemented formation water was used also occurred in the control core system, and, thus, was not a result of the growth of strain F in the core system. The presence of high levels of strain F at the same time that a decrease in the effluent concentrations of sulfide and nitrate was observed indicated that strain F was present and metabolically active.

Acknowledgment

This work was funded by the Gas Research Institute (Chicago, Illinois).

The authors wish to thank the Northern Natural Gas Company of Redfield, Iowa, for the core material and formation water used in this work.

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Table 1 Chemical Composition of Formation Water Collected from the Davis 6 Well in the St. Peter Formation

Component	Concentration (ppm)
Iron	0.6
Sulfide	9
Chloride	420
Sulfate	450
Phosphate	1.8
Hardness	960
Total dissolved solids	718
Alkalinity	660

Samples were analyzed by BTI in late March 1989. The pH of the sample was 7.2.

Table 2 Sulfide Production by *D. desulfuricans* Grown with and without the Wild-Type or Mutant Strains of *T. denitrificans*[a]

Culture[b]	*T. Denitrificans* Inoculum Size (ml)	14 Days (mg/l)	19 Days (mg/l)
DD alone	0	47.4	42.2
DD + wt	0.1	28.0	28.8
	0.2	51.5	43.5
	0.3	22.5	19.5
DD + F	0.1	4.0	< 0.1
	0.2	< 0.1	< 0.1
	0.3	< 0.1	< 0.1

[a] Cultures were anaerobically grown in the modified medium without thiosulfate. The inoculum size of *D. desulfuricans* was 0.1 ml. Data are averages of two individual cultures.

[b] Abbreviations are: DD—*D. desulfuricans,* WT—*T. denitrificans* wild-type strain, and F—sulfide-resistant strain of *T. denitrificans.*

Table 3 **Concentration of Substrates and Products of *D. desulfuricans* and Oil Field Brine Enrichment Grown with and without Strain F of *T. denitrificans***

		Concentrations (mM)[c]				
Culture[a]	Growth[b] (A)	Lactate[c]	Acetate[d]	Nitrate[c]	Sulfate[c]	Sulfide[c]
DD	0.30[d]	< 0.1	1.4 ± 0.2	38.7 ± 2.3	5.4 ± 0.9	0.6 ± 0.07
DD + F	0.10 (7)	< 0.01	1.0 ± 0.4	33.0 ± 2.0	7.0 ± 0.5	
Brine enrichment	0.41[d] (9)	< 0.1	< 0.2	37.3 ± 2.3	5.8 ± 0.4	0.9 ± 0.1
Brine enrichment + F	0.12 (12)	< 0.1	< 0.2	33.3 ± 2.1	6.6 ± 1.7	< 0.03
F	0.19 (12)	1.9 ± 0.2	< 0.2	8.0 ± 0.9	26.0 ± 2.0	< 0.03
Uninoculated		1.5 ± 0.0	< 0.02	45.0 ± 0.0	6.5 ± 0.0	< 0.03

[a] Inoculum size for DD and brine enrichment was 0.1 ml. For strain F, 0.3 ml inoculum was used.

[b] Mean of the absorbance of three individual cultures with the number of days required to reach the absorbance given in the parenthesis.

[c] Mean of the values obtained from three individual cultures ± the standard deviation.

[d] Black precipitate present.

Table 4 The Effect of Nitrate and Inoculation with *T. denitrificans* (Strain F) on the Concentration of Sulfide in the Core System with a Hydraulic Retention Time of 3.2 hr (Preliminary Core Experiment)

Additions to Formation Water[1]	Sample Location[2]	Sulfide μM	Sulfate mM	Nitrate mM	SRB cells/mL	APB cells/mL	Strain F cells/mL
None	IN	170	4.8	0.5	10^5	10^3	<10
	EF	160	4.0	0.5	10^5	10^5	<10
Nitrate							
1st treatment	IN	170	1.4	39.0	10^5	10^3	
	SP-1	ND	1.4	28.5	10^7	10^7	
	SP-2	ND	1.4	19.3	10^5	10^5	
	EF	110	1.4	19.2	10^5	10^5	
2nd treatment	IN	190	4.8	39.5	10^7	10^7	<10
	SP-1	ND	1.1	36.1	10^7	10^7	<10
	SP-2	ND	2.5	30.1	10^5	10^5	<10
	EF	110	7.8	21.9	10^5	10^7	<10
Strain F + nitrate + nutrients							
1st treatment	IN	160	2.6	10.6	10^7	10^7	10^7
	SP-1	140	3.5	8.4	10^7	10^7	10^7
	SP-2	75	5.2	6.8	10^7	10^5	10^7
	EF	25	5.8	3.5	10^7	10^7	10^7
2nd treatment	IN	150	2.3	9.2	10^7	10^7	10^5
	SP-1	47	3.3	7.8	10^7	10^7	10^7
	SP-2	25	3.7	5.2	10^7	10^7	10^7
	EF	16	4.7	4.7	10^7	10^7	10^7

SRB = sulfate-reducing bacteria; APB = acid-producing bacteria; ND = not determine; IN = influent; SP-1 = sample port–1; SP-2 = sample port–2; EF = effluent.
[1]Formation water was amended with sodium nitrate, certain inorganic nutrients, and inoculated with strain F as described in Materials and Methods.
[2]See Figure 1 for sampling locations.

Table 5 Effect of Nitrate on Sulfide Production Prior to Inoculation with Strain F (Extended Core Experiment)

Treatment[1]	Core system	Sample Location	Sulfide[2] µM	C.V.[3]	Sulfate mM	%S	Nitrate mM	SRB cells/mL	APB cells/mL	Strain F cells/mL
acetate	control	influent	70	58	4.9		-	10^6	10^7	0
		effluent	224	8	4.5	97	-	10^7	10^7	0
acetate and nitrate		influent	65	7	4.9		5	10^6	10^7	0
		effluent	229	3	4.8	103	5	10^7	10^6	0
acetate	test	influent	70	34	4.8		-	10^6	10^6	0
		effluent	247	11	4.7	106	-	10^7	10^6	0
acetate and nitrate		influent	54	22	4.9		5	10^6	10^6	0
		effluent	216	4	4.8	102	5	10^7	10^7	0

[1]Reservoirs were amended with 10 mM sodium acetate or 10 mM sodium acetate and 5 mM sodium nitrate.

[2]Values reported are means of 3 samples taken over a 7-day period.

[3]Coefficient of variation.

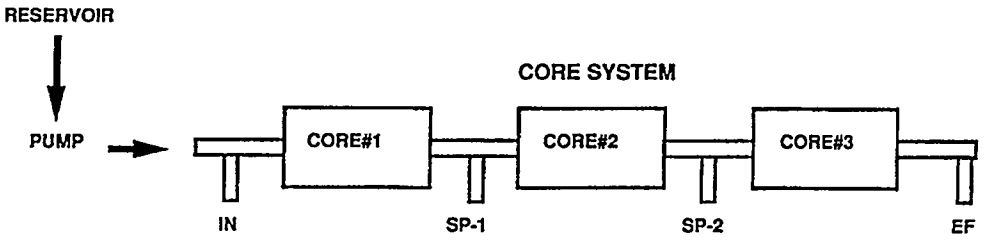

Figure 1 Core System Design

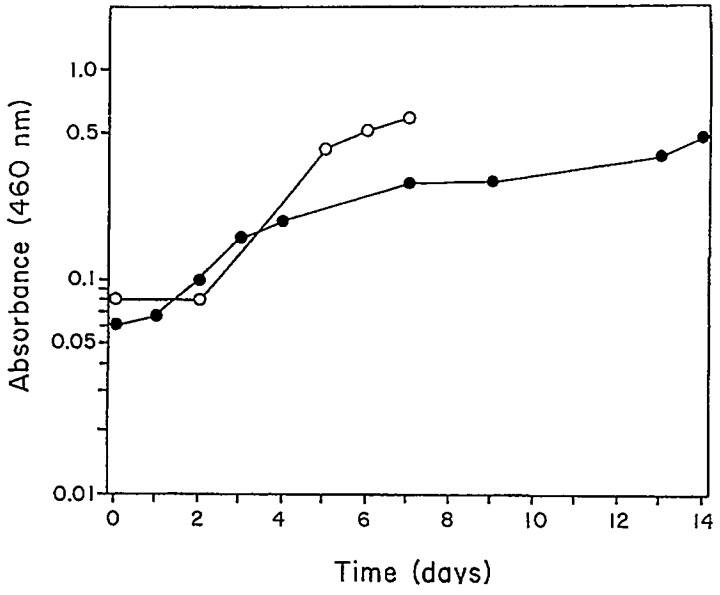

Figure 2 Growth of *D. desulfuricans* (o) or *T. denitrificans* (•) in Pure Culture in Modified Medium

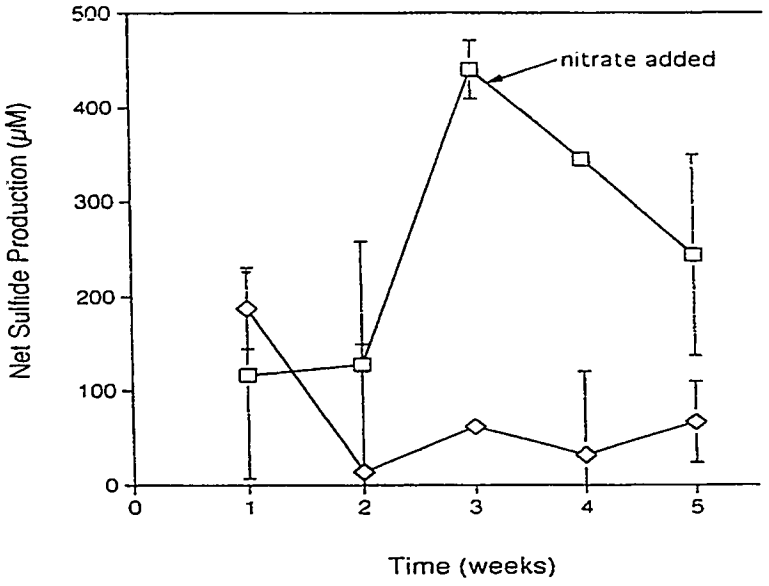

Figure 3 **Effect of Strain F Inoculation on Sulfide Production When Formation Water with 10 mM Acetate and 5 mM Nitrate Was Used. The Control Core System Received Acetate-Amended Formation Water without Nitrate for the First 3 Weeks. Control Core System, □; Test Core System, ◊. (Standard Deviations Included)**

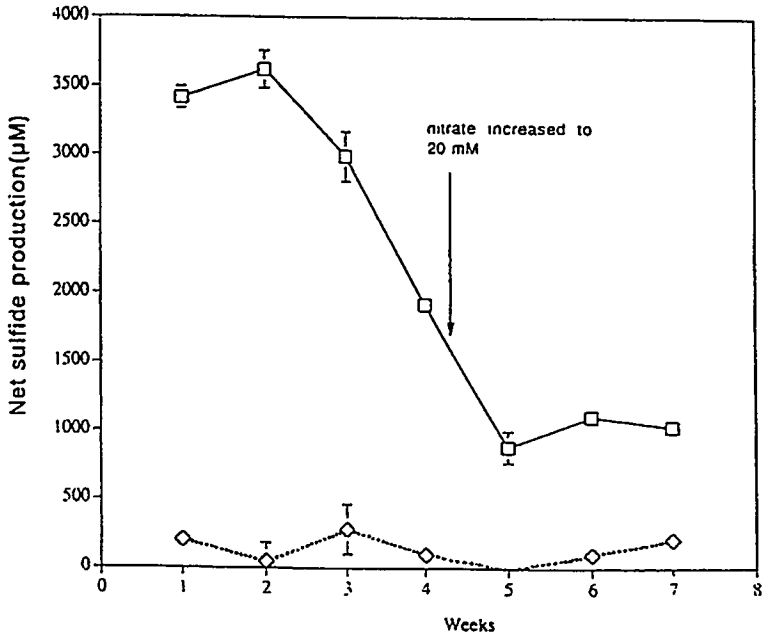

Figure 4 **Effect of Strain F Inoculation on Sulfide Production from Formation Water with Lactate, Nitrate, and Inorganic Nutrients. Nitrate Concentration Was Increased from 10 mM to 20 mM after 5 Weeks. Control Core System, □; Test Core System, ◊. (Standard Deviations Included)**

Microbial Oxidation of Soluble Sulfide in Produced Water from the Bakken Sands

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Abstract

The presence of soluble sulfide in produced water results in problems for the petroleum industry due to its toxicity, odor, corrosive nature, and potential for wellbore plugging. Sulfide oxidation by indigenous nitrate-reducing bacteria (NRB) present in brine collected from wells at the Coleville Unit (CVU) in Saskatchewan, Canada, was investigated. Sulfide oxidation took place readily when nitrate and phosphate were added to brine enrichment cultures, resulting in a decrease in sulfide levels of 99–165 ppm to nondetectable levels (< 3.3 ppm). Produced water collected from a number of producing wells was screened to determine the time required for complete sulfide oxidation, in order to select candidate wells for treatment. Three wells were chosen, based on sulfide removal in 48 hours or less.

These wells were treated down the backside of the annulus with a solution containing 10 mM KNO_3 and 100 μM NaH_2PO_4. Following a 24- to 72-hour shut-in, reductions in pretreatment sulfide levels of greater than 90% were observed for two of the wells, as well as sustained sulfide reductions of 50% for at least two days following startup. NRB populations in the produced brine were observed to increase significantly following treatment, but no significant increases in sulfate-reducing bacteria were observed. These results demonstrate the technical feasibility of stimulating indigenous populations of NRB to remediate and control sulfide in produced brine.

Introduction

Produced water from oil reservoirs frequently contains soluble sulfide (H_2S, HS^- and S_2^-) as a consequence of the activities of sulfate-reducing bacteria (SRB). The presence of soluble sulfide in produced brines creates serious problems for the petroleum industry due to toxicity, odor, metal corrosion, and potential for plugging of injection wells by formation of iron sulfides. Removal of sulfide from sour produced water can be accomplished either by physical or chemical means, e.g., stripping with steam or flue gas, oxidation with air or permanganate, or by precipitation as a metal salt. The drawbacks associated with these methods include the need for specialized facilities, waste disposal, hazardous chemical usage, and cost.

An alternative method by which sulfide can be removed from reservoir brines is through biologically mediated processes. Jenneman et al.[1] demonstrated that the ecology of a sulfide-laden environment could be manipulated by changing the dominant electron acceptor from sulfate to nitrate, such that nitrate-reducing bacteria (NRB) could oxidize the sulfide present and then out-compete the SRB for common electron donors, e.g., organic acids. McInerney et al.[2] injected ammonium nitrate into the petroleum reservoir of the Southeast Vasser Vertz Sand Unit in Payne County, Oklahoma, and reported a 40% to 60% reduction in soluble sulfide at three adjacent producers that was attributed to indigenous NRB. Similarly, the efficient oxidation of sulfide by the bacterium, *Thiobacillus denitrificans* strain F, has been demonstrated in rock cores,[3] bioreactor systems,[4] and in a field pilot[5] to remediate sour produced water. We have been examining sulfide oxidation by an indigenous NRB population in produced brine from wells at the CVU in Saskatchewan, Canada, and have observed the complete oxidation of 99–165 ppm soluble sulfide in laboratory brine enrichments amended with 5 mM KNO_3 and 100 μM NaH_2PO_4. In addition, batch treatment of three producing wells at the CVU, containing between 52 and 160 ppm sulfide with nitrate and phosphate resulted in reductions in sulfide of greater than 90%. This paper presents the results of these well treatments, along with the laboratory investigations used to select candidate wells for treatment.

Reservoir Description

The Coleville Field located near Kindersley, Saskatchewan, Canada, is owned and operated by Phillips Petroleum Company. The field was discovered in 1951 and has been waterflooded since 1958. The wells produce out of the Bakken Sandstone at a depth of 2,700 ft. The bottomhole temperature is about 30°C. The permeability of the formation averages between 500 and 1,000 md, with some streaks of 2 to 3 darcies. The oil is heavy, asphaltic crude with an API gravity of 13. Currently, the

produced water is very low in total dissolved solids (i.e., < 8,000 mg/l). Chloride, bicarbonate, and sulfate are the principal anions and sodium, calcium, and magnesium are the major cations. The pH of the produced water, measured at atmospheric conditions, is between 7.0 and 8.5. The produced water is sour, containing soluble sulfide concentrations ranging from a few ppm to greater than 160 ppm. Water injection rates at the time of testing were about 30,000 barrels of water per day (BWPD), of which greater than 90% was reinjected produced water.

Materials and Methods

Laboratory Testing

Brine Collection. Brine was collected from the field under oxygen-limited conditions in one-liter, sterile glass bottles which were equilibrated under anaerobic conditions prior to sampling. Bottles were shipped from the field to the laboratory immediately after collection, and stored in a Coy anaerobic chamber (Coy Products, Ann Arbor, Michigan) prior to use. Brine was collected from the free water knockout (FWKO) tank, in addition to collection of wellhead samples of coproduced oil and water.

Enrichment Conditions. Enrichments were prepared in triplicate under anaerobic conditions in serum bottles. Serum bottles (50 ml) were autoclaved and allowed to equilibrate overnight in the Coy chamber. Potassium nitrate and sodium phosphate (monobasic) were added to the bottles from sterile stock solutions, followed by 50 ml of brine. Bottles were stoppered with butyl rubber stoppers, crimp sealed, and incubated at 30°C.

Sulfide Assays. Sulfide was determined colormetrically by a modification of the method of Fogo and Popowski,[6] where sulfide was trapped by reaction with zinc acetate (0.02% final concentration).

Field Testing

Nitrate / Nitrite Analysis. Nitrate content was analyzed using a Compact Ion Meter (Horiba) equipped with a nitrate sensor. Sulfide was removed from the sample prior to nitrate analysis by precipitation with zinc acetate and filtration through a 0.45 μm membrane filter. Nitrite analysis was performed using a Nitrite CHEMets Kit (CHEMetrics, Inc., Calverton, Virginia).

Sulfide Analysis. Sulfide was measured using a commercial sulfide detection kit (Aquaquant, E. M. Sciences). Sulfide levels in samples were measured within an hour of the time they were collected in the field. For samples containing coproduced water and oil, the water sample was drawn off the bottom of the bottle with a long, cannula needle attached to a syringe. If necessary, the oil and suspended particles were removed by filtration with a 0.45 μm syringe filter before assaying.

Determination of Cell Numbers. Enumeration of SRB and NRB was carried out by performing tenfold serial dilutions in bottles of the appropriate culture medium. Each positive bottle above represents a tenfold increase in cell number. SRB were cultured in API RP38 medium (C&S Laboratories, Tulsa, Oklahoma) and NRB were cultured in CVU brine containing 5 mM KNO_3, 100 μM NaH_2PO_4, and 0.001% resazurin. This brine was collected at the FWKO tank and sterile filtered through a 0.22-μm membrane filter prior to use.

Treatment Chemicals. A 50 lb bag of ammonium nitrate (34:0:0) was purchased from Hildabrand's Farm & Ranch Supply in Bartlesville, Oklahoma. The ammonium nitrate was added to deionized water to yield a final concentration of 5 M (400 g/l). This solution was allowed to stand until the clay settled, which was subsequently removed by filtration through a 0.22-μm membrane filter. Sodium phosphate (monobasic) was added to the nitrate solution to yield a final concentration of 50 mM (6 g/l). The concentrate was dispensed in plastic polyethylene bottles and shipped to the Coleville Field.

Well Treatment Procedure. Corrosion inhibitor for all wells treated in this study was discontinued on September 20, 1994. All treated wells were on beam pumps. The annulus of each well is cased to depth and open to the atmosphere. Other properties are listed in Table 1.

On Tuesday, September 27, 1994, wells 9-24, D7-21, and 59-20 were treated down the backside of the annulus with 3.44, 2.18, and 3.25 gal of the nitrate and phosphate solution, respectively. Afterward, the chemical was flushed with 4 barrels (bbl) of brine collected at the FWKO tank to yield a downhole concentration of 682 ppm nitrate and 1.05 ppm phosphate. Wells 9-24, D7-21, and 59-20 were shut-in at 9:09 a.m., 9:50 a.m., and 10:07 a.m., respectively. Immediately prior to treatment, two 1-liter bottles were filled with approximately 500 cc of coproduced fluids collected at the wellhead of each well. One of these samples was used to determine baseline levels of sulfide, nitrate, nitrite, SRB, and NRB. The other bottle was inoculated with 1 cc of the treatment chemical solution and transported back to the laboratory, where it was incubated at room temperature. This bottle was used as a laboratory control for comparison to field oxidation rates.

Results

Laboratory Testing / Screening

General Conditions. The key factors required for sulfide oxidation to occur were the presence of nitrate and indigenous bacteria (data not shown). Supplementation of brine with nitrate and phosphate were the only amendments necessary for sulfide removal. Figure 1 demonstrates sulfide disappearance after the addition of 5 mM KNO_3 and 100 μM NaH_2PO_4 in enrichments prepared with FWKO brine. Sulfide disappearance to nondetectable levels took place within 48 hours. No change took place in controls without added nitrate.

Nitrate-Dependent Sulfide Oxidation. Nitrate concentrations ranging from 0 to 10 mM (0–620ppm) were tested to determine the minimum concentration needed for complete removal of the sulfide present. In this case, 100 μM NaH_2PO_4 was added to all of the enrichment bottles. A nitrate level of 2.5 mM (155 ppm) was sufficient to allow complete removal of the sulfide (120 ppm) within 48 hours (see Figure 2). Within 24 hours of incubation, the enrichments turned a transparent yellow color and then, by 30 hours, the medium contained a yellowish-white precipitate that settled to the bottom of the bottle upon standing. This precipitate was later determined to be elemental sulfur and calcite (results not shown). This suggested that the sulfide was being oxidized at the expense of nitrate reduction. The pH of the enrichment increased from 7.4 to 8.5, also indicative of nitrate reduction.

Screening of Produced Brines. Brines collected from several producing wells were screened for the presence of sulfide oxidizers by monitoring the disappearance of sulfide to a nondetectable level. Brine collected from 6 producing wells was tested. Table 2 shows the number of times each well was sampled, the average initial sulfide concentration, and the time required for sulfide oxidation. The criteria used to select candidate wells were (1) relatively consistent initial sulfide levels, (2) complete sulfide removal in 48 hours or less, and (3) total fluid production of around 100 bbl fluid/D (BFPD) (see Table 1). All wells tested, with the exception of one sample obtained from well 56-24, showed oxidation of sulfide within 48 hours. The wells selected for treatment were 9-24, 59-20, and D7-21.

Field Tests

Shut-in Test. In order to determine the effect of a shut-in period on the natural levels of soluble sulfide in the produced brine, well 9-24 was shut in for 24 hours and then restarted. After restarting the well, samples of produced brine were

collected and tested for sulfide after one and three hours. The sulfide level prior to shut-in was 177 ppm, while the sulfide level after restarting the well was 199 ppm after one hour, and 204 ppm after three hours.

Treatment of Well D7-21. Following the 24-hour shut-in, well D7-21 was placed back on pump, and after 15 minutes of production, a wellhead sample was collected and assayed for sulfide. The sulfide concentration at this time was 40 ppm, or about 20% less than the pretreatment level of 52 ppm (see Fig. 3). The well was shut in for another 2.5 hours, restarted, and sampled again. The sulfide level this time remained at 20% of the pretreatment level; however, following another 2 hours of production, the sulfide level declined to 98% of the baseline amount (see Fig. 3). The nitrate concentration in the produced brine at this time was about 930 ppm (15 mM) and the nitrite concentration was 64 ppm (1.4 mM). The well was shut in again and restarted the next morning, at which time the sulfide level increased to 13 ppm. By 54 hours following treatment, the sulfide concentration increased to 20 ppm or roughly 40% of the pretreatment level. No nitrate or nitrite was detected in any of the samples taken during this time. The sulfide level remained at 20 ppm for at least another 18 hours of production, corresponding to a cumulative volume of 40 bbl of fluid pumped since the startup of the well. Thirteen days later, the sulfide levels had returned to the pretreatment level.

SRB and NRB populations were monitored before and after treatment to examine specific changes in these populations. The results indicate that NRB populations increased dramatically following the 24-hour shut-in, that is, an increase from 3 positive bottles before treatment to at least 8 positive bottles following treatment (see Fig. 3). NRB populations remained as high as 8 bottles for 31 days following the start of the treatment, whereas SRB populations ranged between 3 and 5 positive bottles throughout this entire time.

Treatment of Well 9-24. Well 9-24 was shut in for 47 hours following chemical treatment (see Fig. 4). After restarting the well, the sulfide level of the produced brine was 20% below the pretreatment level of 160 ppm. The sulfide level remained fairly constant until about 6 hours following startup, when the sulfide concentration decreased to 50% of the pretreatment value and continued to drop steadily over the next hour or so, reaching a minimum of 16 ppm or 10% of the pretreatment level. The sulfide concentration then increased rapidly over the next two hours and eventually leveled off at 80 ppm or 50% of the pretreatment amount, remaining there for at least the next 8 hours. This means that 61 bbl of fluid were produced at a sulfide level that was at least 50% lower than the pretreatment level, nearly 60% more fluid than was treated in the wellbore prior to shut-in. Sixteen days later, the sulfide level returned to its pretreatment concentration. No nitrate or nitrite was detected in any of the samples tested.

SRB populations remained at 3 or 4 positive bottles throughout the test period, whereas NRB increased from 4 positive bottles before treatment to at least 8 bottles following treatment (see Fig. 4). Sixteen and 31 days following treatment the NRB populations were still as high as 6 bottles.

Treatment of Well 59-20. Well 59-20 was shut in for 70 hours following treatment (see Fig. 5). A sample of the produced fluid collected at the wellhead within 15 minutes of startup contained 80 ppm sulfide, or 50% of that measured prior to treatment. Over the next hour of production, the sulfide level rose sharply to 160 ppm and then decreased to 64 ppm during the following hour. At this time, 1,700 ppm (27 mM) nitrate and 16.6 ppm (0.36 mM) nitrite were detected in the produced fluid. Over the next couple of hours, the sulfide concentration remained steady at 40% to 60% of the pretreatment level, but no additional nitrate or nitrite was detected. Sixteen days following treatment, the sulfide level had increased back to pretreatment levels.

SRB populations remained at 2 to 3 positive bottles throughout the test, whereas, NRB populations rose significantly from 6 to as many as 8 bottles (see Fig. 5). By 16 and 31 days following treatment, the NRB populations had declined to 7 and 6 bottles, respectively.

Discussion

Laboratory Enrichments

Laboratory experiments demonstrated the ability of indigenous NRB present in CVU-produced brine to completely oxidize sulfide upon addition of nitrate and phosphate. The indigenous NRB are apparently quite resistant to sulfide toxicity, tolerating as much as 160 ppm sulfide. It has been reported that wild strains of *Thiobacillus denitrificans,* autotrophic NRB, have sulfide tolerances that are much lower, ranging from 80 to 100 ppm sulfide.[7,8]

Examination of the relationship between nitrate concentration and the amount of biological oxidation in CVU brine indicated that it takes approximately 2.5 mM (155 ppm) nitrate to oxidize 3.1 mM (102 ppm) sulfide or 0.8 moles of nitrate per mole of HS- (see Fig. 2). The only detectable product of sulfide oxidation in CVU enrichments was elemental sulfur. Elemental sulfur has been reported as a by-product of sulfide oxidation for *Thiobacillus denitrificans* strain F; however, Sublette and Sylvester[9] attributed this phenomenon to stress imposed on the bacteria by high levels of sulfide. Similarly, elemental sulfur was produced as a

product of sulfide oxidation by *T. thioparus* under conditions of oxygen limitation.[14]

Screening of Wells

One criterion for the selection of wells to be treated was that the wells have a total fluid production of around 100 BFPD or less, which would allow us to collect enough samples of treated produced fluids for proper evaluation of the treatment. Also, we felt that batch treatment would not be as effective for the higher volume producers, since they would likely have to be treated more frequently. Other criteria selected included a stable baseline of sulfide in the produced water over time, and the complete removal of sulfide within 48 hours. These criteria were selected so that reductions in sulfide concentrations would not be confused with natural fluctuations in sulfide levels, and producing wells would not need to be shut in longer than 2 days.

Field Tests

Following chemical treatment, produced brine from all three wells displayed significant reductions in sulfide levels, which was attributed to in-situ biological sulfide oxidation by indigenous sulfide-oxidizers. Recently, several other investigators have reported the ability of indigenous sulfide oxidizers to reduce sulfide concentrations during field tests;[2,5,10] however, in only two of the cases[2,10] was nitrate found to be a useful electron acceptor.

The slightly elevated sulfide concentrations measured one and three hours following the shut-in test at well 9-24 suggested that the sulfide reductions following treatments were not the result of shut-in alone (e.g., dilution by stratal waters or air oxidation). Furthermore, laboratory tests run prior to the treatments revealed that natural fluctuations in sulfide levels for all three wells varied by only 7% to 26% upon repeated sampling (see Table 2). Also, a dramatic increase in NRB populations of 100- to 100,000-fold in wells D7-21 and 59-20 (see Figs. 3 and 5) and the presence of nitrite, a by-product of biological nitrate reduction, in the produced brine following treatments suggested the sulfide reductions were biologically mediated. It has been suggested that biological sulfide oxidation in the presence of nitrate may consist of chemical as well as biological mechanisms, since nitrate reduction can result in the production of nitrite, which in turn chemically oxidizes sulfide to elemental sulfur.[10] Controls using nitrite in the presence of sterile-filtered CVU brine indicated no chemical oxidation of sulfide took place over a 48-hr period at 30°C (results not shown).

The time interval over which the maximum reduction in sulfide level occurred was very narrow in all wells, suggesting that the nitrate did not mix well downhole, but instead remained as a concentrated slug. This was further indicated by the presence of higher than expected concentrations of nitrate and nitrite in the produced brine of wells D7-21 and 59-20 soon after restarting the wells. Circulation, squeeze treatments, and premixing of the chemicals with the brine could all be used to increase mixing in the wellbore and in the tubing string.

The treatments described effectively reduced sulfide concentrations by as much as 50% in a volume of produced water that was at least twice the size of that contained in the wellbore. This suggests that either some of the nitrate dispersed beyond the wellbore region, or that the sulfide levels distal to the wellbore were lower than those in the immediate vicinity, perhaps due to an active, localized SRB population in the wellbore. Dispersion of the chemical downhole would seem unlikely, given the apparent poor mixing of the chemical in the wellbore. The latter explanation of sulfide reductions, however, is intriguing, since it implies that the NRB population, which was stimulated by the addition of nitrate, out-competed the local SRB biofilm population and delayed further sulfide production. This suggests that the nitrate treatment, at least in these instances, had some residual activity on the local biofilm population in controlling the return of biogenic sulfide. Mc Kinley et al.[11] found when back flowing injection wells that most of the biomass was contained in the first couple of wellbore pore volumes, suggesting concentration of microorganisms in the wellbore vicinity. Although their study was performed in injection wells and not producers, it is possible that SRB could have been concentrated in the wellbore of producers at CVU during drilling operations. McInerney et al.[2] also suggested that SRB may be localized near the wellbore (of injection wells), based on slow penetration rates of SRB in Berea sandstone cores.

Monitoring of the NRB population indicated that increasing concentrations of planktonic NRB were observed for as long as one month following treatment, without significant increases in the SRB population. Therefore, it appears that a single application of nitrate and phosphate can stimulate and maintain a significant NRB population downhole without significantly increasing numbers of planktonic SRB. The increased concentrations of NRB following treatment further suggests that subsequent treatment of these wells should result in faster sulfide biooxidation rates and shorter shut-in times. The neutral effect of the treatment on the SRB population is important, since it is well known that certain SRB can use nitrate in addition to sulfate.[12] Jack et al.[13] observed a significant change in the biofilm (i.e., attached cells) community following addition of nitrate to a sulfide-laden brine, and also observed an increase in corrosion. It is possible that nitrate addition stimulated a population of nitrate-reducing SRB; however, no description of the nature of the bacteria was given.

Survival of NRB downhole in the CVU brine at such high cell densities in the absence of injected nitrate is intriguing. It is possible that NRB derive energy from processes other than nitrate-dependent sulfide oxidation, or form a very persistent biofilm during treatment. We have some evidence indicating that these bacteria readily attach to particles such as calcite and sand grains (results not shown).

Economics. A total of 30 lb of ammonium nitrate was used to treat all three wells at a chemical cost of $0.16 per lb. Therefore, the total cost of the ammonium nitrate used was $4.80, or an average cost per well of $1.60. The cost of the phosphate was not included, since it was used at only a fraction of the amount of nitrate used (i.e., 11 mM nitrate vs. 0.11 mM phosphate). Based on the laboratory-derived stoichiometry for this reaction, the amount of chemical used in these tests exceeded the amount needed by 3- to 8-fold, depending on the well treated, suggesting that the chemical costs per well per treatment could be as low as $0.20 to $0.53. Since we cannot calculate the actual amount of sulfide oxidized, it is not possible to calculate the cost per unit of sulfide oxidized; however, based on a presumed stoichiometry of 0.8 M nitrate needed to oxidize 1 M of sulfide, it would require about $0.014 of nitrate to oxidize 1 M of sulfide to elemental sulfur. Economics based on this reaction should compare favorably to a sulfide oxidation process using the chemoautotroph, *Thiobacillus denitrificans,* which requires 1.6 M of nitrate to oxidize 1 M of sulfide to sulfate.[2]

Therefore, the successful application of a treatment to promote in-situ sulfide bio-oxidation is advantageous because it eliminates the cost associated with growth of exogenous bacteria and the need for specialized treatment facilities. Furthermore, adaptation of exogenous bacteria to field conditions is unnecessary and, although by no means optimized, the potential simplicity of the field experiments illustrates that positive results can be obtained with minimal equipment design.

In conclusion, we feel that the results of these studies suggest that CVU is a good reservoir in which to develop and implement methodologies for the reduction and control of sulfide through the use of indigenous NRB. It is hoped that implementation of this technology will result in a more cost-effective means of corrosion control and assist in environmental protection.

Acknowledgments

The authors acknowledge the assistance of Andrew Arksey, who coordinated the sampling and shipping of brine, as well as the inventorying of supplies and equipment needed at the CVU for the field tests. We are also grateful to Phillips Petroleum Company for support of this project and especially to Peter Nichols (Phillips Petroleum Company, Calgary, Alberta), who has been very supportive

and helpful in our efforts to transfer this technology to the field. A special thanks to LaVonne Melheim for reviewing this manuscript.

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Table 1 Properties of Wells Selected for Treatment

Well #	Fluid Level ft	Tubing Depth ft	Col. Height ft	Casing I.D. in	Wellbore Storage bbl	Fluid Production		
						BOPD	BWPD	BFPD
9-24	1800	2739	939	6.37	37.0	6.29	50.3	56.6
59-20	1800	2682	882	6.37	34.7	6.29	94.3	101
D7-21	1800	2695	895	4.95	21.3	3.14	31.4	34.5

BOPD, barrels of oil per day; BWPD, barrels of water per day; BFPD, barrels of fluid per day

Table 2 Results of Sulfide Oxidation Screens for Selected Wells at the CVU Prior to Treatments

Well #	Number of Times Sampled[a]	Average Initial Sulfide Conc. (ppm)[b]	Complete Oxidation (%)[c]	
			24 Hours	48 Hours
5-24	2	167	0	100
9-24	5	152 ± 6.6	80	100
56-24	2	179	50	50
59-20	3	119 ± 27	67	100
D5-22	3	37 ± 21	100	100
D7-21	3	45 ± 20	67	100

a Samples collected on different dates; three replicate enrichments prepared from each sample.

b Average for all three replicates for particular well at time zero.

c Reduction of the sulfide level in all three replicates of a single sample to a nondetectable level (<3.3 ppm). The values above are expressed as the time required for complete oxidation of each sample, expressed as a percentage of all samples collected (e.g., for well 5-24, all of the replicates in the two samples displayed complete oxidation within 48 hours).

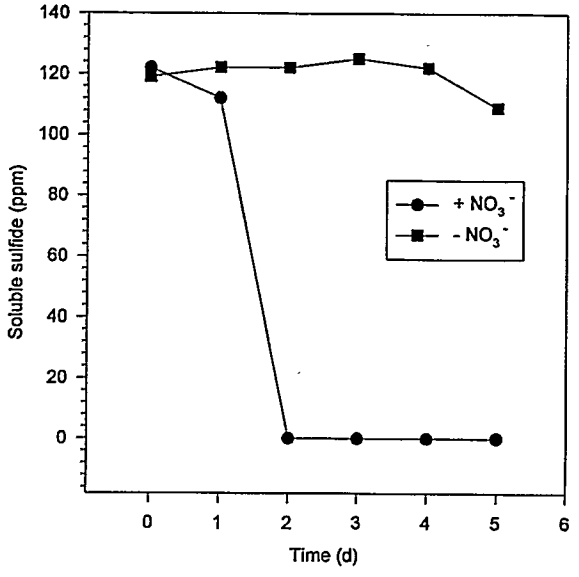

Figure 1 Effect of Nitrate on Removal of Sulfide in CVU-produced Water

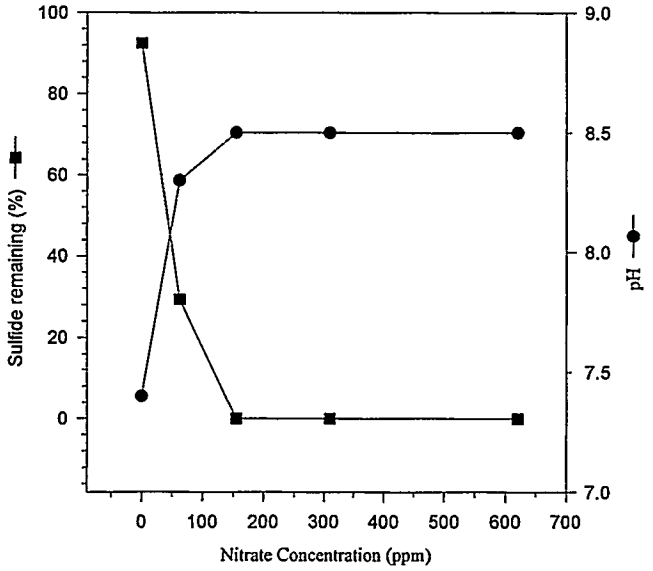

Figure 2 Effect of Nitrate Concentration on Sulfide Removal in CVU-Produced Brine

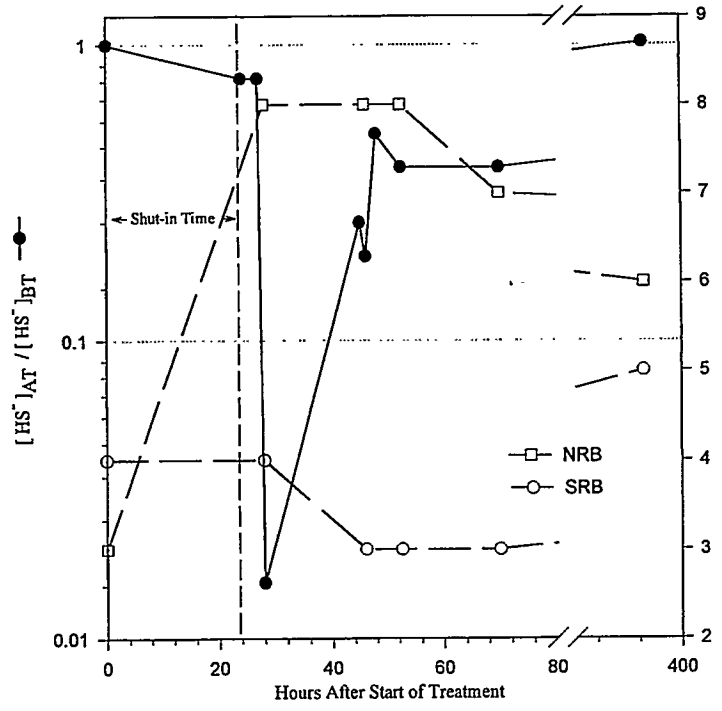

Figure 3 Effect of Treatments on Sulfide, SRB, and NRB levels in Produced Brine at Well D7-21; HS^-_{BT} (Sulfide Level before Treatment) and HS^-_{AT} (Sulfide Level after Treatment)

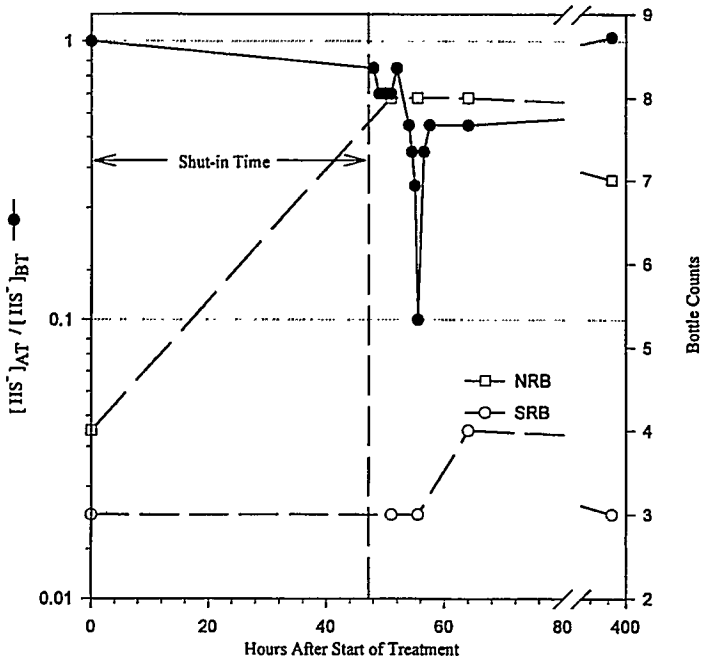

Figure 4 Effect of Treatments on Sulfide, SRB, and NRB Levels in Produced Brine at Well 9-24; HS^-_{BT} (Sulfide Level before Treatment) and HS^-_{AT} (Sulfide Level after Treatment)

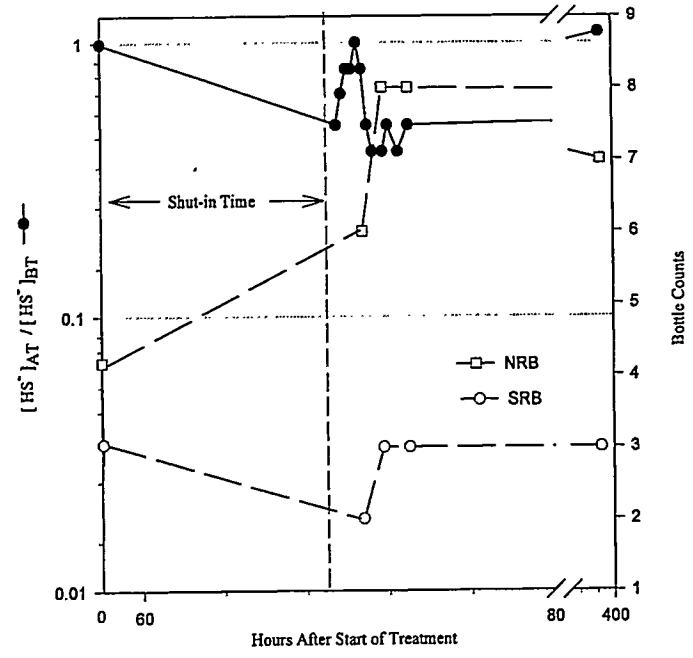

Figure 5 Effect of Treatments on Sulfide, SRB, and NRB Levels in Produced Brine at Well 59-20; HS^-_{BT} (Sulfide Level before Treatment) and HS^-_{AT} (Sulfide Level after Treatment)

Biocompetitive Exclusion Technology: A Field System to Control Reservoir Souring and Increase Production

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Abstract

Biogenic formation of sulfide in reservoirs by Sulfate Reducing Bacteria (SRB) causes serious plugging, corrosion, and environmental safety problems. The production of sulfide can be decreased, and its concentration reduced, by the establishment and growth of an indigenous microbial population which results in a replacement of the SRB population. This approach to modify the reservoir ecology utilizing preexisting carbon sources coupled with the introduction of an alternate electron acceptor forms the basis of a new Biocompetitive Exclusion technology which has the potential to enhance oil recovery and decrease paraffin deposition and corrosion. Preliminary field results from an ongoing DOE-sponsored research program will be discussed.

Brief Objective

The objective of this DOE/BDM contract which makes use of the "Biocompetitive Exclusion Technology" developed by GMT is the field demonstration of prevention and treatment of H_2S, FeS, paraffin, and scale in producing wells (oil and some gas wells) and the demonstration of similar treatments for injection wells. A further objective of this field project is to demonstrate the cost effectiveness of the program, thereby preventing the premature abandonment of economically marginal wells.

Project Description

Background

This project involves the field demonstration of amelioration of problems (H_2S, FeS, paraffin, and scale) that lead to premature well abandonment. The treatment of wells which have become economically marginal to operate due to these problems is based in part on the alteration of the microbial ecology of the downhole environment. By taking advantage of the fact that there are different levels of energetic efficiencies of various anaerobic respiration processes, the growth of sulfide generating bacteria (also called sulfate-reducing bacteria or SRB) can be inhibited by a more efficient, beneficial replacement population. This is the main basis of the GMT "Inject-Chek" treatment process; the replacement of the detrimental SRB population with a population of bacteria that use nitrate for respiration, the denitrifying bacteria (DN). The GMT "Inject-Chek" treatment process replaces the detrimental SRB population downhole with a beneficial DN population by the mechanism of biocompetitive exclusion.

In addition to supplying the alternative electron acceptor, nitrate, that allows the previously dormant but more efficient, beneficial, denitrifying bacteria to outcompete the SRB, the Inject-Chek treatment process makes use of two other inorganic salts. One salt is nitrite, which acts as a chemical scavenger of sulfide and the other anionic, inorganic salt is molybdate. Molybdate is frequently used to inhibit the action of SRB, but it was discovered that very low concentrations of molybdate exhibited a biocidal effect upon SRB when used in combination with either nitrate or nitrite. It was this serendipitous and unexpected result which led to the April 1995 issue of a patent for the Inject-Chek treatment process. It should be noted that the concentrations of the three inorganic salts can be tailored to different individual well chemical parameters. For example, some wells may contain very little sulfide, and the transitory production of nitrite from nitrate during denitrifier

growth downhole could supply enough nitrite for sulfide scavenging purposes in a well of that nature.

Treatment Protocol

Two treatment protocols have been developed, and other various treatment protocols are under development. One of the developed protocols involved the introduction of the Inject-Chek chemical treatment down the annulus of the well, at which time the well was shut in for 3 days. The second protocol was similar, but made use of a 10-gallon water chase on top of the 2 gallons of Inject-Chek used. Both protocols relied upon visual observations of the operators to judge success or failure of the treatment because the wells tested were not isolated and fed production into common battery systems.

GMT has purchased and refurbished a Port-A-Chek instrument that will become an integral part of future protocols. This machine can be used to monitor an individual well, and this allows measurement of total production and programmed autosampling of the oil/water ratio for an isolated well separate from other lease production. This machine is currently in use for a protocol under development. In this protocol, baseline data for an isolated well is being collected and when sufficient data is collected, the well will be shut in without treatment for 3 days. Then the well will be put back into production and data collected for comparison to the period prior to shut-in. When the well returns to a stable, baseline production mode, the well will again be shut in for 3 days, but after Inject-Chek treatment. Data can then be collected again after the 3-day shut-in period to judge the effectiveness of the treatment. The chemical composition of the Inject-Chek treatment might then be altered or the shut-in period changed to maximize the effectiveness of the treatment. The chemical composition of the initial Inject-Chek treatment is currently being governed by the results of laboratory analysis of the chemical parameters and microbiology of the isolated well. Additional protocols will be developed based upon the accumulation of field test results.

Currently, additional treatments are being performed on selected injector wells situated in the National Petroleum Reserve No. 3 field in Caspar, Wyoming. In these current tests, the prime aim is to mitigate sulfide production at producing wells that are in formation communication with production wells.

The preliminary results from field treatments are summarized in Tables 1 and 2. Figure 1 is a photograph of the Port-A-Chek system which will be employed to help judge the effectiveness of field treatments. Table 3 summarizes data from a typical experiment using Inject-Chek to treat flooded Berea Sandstone Cores.

Status of Deliverables and Accomplishments

To Date

Deliverables to date include:

1. The issue of a summary report
2. The issue of monthly and quarterly status reports
3. The completion of a technology transfer plan
4. The selection of a preliminary field treatment process
5. The field treatment of nonisolated wells
6. Continued laboratory examination of simulated treatments
7. The preliminary identification of test wells which has resulted in the selection of the wells/leases indicated:
   a) Matoaka lease, Washington County, Oklahoma
   b) Dunbar lease, Montgomery County, Kansas
   c) Goss lease, Humble, Texas
   d) National Petroleum Reserve No. 3, Casper, Wyoming
   e) Phillips Petroleum Co. NPU lease, wells no. 28, 59, and 114
   f) PP Co. Gandu lease, wells no. H-19, N-7, and P-6
   g) PP Co. gas lease, Moore County, Texas, The Clevy, Barre, Arlene A
   h) Various Independents in Creek, Tulsa, Nowata, and Washington Counties
8. The chemical and microbiological analyses of a, b, c, d, g, and h
9. Preliminary field treatments of a, b, and d

Future

Future deliverables include:

1. The issue of annual, quarterly, and monthly reports
2. The completion of an economic analysis
3. The refinement of laboratory protocols
4. The refinement and further testing of field protocols on isolated single wells on different leases and in different formations, and the testing of treatments on injector wells
5. The issue of a final report
6. Implementation of technology transfer through publications, meetings, press releases, trade exhibits, and Internet postings

Potential Application or Utility of Results

The continuing discovery of the principles that govern manipulation of natural microbial populations opens a new field for applied biotechnology. The biocompetitive exclusion technology being developed for this project is one example. New opportunities which exploit this innovative technology are being developed as ramifications of its potential are revealed.

The biocompetitive exclusion mechanism of the Inject-Chek technology fosters the growth of a favorable microbial consortium at the expense of a detrimental microbial population. This selective alteration and manipulation of microbial populations are achieved by an improved understanding of the subsurface microbial ecology and its interrelationship with the natural fluid composition and characteristics. In particular, the Inject-Chek technology is designed to take advantage of and to incorporate the geochemical factors and nutrients already present in the environment. Thus, by subtle changes and additions, the modification and control of such factors will create a positive result. A partial list of the immediate and realistic targeted commercial opportunities is summarized below for illustrative purposes.

1. Removal and prevention of H_2S in producing wells
2. Increased production by iron sulfide (pyrite) removal

3. Increased oil production by tertiary oil recovery mechanisms

4. Treatment of gas storage reservoirs

5. Oil-water treatment in surface facilities

6. Improved steamflood operations

7. Oil-related contaminate removal

8. SRB-free anaerobic bioremediation applications

Benefits and Value

The use of the Inject-Chek treatment process for producing oil wells will lead not only to a reduction in the levels of sulfate-reducing bacteria (SRB), but also to a removal and prevention of H_2S and FeS, reducing corrosion. If the FeS is complexed with scale and/or paraffin, the use of the Inject-Chek treatment process can mobilize these well-plugging agents. Additionally, the beneficial replacement population, fostered by biocompetitive exclusion at the expense of the SRB population after Inject-Chek treatment, has the potential to increase oil production by traditional MEOR (microbial enhanced oil recovery) mechanisms.

It is estimated that the commercial impact and economic benefits resulting from the use of the Inject-Chek treatment process will be realized by the end of 1995.

Problems Encountered

A few problems have been encountered in the implementation of this field demonstration project. The most vexing problem initially was determining a means by which to judge the success of the Inject-Chek treatment process. This problem was overcome through the acquisition of a Port-A-Check instrument. This machine, which only very recently was made operational, has the ability to accurately measure total fluid production as well as the oil/water ratio for an individual, isolated well. This instrument gives far more accurate data than the traditional "bucket test."

The independent lease operators appear to be very cooperative, but often their field data are incomplete. The researchers at Phillips Petroleum Co. have been most helpful, and the only problem that remains is to convince their field operators that the Inject-Chek treatment process should be tried. The main obstacle/problem is

their unwillingness to forsake 3 days of production that would result from the 3-day shut-in period recommended for the treatment protocol.

Initially, GMT was also troubled by liability concerns so a "hold harmless" agreement is now instituted before field testing starts.

Conclusions

Although much work remains to be done in this 2-year field testing program, some conclusions can be made from prior laboratory work and the experience gained from the preliminary field testing that has already been performed. First, and most importantly, it should be noted that successful field treatments that ameliorate problems associated with sulfate-reducing bacteria and plugging agents will lead to the prevention of premature well abandonment. Also, it is clear that the Inject-Chek treatment process can promote MEOR mechanisms at less cost than traditional MEOR because the process requires no added microorganisms or added energy sources. Finally, when the Inject-Chek treatment process is successfully field proven, there will be a substantial commercial impact not only in marginally economic stripper wells, but also in other energy and environmental applications.

Table 1 Matoaka Lease—Lab Data Well #5

Salinity—**21%**	
VFA—Acetate, **30** ppm (2/25/95); **11** ppm (4/21/95)	
Sulfate—**99** ppm	
Microbiology—3/23/95	
SRB	10^1/ml
DN	$<10^1$/ml
GAB	10^1/ml
Microbiology—4/21/95	
SRB	10^1/ml
DN	$<10^1$/ml
GAB	10^1/ml

Table 2 Dunbar Lease-Lab Data Well #5

Date	Comment Well #5	Comment Injector
3/24/95	2 gallons IC/F backside, shut in	1 gallon IC/F
3/25/95	shut in	1 gallon IC to SWT
3/26/95	shut in	1 gallon IC/F
3/27/95	well turned on-line	1 gallon IC/F
3/28/95	increase in OWR: possible IC effect	1 gallon IC/F
3/29/95	shut in: 4 gallons IC/F backside	1 gallon IC/F
3/30/95	shut in	1 gallon IC/F
3/31/95	shut in	1 gallon IC/F
4/1/95	shut in	no treatment=NT
4/2/95	well on-line	NT
4/3/95	well on-line: OWR+, gas+	NT, no dye in SWT
4/4/95	oil cut approx. 30%, gas sat, hitting low	NT
4/5/95	good oil cut (10%), some gas, hitting low	1 gallon IC
4/6/95	5% cut, hitting low	1 gallon IC
4/7/95	well on-line	1 gallon IC
4/8/95	back to historical prod. 3 gal IC not SI	1 gallon IC
4/9/95	on-line	NT
4/10/95	on-line	NT
4/11/95	on-line, historical production	NT
4/12/95	well shut in no treatment, lease down	NT
4/19/95	lease back on	2.5 gallons IC daily to 6/6/95
5/12/95	lease shut in	
5/16/95	lease back on	
6/6/95		5.0 gallons IC daily to 6/24/95
6/24/95	Lease production halted indefinitely	IC halted
	IC = InjectChek	IC/F = InjectChek/Fluorescein

Laboratory Analysis

Salinity—13%

Acetate—64 ppm (2/25/95); 5 ppm (5/23/95); 0 ppm (6/21/95)

Sulfate—23 ppm (2/25/95); 74 ppm (6/24/95)

Microbiology—3/23/95

SRB	10^1/ml
DN	$<10^1$/ml
GAB	10^3/ml

Microbiology—4/21/95

SRB	10^1/ml
DN	10^3/ml
GAB	10^4/ml

Table 3 Berea Sandshore Coreflood IC Added 5/17/95

	Date	Sulfide In/Out	SO_4 In/Out	Flow Rate ml/min
	5/15		0.02	
	5/16	12.1/56.6	235/66	0.02
Start	5/17		258/50	0.02
	5/18		209/179	0.13
	5/19		276/185	0.05
Stop	5/20		279/279	0.05
	5/23	10.1/5.3	273/268	0.05
	5/26		267/245	0.05
	5/27		271/232	0.05
	5/31		234/146	0.05

	Date	SRB In/Out	GAB In/Out	Acetate In/Out
	5/15	$10^2/10^7$	$10^2/10^5$	265/195
	5/20	10^2/-	$10^2/10^6$	271/140

Figure 1 Port-a-Chek System

Strategies to Diagnose and Control Microbial Souring in Natural Gas Storage Reservoirs and Produced Water Systems

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Abstract

Hydrogen sulfide production (souring) in natural gas storage reservoirs and produced water systems is a safety and environmental problem that can lead to operational shutdown when local hydrogen sulfide standards are exceeded. Systems affected by microbial souring have historically been treated using biocides that target the general microbial community. However, requirements for more environmentally friendly solutions have led to treatment strategies in which sulfide production can be controlled with minimal impact to the system and environment. Some of these strategies are based on microbial and/or nutritional augmentation of the sour environment.

Through research sponsored by the Gas Research Institute (GRI) in Chicago, Illinois, methods have been developed for early detection of microbial souring in natural gas storage reservoirs, and a variety of mitigation strategies have been evaluated. The effectiveness of traditional biocide treatment in gas storage reservoirs was shown to depend heavily on the methods by which the chemical is applied. An innovative strategy using nitrate was tested and proved ideal for produced water and wastewater systems. Another strategy using elemental iodine was effective for sulfide control in evaporation ponds and is currently being tested in microbially sour natural gas storage wells.

Introduction

Hydrogen sulfide production can occur in a system through two fundamentally different mechanisms. Microbial souring results from current biological activity, most commonly involving sulfate-reducing bacteria (SRB). Microbial souring may also result from the breakdown of organic matter containing sulfur and through the activities of sulfur-reducing bacteria. In contrast, nonbiological souring is not due to current microbiological activity but is a result of chemical or geochemical reactions, or possibly the release of hydrogen sulfide that was microbially produced in ancient times.

To remediate souring, it is important to diagnose the underlying cause of the problem. Typically, microbial souring increases in severity with time. Therefore, remedies based on sulfide removal, such as oxidants and scavengers, may serve only to mask the problem while sulfide-producing microbial communities proliferate in the system. In contrast, if the problem is nonbiological, remedies based on microbiological control would be a waste of resource.

Microbial souring may be encountered in a variety of industrial environments. Economically, souring in subsurface oil and gas reservoirs may be the most serious of the problems encountered due to the difficulties associated with effective treatment and the potential loss of revenue due to compliance-related shutdown. Other industrial systems that have suffered from microbial souring include wastewater retention lagoons, evaporation ponds and tanks,[1] sewage treatment and carriage systems,[2] and potable water aquifers.[3]

Diagnosis of Microbial Souring

A proper diagnosis of microbial souring should be based on the detection of microbes associated with hydrogen sulfide production and a chemical characterization of the affected environment.[4] A confirmed diagnosis is particularly important in applications where the souring mechanism can be easily misunderstood. For example, souring in subsurface environments may be biological, nonbiological, or a combination of both mechanisms. The SRB are frequently found in sweet and sour environments. Therefore, their presence in subsurface reservoirs cannot be the sole determinant for souring. Surface systems also need biological and chemical characterization to determine the souring mechanism; however, the diagnostic process may be relatively simple as compared with a reservoir problem. Finally, in systems where microbial souring is obvious (e.g., sewage applications), a formal diagnosis of the souring mechanism may not be as important as finding ways to mitigate the problem while staying within

allowable system operating parameters. In the following discussion, some strategies for diagnosing microbial souring in natural gas storage reservoirs and surface-operated produced-water systems are described.

Subsurface Natural Gas Storage Reservoirs

Microbial souring is common in subsurface natural gas storage reservoirs which store gas in aquifers. The movement of groundwater in the reservoir as gas is injected and withdrawn is thought to play an important role in supplying fresh nutrients to the microbial community that produces sulfide.[5] Natural gas storage in depleted oil reservoirs which often have had water introduced for secondary oil recovery are also prone to microbial souring. Reservoirs in which gas is injected and withdrawn seasonally (i.e., summer injection and winter withdrawal) are more straightforward to diagnose than those in which gas is injected and withdrawn upon demand.

The first step in diagnosing the cause of souring in a subsurface natural gas storage reservoir is to review available information to determine if microbial activity in the reservoir is probable. Fundamental information includes the reservoir temperature and sulfide levels in the aqueous and gas phases. High temperatures, 250°F or greater, are not consistent with the occurrence of microbial souring. In investigations of hyperthermophilic bacteria from natural environments, SRB have been identified that can tolerate temperatures up to 203°F with optimal growth occurring at 181°F.[6] Sulfide is toxic to most bacteria, including SRB. Therefore, reservoirs that produce high concentrations of hydrogen sulfide (e.g., > 10%) are not likely due to SRB activity, because the SRB would become inhibited at these levels. Chemical data on the natural formation water can indicate whether the reservoir is prone to microbial souring. Specifically, high levels of sulfate, iron, and bicarbonate alkalinity are conducive to the growth of microbial colonies which can include SRB.

Operational information can be used to further assess the likelihood that microbial souring is occurring in a reservoir. The use of nontreated production fluids, such as drilling mud or fracturing agents, and the practice of injecting nontreated water may introduce bacteria and nutrients into the reservoir that can result in microbial souring.

Sulfide-production data of individual wells can be useful in determining if the souring problem is microbial and, if so, indicate how advanced the condition has become. In gas-storage reservoirs, microbial souring is initially local to affected wells and gradually spreads out into the formation. In the early stage condition, it is common to find sweet and sour wells of comparable completion depths in close

surface proximity (see Fig. 1). In contrast, reservoirs having nonbiological souring are often characterized by having all wells sour, with each well producing comparable levels of sulfide each year. If microbial souring reaches an advanced stage, all wells in the reservoir may be sour (as in the nonbiological case), but the sulfide production will continue to increase each year (see Fig. 2). Upon determining that microbial souring is possible, the operator should proceed with an organized field investigation as described below.

Field Data Collection. A souring assessment should be based on data that are as representative of the environment sampled as possible. The person doing the assessment should be cautious of any outside influences at the site that may confound the results. In natural gas storage reservoirs, the best available sampling points are usually at the wellheads, although downhole samples may occasionally be collected and compared to the wellhead data. If both sweet and sour wells are available for sampling, a minimum of 10 wells of each type is recommended for a statistically valid data set. If all wells are sour, a minimum of 20 wells should be assessed to determine if elevated sulfide levels are associated with the presence of SRB.

In sweet and sour environments of otherwise similar characteristics, the bacteria levels are usually higher by at least one order of magnitude in the sour environment. The acid-producing bacteria (APB) are usually detected with SRB because the organic acids they produce are utilized by the SRB as nutrients. The criteria for classifying sweet and sour wells is somewhat arbitrary. The authors have established and followed a guideline of sweet wells having, on a seasonal average (four months of gas withdrawal), less than one ppm hydrogen sulfide in the gas and less than 50 mg/l total sulfide in the water. Wells not meeting this criteria are classified as sour for the purposes of the assessment. Wells which are sour solely due to sulfides in the water may exhibit some downhole hydrogen sulfide scavenging capability, commonly afforded by metals such as iron and manganese. If the metals are from the well components, the scavenging capability will likely be short-lived. If there is a naturally occurring source of metals, the scavenging capability may last for years until the sulfide production grows to exceed the scavenging capability of the reservoir. When this happens, the transition to an advanced condition of microbial souring may occur relatively quickly.[4]

Sampling of the wells should be done at least once per month for an entire gas-withdrawal season, or for four months if gas withdrawal is nonseasonal. Microbial analyses should include tests for viable SRB and APB. The samples for viable bacteria should be processed on site to avoid changes which may otherwise result while in transit. The samples can be easily processed on site using commercially available microbiological test kits. Quantitative chemical analyses for acid-soluble sulfate and total phosphate, sulfide, iron, and manganese should be performed on

the water samples. The hydrogen sulfide level of the gas should be measured on site using a commercially available gas detector.

Data Analyses and Interpretation. The first step in the analyses is to review the sulfide results and sort the viable bacteria data into sweet and sour groups. The bacteria levels can best be evaluated in $\log_{10}$. If the reservoir has microbial souring, the mean bacteria level of the sour wells should be at least one order of magnitude higher than the level of the sweet group. If all of the wells are sour, an assessment can be done based on the presence or absence of SRB. If all wells have SRB, the chemical results will have to be evaluated to determine the souring mechanism. If all wells are sour, but a significant number have no SRB, and the levels of SRB are not positively correlated with sulfide levels, the problem is likely to have significant nonbiological component. In this case, the sulfur isotope test can provide confirmation.

The chemical results are best interpreted with respect to time, in conjunction with the viable bacteria levels. Figure 3 shows the correlations among water production, SRB, nutrients, and sulfide. The onset of water production, which indicates ingress in the subsurface environment, has an important role in stimulating bacterial growth. However, once the water is no longer limiting to their growth, it has little residual effect. As the SRB reach a peak in level, the sulfate and phosphate, which are consumed by the bacteria, diminish in level. The sulfide level in the water is highly correlated with the SRB level. The peak in hydrogen sulfide level in the gas follows the sulfide in the water by one month, possibly due to the natural sulfide scavenging capability in the reservoir. The tight correlations seen in Figure 3 are indicative of a reservoir that is predominately affected by microbial souring. In reservoirs where microbial and nonbiological souring may be working in concert, these correlations may be observed to a lesser degree.

Sulfur isotope analyses can be done to confirm or refute the results of microbiological and chemical analyses. The test is based on the fact that SRB, given an abundance of sulfate containing a mixture of sulfur isotopes, will select for the lighter ^{32}S. This phenomenon, called biological fractionation, results in depleted levels of ^{34}S in the gas phase, relative to the water phase. Abiotic chemical reactions that reduce sulfate to sulfide are not similarly affected. This test is relatively time-consuming and expensive, and therefore, is not normally considered necessary when the other data clearly show that microbial souring is the predominate mechanism. The sulfur isotope analysis is best utilized when little correlation is found in the data and the mechanism appears to be nonbiological.

Surface Systems

The diagnosis of microbial souring is relatively straightforward in surface systems as compared to natural gas storage reservoirs; nevertheless, the problem is frequently encountered in the industry. Some public wastewater facilities have stringent regulations on the total sulfide content of wastewater discharged to the system by the user. High-sulfate waters retained in closed tanks without microbial control are prone to microbial souring as system conditions go anaerobic. As in reservoir souring, it is important to consider the obvious factors that would preclude microbial souring as a possibility: system temperature, pH, and any other factors (e.g., chemical treatment) that would inhibit microbial growth.

Surface systems suspected of microbial souring should be tested at influent, mid-system, and effluent points for viable levels of SRB, sulfate, and sulfide. If microbial souring is occurring, a drop in sulfate and an increase in sulfide, from influent to effluent, should be measurable. In addition, viable SRB should be present in the mid-system effluent, and possibly in the influent. Sometimes systems receive sulfide-laden water from points upstream. In such cases, the SRB, sulfide, and sulfate levels may not materially change from influent to effluent of the system. To properly diagnose this scenario, the assessment must be taken closer to the source of the sulfide.

Mitigation of Microbial Souring

Microbial souring may be mitigated using a variety of approaches. The accessibility of the affected portions of the system and the restrictions on types of chemicals that can be applied are fundamental considerations in choosing a mitigation strategy. When the source of the problem is too remote for effective treatment, the approach may focus on treating symptoms rather than the cause and may involve sulfide scavengers or oxidants. In subsurface reservoirs, detecting the problem early, before colonization spreads beyond the immediate regions of the wellbores, is critical to success. In systems where toxicity is restricted, chlorine, bromine, or iodine may be prescribed in a manner in which the free residual of chemical is maintained at safe levels while achieving microbial control. In the discussion that follows, mitigation strategies employed in natural gas aquifer-storage reservoirs and produced-water tank farms are described.

Natural Gas Aquifer-Storage Reservoir

An aquifer-storage reservoir was diagnosed as having early stage microbial souring, and downhole biocide treatment had not previously been practiced. The

formation water produced during gas withdrawal was discharged to a municipal wastewater plant having toxicity limitations on the influent. If a biocide was used, it would be important to deactivate any toxic residual in the produced water prior to discharge. The compatibility of the biocide with the formation was also important, to prevent formation plugging as a result of polymer formation or precipitation. Finally, the effectiveness of the biocide for controlling the specific types of bacteria in the reservoir needed to be proven prior to starting the treatment program.

Laboratory tests were done on a biocide formulated of 25% glutaraldehyde to determine the compatibility, effective kill, and persistence in the formation water. The compatibility test was essentially a check for loss in filterability, done according to the National Association of Corrosion Engineers' NACE TN-01-73 method. Effectiveness testing had previously been done at an analog reservoir (same formation, types of bacteria, and microbially sour) whereby levels of 50 ppm active glutaraldehyde were shown to provide effective kill. The persistence test was also done at the analog reservoir, and the results showed that active glutaraldehyde could persist for more than six months in the formation water.

The persistence of glutaraldehyde in the formation water created a need for an in-field deactivation method. Tests of several methods proved that elevating the pH to above 11 using caustic and mixing for at least 48 hours, followed by neutralization with mineral acid, was the most practical and economical approach.[7] The deactivated wastewater contained a nontoxic condensation-reaction product of glutaraldehyde which is essentially nonreversible.

First-Year Treatment. The initial treatment was done in the last month of the summer gas-injection season. Gas withdrawal generally occurs two to three months thereafter. The wells received one to two barrels (55 gal) of the 25% glutaraldehyde formulation as per the instructions of the biocide vendor. Wells which had higher than average sulfide and water production were given the larger amount. The biocide was followed by a quantity of potable water (overflush) to disperse the chemical into the formation. The amount of overflush was arbitrarily set to 500 gal per well. In the subsequent gas-withdrawal season, produced water samples collected from the wellheads of treated and nontreated wells were tested for levels of viable APB and SRB, glutaraldehyde residual, and sulfide. Mean seasonal levels of hydrogen sulfide in the gas in the year prior to treatment were compared to the year after treatment to assess the overall effectiveness of the treatment program.

The results from wellhead monitoring showed that the treatment was not effective in reducing sulfide production. Figure 4 shows that the mean seasonal hydrogen sulfide levels in the gas of both treated and nontreated wells increased after biocide treatment. However, in water samples where the glutaraldehyde level was more than 50 ppm, good microbial control was achieved. Based on these results, it was

concluded that the biocide, although highly effective against the bacteria, was not dispersed far enough from the wellbores to effectively control souring in the reservoir. A modified treatment plan was implemented in the subsequent year.

Second-Year Treatment. The treatment approach in the second year focused on getting better dispersal of the biocide into the formation. Physical parameters including perforation thickness, rathole depth, and tube or casing diameter were accounted for in determining the amount of biocide and overflush to apply. To provide for greater dispersion and longer contact time, the treatment was applied three times during the gas-injection season; early, middle, and late summer. The amount of biocide applied per treatment was reduced to one-third (i.e., 18–36 gal) of the previous year, while the amount of overflush was increased by a factor of four. To assist in moving the treatment solution away from the wellbore, gas was injected into the treated wells with surrounding wells blocked in. Monitoring of treated and nontreated wells during the subsequent gas-withdrawal season was done as in the prior year.

Figure 5 shows the progressive increase in hydrogen sulfide levels in the nontreated control wells over the three-year period which encompasses the year before treatment, the year after the first treatment of the test wells, and the year after the second treatment. The progression in hydrogen sulfide level is consistent with the expanding microbial community in the subsurface. These data serve as a baseline from which to compare the treated wells.

Figure 6 shows the effects of both biocide treatments of the test wells as compared to the year before treatment. The first treatment was ineffective and was followed by an increase in hydrogen sulfide levels, comparable to that seen in the nontreated group. The year following the second treatment was characterized by a drop in hydrogen sulfide levels in most wells, with only one well (well #10) showing a significant increase. Well #10 was anomalous in that it did not produce enough water to sample, and therefore, the level of biocide present in the downhole environment could not be determined. These results, coupled with reduced aqueous sulfide levels and positive microbial control, proved the amended approach to treatment successful. In addition, the increased overflush and reduced amount of biocide per treatment helped reduce the levels of biocide waste generated during gas withdrawal.

Iodine Treatment. The use of elemental iodine proved successful for remediating microbial souring in evaporation ponds containing produced water.[1] In subsequent laboratory tests, a potential was realized for the use of iodine for remediating microbially sour natural gas storage wells. The chemical basis for the strategy is as follows:

$$I_2 + H_2S \rightarrow S + 2HI \quad (1)$$

An advantage to this approach is iodine's low toxicity to higher organisms at its solubility limit in water (330 mg/l). However, dissolved iodine and iodide (I^-) offer antimicrobial qualities at much smaller concentrations. Due to the limited solubility in water, solid iodine can have a relatively long persistence as the particles slowly dissolve. Hydrogen sulfide increases the solubility of iodine which serves to expedite its delivery to the environment when needed.

Two natural gas storage wells were treated using a solution of 3% iodine dissolved in isopropyl alcohol. The iodine treatment was designed to be a cost-equivalent alternative to the glutaraldehyde treatment used in other wells in the same field. The solution was applied downhole and immediately followed with fresh water. When the iodine/alcohol solution contacts water, ultra-fine particles of elemental iodine drop out and are deposited into the formation. The treatment was done twice during the summer gas-injection season. These particles act as a time-release mechanism for delivering iodine to the sour environment. Monitoring of produced water collected at the wellheads showed that iodide, the product of iodine and hydrogen sulfide, was detectable for up to six months after the treatment.

Figure 7 shows the history of hydrogen sulfide production in the two wells treated with iodine. In the years prior to 1994, the wells had been treated with glutaraldehyde. The significant reduction in mean hydrogen sulfide levels after the iodine treatment prompted a second year of testing.

Produced-Water Tank Farm

A natural gas storage facility utilized a depleted sour-crude oil reservoir for natural gas storage. The formation temperature was in excess of 220°F, and no viable bacteria were recoverable, which indicated that the souring mechanism was nonbiological. During gas withdrawal, sulfide-laden water was produced and treated with an oxidant (chlorine dioxide) to lower the aqueous sulfide levels to no greater than 0.1 mg/l as required by the receiving wastewater facility. However, upon retention of the treated water in closed tanks, bacteria and recurrent sulfide production were encountered. Thus, two mechanisms were identified which resulted in the water exceeding the sulfide specification: 1) incoming sulfide from

the depleted oil field, and 2) microbial souring in the retention tanks. Biocides were not a feasible solution to this problem due to the nonbiological component of the problem and the toxicity restrictions imposed by the receiving wastewater plant. Figure 8 shows the layout and flow associated with the tank farm.

The problem was solved by adding sodium nitrate to the retention tanks on the effluent side of the tank farm. The nitrate stimulated the growth of nitrate-reducing (denitrifying) bacteria which have a competitive advantage over SRB.[8] As the denitrifying bacteria outcompete the SRB, the microbial community shifts from a mode of sulfate reduction to nitrate reduction. In the process of reducing nitrate, nitrite is formed. Nitrite is an oxidant that is effective in lowering sulfide levels. In addition, a naturally occurring denitrifying bacterium is also a sulfide oxidizer (*Thiobacillus denitrificans*) and can be effective in remediating souring problems.[9] In short, the nitrate-based treatment strategy proved effective in controlling microbial and nonbiological souring without increasing the toxicity of the wastewater.

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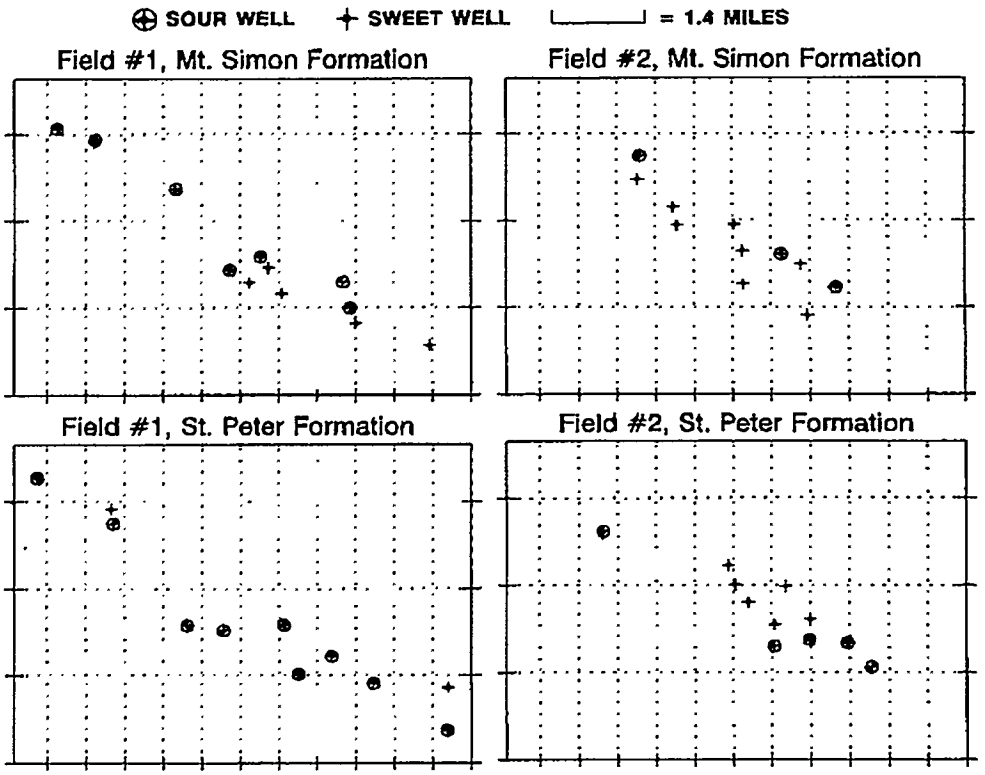


Figure 1 Sweet and Sour Wells in Reservoir with Early Stage Microbial Souring

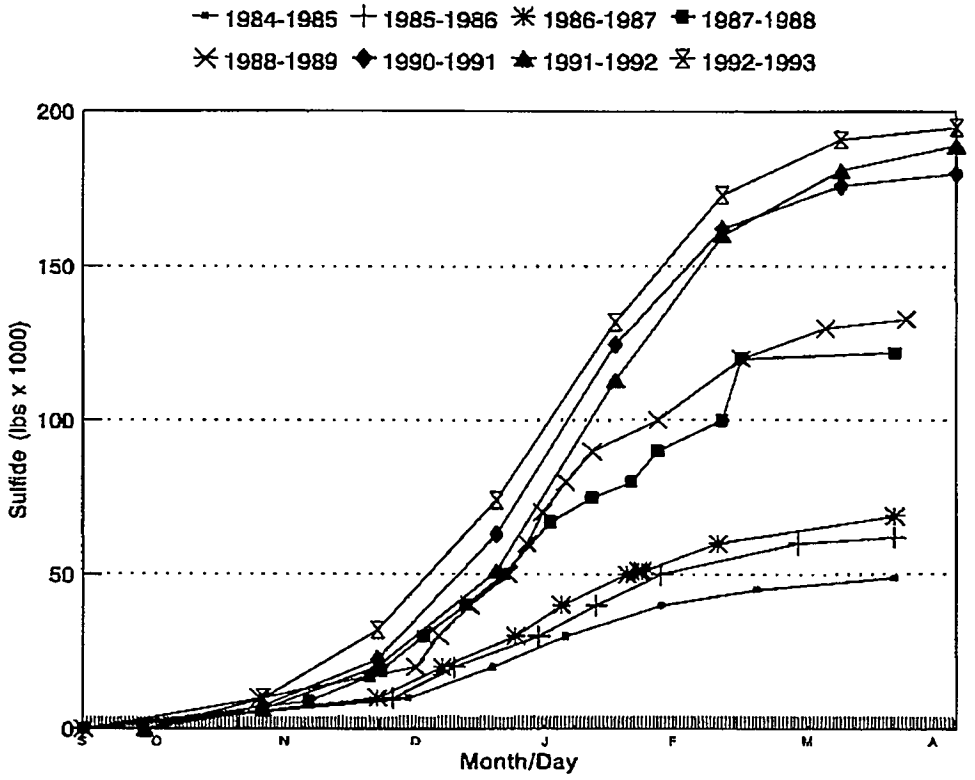


Figure 2 Increase in Sulfide Production in Well with Advanced Microbial Souring

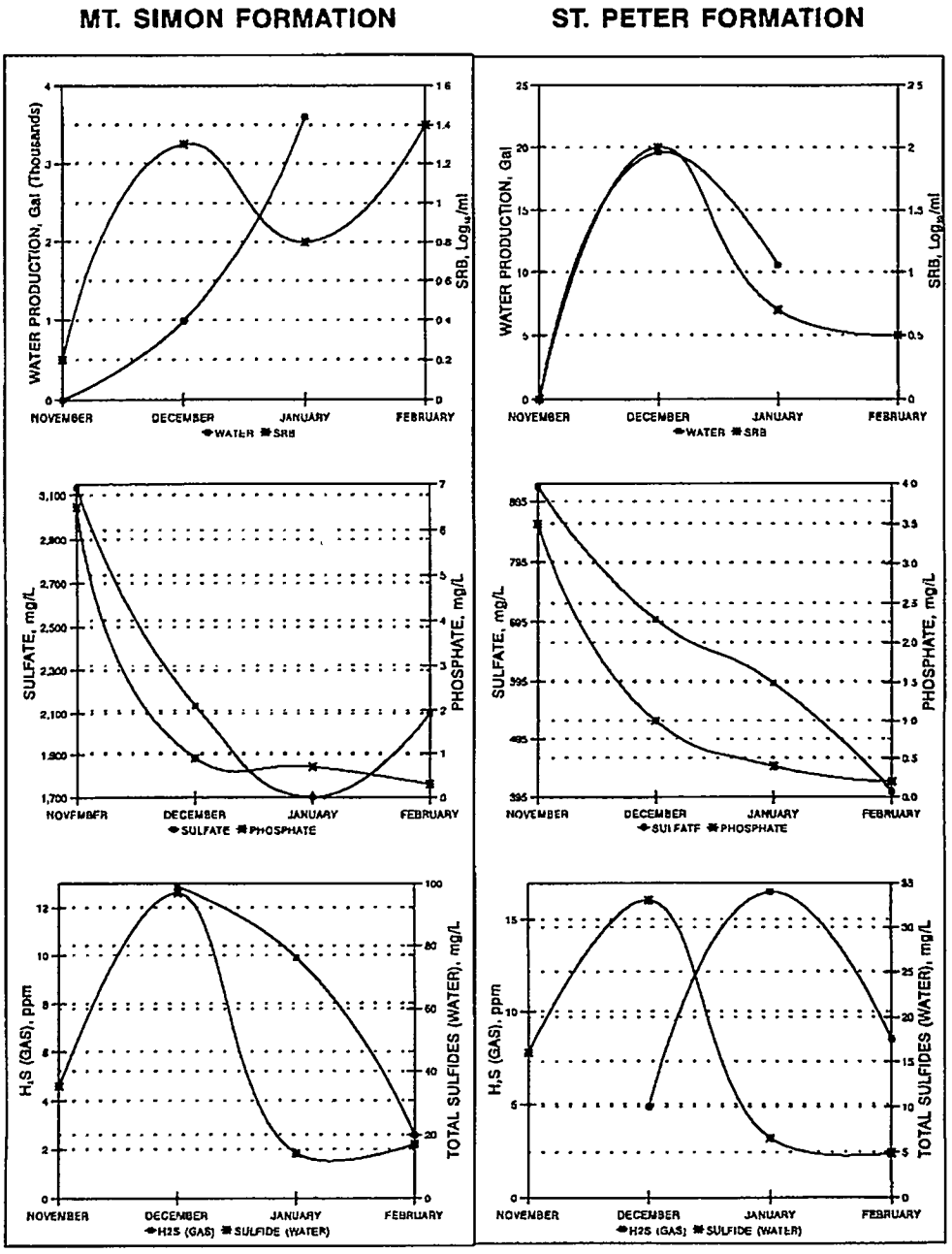


Figure 3 Correlating Parameters in Microbial Souring

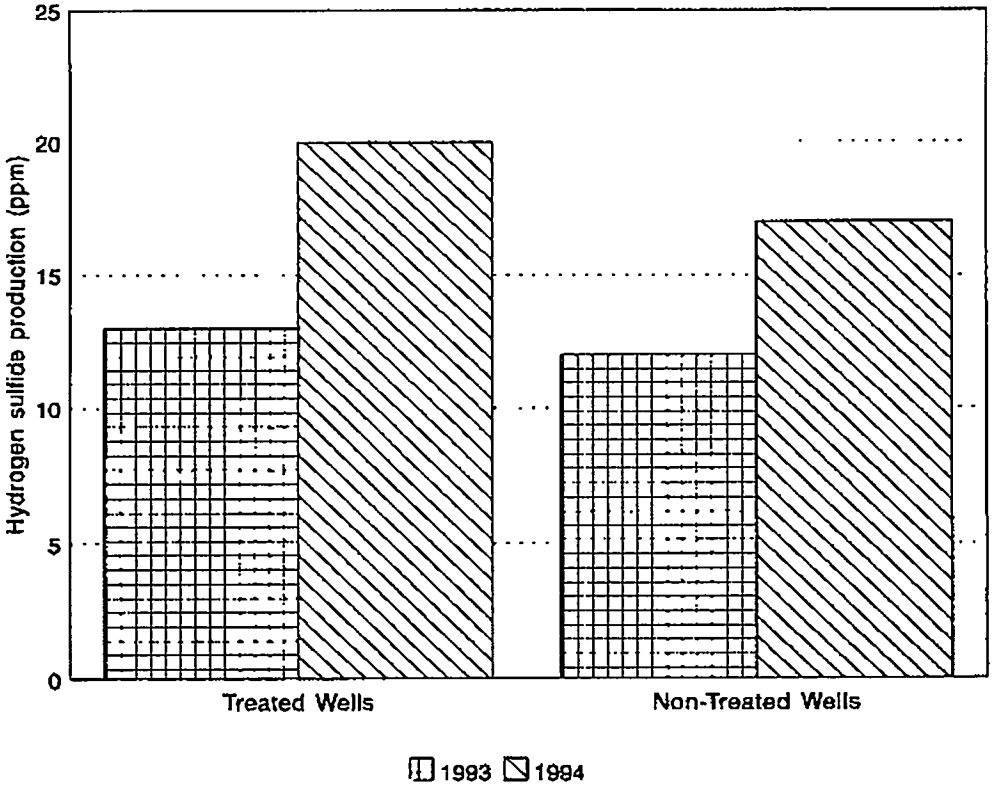
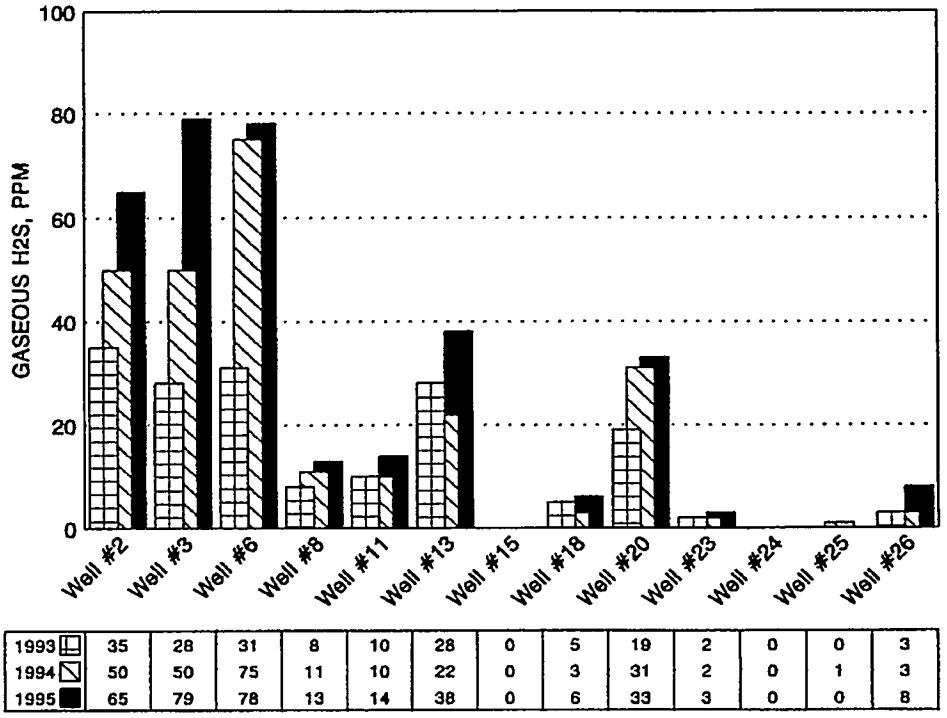


Figure 4 Change in Mean Hydrogen Sulfide Levels in Treated and Nontreated Wells before and after Initial Biocide Treatment



	Well #2	Well #3	Well #6	Well #8	Well #11	Well #13	Well #15	Well #18	Well #20	Well #23	Well #24	Well #25	Well #26
1993	35	28	31	8	10	28	0	5	19	2	0	0	3
1994	50	50	75	11	10	22	0	3	31	2	0	1	3
1995	65	79	78	13	14	38	0	6	33	3	0	0	8

Figure 5 Increase in Mean Hydrogen Sulfide Levels in Nontreated (Control) Wells, 1993–1995

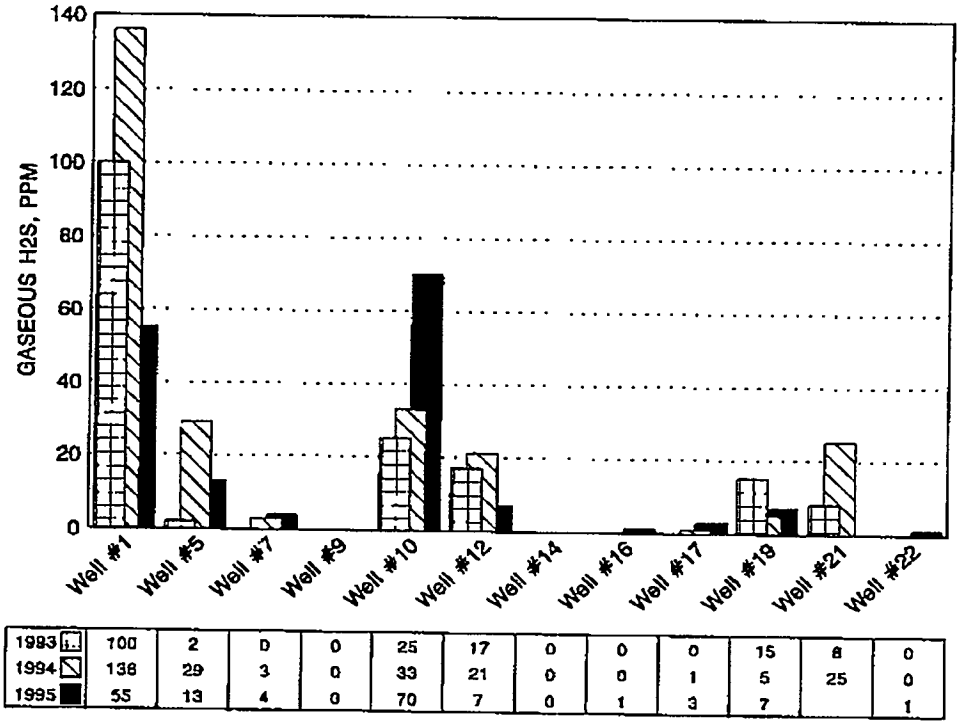


Figure 6 Mean Hydrogen Sulfide Levels in Treated Wells. 1993—No Treatment, 1994—after First Treatment, 1995—after Second Treatment

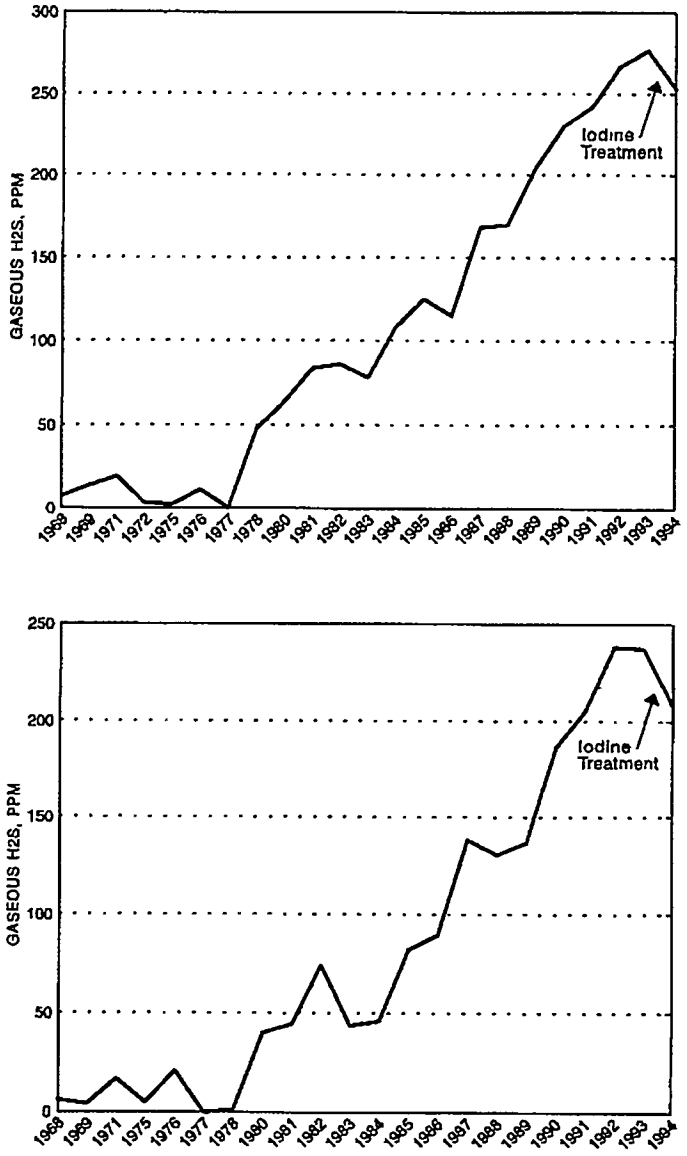


Figure 7 History of Hydrogen Sulfide Production in Iodine Test Wells

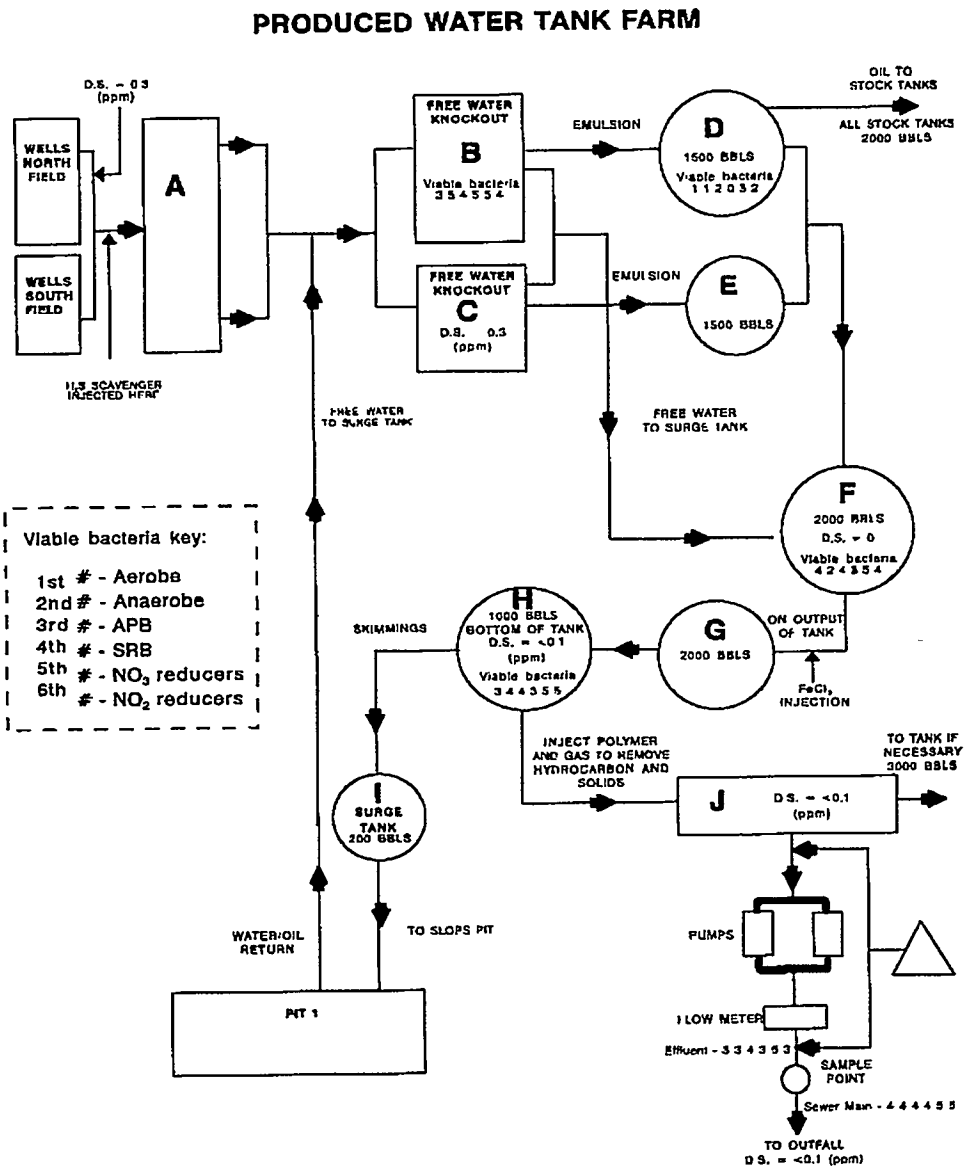


Figure 8 Produced Water Tank Farm

Control of Microbially Generated Hydrogen Sulfide in Produced Waters

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Abstract

Production of hydrogen sulfide in produced waters due to the activity of sulfate-reducing bacteria (SRB) is a potentially serious problem. The hydrogen sulfide is not only a safety and environmental concern, it also contributes to corrosion, solids formation, a reduction in produced oil and gas values, and limitations on water discharge. Waters produced from seawater-flooded reservoirs typically contain all of the nutrients required to support SRB metabolism. Surface processing facilities provide a favorable environment in which SRB flourish, converting water-borne nutrients into biomass and H_2S. This paper will present results from a field trial in which a new technology for the biochemical control of SRB metabolism was successfully applied. A slip stream of water downstream of separators on a produced water handling facility was routed through a bioreactor in a side-steam device where microbial growth was allowed to develop fully. This slip stream was then treated with slug doses of two forms of a proprietary, nonbiocidal metabolic modifier. Results indicated that H_2S production was halted almost immediately and that the residual effect of the treatment lasted for well over one week.

Introduction

The activity of sulfate-reducing bacteria (SRB) in oil field installations has been associated with operational problems, including enhanced corrosion rates, production of oil-wet iron sulfide sludge, and possible exposure of personnel to the toxic hydrogen sulfide (H_2S) gas.

An example of a site with these problems is an onshore oil terminal which processes oil, water, and gas from offshore production platforms. Figure 1 is a schematic diagram of the terminal showing the facility components required to separate the three-phase fluid prior to sales (oil and gas) or discharge (water). This terminal treats about one million barrels of oil plus about 20,000 barrels of produced water per day. Most of the water produced from wells with the oil had been separated from the produced fluids and discharged on the offshore platforms prior to transport to shore. The remaining produced water is treated in three stages of separators and then filtered to remove trace amounts of hydrocarbons to below the specified discharge consent level. Throughout the water phase in the terminal system, viable SRB and H_2S have been detected.

The presence of H_2S in separated water has been an operational problem at the terminal for some years. Several attempts have been made to control the problem by treating with biocides such as glutaraldehyde and isothiazalones in an effort to kill sulfate-reducing bacteria (SRB), presumably the agent of H_2S generation. Such treatments have had limited, if any, effect upon H_2S levels in the plant. The final discharge of the separated and diluted produced water to a local river has imposed constraints on the selection of the chemical treatments used to attempt control. In the case of glutaraldehyde, for example, a final neutralization stage was adopted prior to final discharge.

The aims of the present work were to:

- Quantify the role of SRB in generating H_2S in the terminal
- Assess the efficacy of a novel chemical nonbiocidal SRB inhibitor which would minimize the environmental impact of the final discharge

Previous research has shown that sulfate respiration in sulfate-reducing bacteria can be inhibited by unsubstituted 9,10-anthraquinone (referred to in this report as anthraquinone) and many of its derivatives, resulting in the cessation of H_2S production.[1] The underlying mechanism of inhibition appears to be the uncoupling of electron transfer from adenosine triphosphate (ATP) synthesis via

anthraquinone-mediated electron transfer reactions.[2] This concept, using products containing different forms of anthraquinone, was tested in the present study.

One product contained a water-dispersion of the insoluble anthraquinone while the second contained a soluble form stable in a caustic solution, but which ultimately reverts back to biochemically active anthraquinone particles under reduced pH and/or oxidizing conditions. These products are marketed by Environmental Biocontrol, International as Sulf Control® and Super Sulf Control®, respectively.

Anthraquinone is relatively benign compared to typical oil field biocides. Results of ecotoxicological studies performed on Super Sulf Control® are summarized in Table 1. Shown also are Nordic and EEC toxicity classifications[3,4] and previously reported toxicity levels for chemicals being used offshore.[5]

Materials and Methods

Side Stream Devices

Side stream devices were installed at the oil terminal in order to quantify microbiological sulfide generation and the effects of anthraquinone treatments. Previous work had demonstrated that the effluent from the terminal's surge tanks would be a good location for the side stream devices, since downstream from this point sulfide concentration and bacterial populations increased. As shown in Figure 1, three side stream devices were installed in parallel, each taking a water supply from the surge tanks and discharging to the API separator via the site drainage system. One side stream was reserved as an untreated control to which the two chemically treated side streams could be compared.

The side streams were mounted on a skid as shown in Figure 2. Each consisted of a flow control and chemical injection port, a holder for cylindrical microbiological test surfaces (mild steel coupons), an upward flow packed bed bioreactor and an effluent sampling port. The bioreactors consisted of 15 cm diameter, 1.8 m high food grade plastic pipe packed with 1.6-cm polypropylene pall rings. This packing provided a surface area of 319 m^2/m^3 of reactor volume. Each bioreactor had a volume of 27 liters, a porosity of 90%, and a calculated total internal surface area of 9.48 m^2. The water from the surge tanks, mainly produced water and site drainage water, flowed at a rate of 0.65 liters/m to each side stream device, first through the 2-mm annulus between the microbiological coupons and holder and then to the bioreactor. The hydraulic retention time in the bioreactor was 42 minutes.

Three months were allowed between commissioning the side stream devices and the initiation of tests. During this time water from the surge tanks was flowed continuously through the side streams in order to establish a population of bacteria on the inner surfaces.

Microbiological Monitoring

Monitoring of both planktonic and sessile populations was carried out by serial dilution for SRB in the growth medium shown in Table 2. All culture bottles were incubated for 28 days at 30°C, close to the system temperature (23°C to 37°C), prior to quantifying growth. Positive SRB growth was determined by the appearance of black iron sulfide.

Planktonic monitoring was achieved by inoculating 1 ml of sample directly into the dilution series. Sessile monitoring was achieved by first scraping the cylindrical test surface with a sterile scalpel into 10 ml of sterile maximum recovery diluent (Difco). After vigorous shaking to disperse the bacterial cells, 1 ml of the diluent was inoculated into the dilution series.

Chemical Analyses and Other Measurements

Sulfide. Samples for sulfide analysis were fixed by adding 5 ml of 2% zinc acetate solution to 5 ml of sample. The concentration of sulfide was determined by the methylene blue reaction.[6]

Volatile Fatty Acids. Samples for analysis of volatile fatty acids (VFA) were fixed by adding 1 ml of concentrated hydrochloric acid to 150 ml of sample. The concentration of VFA was determined by ion exclusion chromatography.

Eh, pH, and Temperature. Readings of Eh, pH, and temperature of the feed and effluent from each of the side streams were made on-site using a portable meter (Testo Ltd.) connected to a platinum combination electrode (Russell pH Ltd.) and a temperature-compensated pH electrode (Testo Ltd.). The mV readings from the platinum electrode were converted to Eh values by adding 194 mV.

Preparation of Chemicals for Injection

Super Sulf Control®, containing a water-soluble form of anthraquinone, is pH and oxygen sensitive and must be kept in an anaerobic caustic solution (pH>12) to maintain the anthraquinone in solution. Dilution of the product from the supplied

22% anthraquinone to 0.6% was required due to the pump handling capacity. Dilution was carried out in an aspirator completely filled (5.25 liter) with city water to which 2.1 g sodium hydroxide (to increase the pH) and 0.78 g sodium sulfite (to scavenge dissolved oxygen) had been added. After standing for 30 minutes to ensure minimal dissolved oxygen remained, 145.5 ml of water was removed and 145.5 ml of Super Sulf Control® was added. A large-bore syringe was used for the liquid transfers to minimize oxygen ingress. The aspirator was sealed prior to using the chemical, and the contents were mixed by rolling. The stability of the red coloration of the solution indicated that oxidation had not taken place prior to injection into the side stream.

Regular Sulf Control® is neither pH nor oxygen sensitive. Dilutions were made in city water with no additional precautions other than to shake the aspirator well in order to disperse the chemical before and during injection.

Results

Two trials were carried out. The first, between 17–24 October 1994, was intended to provide preliminary short-term results and to test the sampling and analytical protocols. The second trial, between 7–24 November, provided information on longer term effects and residual effects after the termination of treatments. During each trial one side stream device was used as an untreated control, and one was treated with the anthraquinone products.

Trial 1

Influent Water. During the trial the temperature of the water feeding the side streams (effluent from the surge tanks) varied between 27.2°C and 34.0°C. The pH varied between 5.6 and 5.9, and the Eh ranged between –90 to –25 mV. The typical composition of this water is given in Table 3.

Side Stream Effluents. During treatment periods the pH of the effluent tended to be lower than the inlet, by up to 0.1 pH unit. However, the effluent from the untreated side stream had a pH slightly higher than the inlet, but typically by less than 0.1 pH unit. The Eh of the effluents from all of the side streams was very similar to that of the feed water.

Control Sulfide Productivity. As shown in Figure 3, the sulfide concentration in the outlet of the surge tanks (the feed to the side stream devices) varied between 11.8 and 22.9 mg/l during the trial. The sulfide concentration was always greater in the water which passed through the control bioreactor. This increase in sulfide

concentration is attributed to the activity of the SRB population which had colonized the inner surfaces of the bioreactor.

Anthraquinone Treatments. Two water-soluble anthraquinone treatments were made during Trial 1. The effects of these treatments are shown in Figure 4, where the sulfide productivity is plotted in terms of mg sulfide produced per m^2 of bioreactor surface area per day. The initial treatment with 100 mg/l active anthraquinone for 1 hour was effective in inhibiting sulfide productivity, relative to the untreated side stream, for approximately 25 hours. The maximum inhibition observed was 70%. The second treatment, 200 mg/l active anthraquinone for 2 hr, was effective in inhibiting sulfide productivity for at least 40 hr relative to the untreated side stream. During this time the maximum inhibition was 84%.

Volatile Fatty Acids. Samples of water for VFA analysis were taken on two occasions: 1) prior to any chemical treatments, and 2) near the end of trial 1. Acetate levels in the influent water were 470 and 265 mg/l, respectively, while propionate and butyrate levels were below 10 mg/l during both sampling periods. Analyses of effluent water samples indicated that, with and without treatments, slight increases in VFA concentrations occurred across the bioreactor.

Bacterial Populations. The population density of planktonic SRB in the bulk phase of the influent water to the side streams was 10^6 to 10^7 per ml. The bacterial populations in the effluents were very similar to those in the feed water. The population density of sessile SRB on the coupons in the side stream devices ranged between 2.5×10^6 and 2.5×10^9 per cm^2. During the treatments the average population densities of sessile SRB were similar in the untreated and the anthraquinone-treated side streams.

Trial 2

Influent Water. During Trial 2, the temperature of the feed water to the side stream devices ranged between 23.5°C and 37.3°C, and the pH varied between 5.8 and 6.4. The Eh varied between –9 mV and –96 mV. As in Trial 1, the sulfide concentration in the feed to the side stream devices was not constant, with the inlet sulfide concentration varying from 9.6 mg/l to 31.0 mg/l as shown in Figure 5. The observed variability in sulfide concentration is likely to be a result of fluctuations in inputs into the plant and possibly changes in microbiological sulfide generation in the plant upstream of the surge tank outlet.

Sulfide Productivity. Figure 5 emphasizes that significant bacterial sulfate generation took place in the side stream devices. The sulfide concentration in the outlet of the control side stream was always higher than the influent concentration.

The maximum sulfide production across the control side stream device was 43 mg/l. Note that the rate of sulfide generation was higher in Trial 2 than in the first. The pH of the effluent water was higher than the influent water by up to 0.2 pH units. The Eh readings of the influent and effluent streams were very similar.

Anthraquinone Treatments. During Trial 2, three one-hr treatments with 400 mg/l anthraquinone were made. As shown in Figure 6, the first treatment with water-soluble anthraquinone had a marked effect in inhibiting sulfide productivity. This effect was observed for at least four days, during which the maximum inhibition achieved was close to 100%. A subsequent treatment with the water-dispersible (insoluble) anthraquinone was made four days following the first treatment. As a result of these two treatments, the inhibitory effect lasted for approximately 9 days. When the sulfide had returned to a level close to that in the control side stream, a second treatment with water-dispersible anthraquinone was carried out. The effect of this treatment was slight, suggesting that the soluble anthraquinone treatment was mainly responsible for the previous good result.

The pH of the effluent stream was greater than the influent stream by less than 0.1 pH units, and the Eh of the effluent was very similar to that of the influent stream.

Bacterial Populations. During Trial 2, only the sessile populations were monitored. They were similar to those found in Trial 1: 10^5 to 10^9 per cm^2.

Discussion

The side stream devices were designed and operated to maximize the microbiological production of sulfide. This was necessary to provide a demonstrable baseline of activity to which the effects of chemical treatments could be compared. The observed sulfide generation is believed to be representative of a worst case, with the areal productivity being up to 16 times that reported (180 mg Sulfide per m^2 per day) in another oil field installation.[7]

Jørgensen[8] determined that in a natural sediment environment sulfate-reducing bacteria (SRB) generate about 10^{-16} mole sulfide per cell per day. However, a compilation of laboratory data indicated that pure cultures of SRB growing on suitable medium respire at the higher rate of 10^{-15} to 10^{-14} mole SO_4^{2-} per cell per day.[8] Assuming this latter range for a sulfide generation rate and an SRB cell density (as measured in Trial 1) of 10^6 to 10^9 cells per cm^2, then an areal productivity of 0.32–3200 mg per m^2 per day would be expected. This range encompasses the 1,000–3,000 mg per m^2 per day of sulfide generated in the untreated side stream devices.

The fact that anthraquinone, a known metabolic inhibitor of SRB, was effective in the produced water is additional evidence that the agent of sulfide generation was in fact microbiological. Also, that anthraquinone had an inhibitory effect for several days, long after the chemical had been flushed from the system, suggests that sessile bacteria were mainly responsible for the sulfide production.

Research has shown that microcolonies in a biofilm are separated by water channels through which convective flow can take place.[9] The effectiveness of low dosages of anthraquinone lies in the material's nonreactivity. We believe that anthraquinone is transported into the biofilm, diffuses through the biofilm voids, and then diffuses or is randomly transported by Brownian motion into the bacterial microcolonies without reduction in concentration as a consequence of a reaction with biofilm constituents. The anthraquinone is unaffected by other bacteria or the exopolysaccharide matrix present in the biofilm. In comparison, biocides are very reactive, a property which is likely responsible for their limited effectiveness in penetrating into biofilms at low dosages. It is speculated that the soluble anthraquinone molecules move freely in the biofilm and that decreases in biofilm fluid pH (due to microbial acid production and/or a sweeping of lower pH water past the biofilm) precipitate colloidal anthraquinone particles. Partitioning of the particles into the SRB cell membrane then blocks the organism's ATP production and shuts down the sulfate reduction process.

One aim of this work was to prove the concept of the chemical treatments, rather than to optimize dose rates. Super Sulf Control®, the water-soluble form of anthraquinone, was effective in inhibiting SRB metabolism even in conditions of high microbial activity and in the presence of high concentrations of existing sulfide. Since anthraquinone has no effect upon existing sulfide levels, the obvious place for deployment would be upstream in the system where less sulfide has been generated. However, since anthraquinone is an organic material which will partition into oil, injection into the water phase following oil-water separation is necessary for maximum efficacy.

Conclusions

- Indigenous sessile populations of sulfate-reducing bacteria in the onshore oil terminal have been shown to generate high levels of sulfide. Areal sulfide productivities of 1–3 g per m^2 per day and aqueous phase sulfide concentrations in excess of 50 mg/l have been measured.

- Batch treatments with an environmentally safe water-soluble form of anthraquinone were effective in inhibiting significant microbial sulfide

generation for a duration of over one week in a side stream bioreactor device.

Acknowledgments

The authors thank British Petroleum International for permission to publish this work. The opinions expressed are those of the authors and are not necessarily shared by BP International.

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Table 1 Ecotoxicological Data

	Skeletonema Costatum (marine Alga) EC_{50}, mg/l	*Acartia Tonsa* (marine Copepod) LC_{50}, mg/l
Super Sulf Control®	230	310
Statfjord Field Chemicals(1)		
Scale inhibitor	60–180	
Corrosion inhibitor	0.28	
Emulsion breaker	21–50	
Anti-foam	123–430	
Biocide	0.45	
Flocculant	>1,000	
Threshold Values(2)		
Toxic	≤100	≤100
Very toxic	≤1.0	≤1.0

(1) Georgie and Byrne, 1993.

(2) Lundgren, 1989; EEC, 1990.

Table 2 SRB Growth Medium

Component	Amount
KH_2PO_4	0.05 g
$MgSO_4 \cdot 6H_2O$	0.2 g
Sodium Lactate	4 ml
NaCl	10 g
Ascorbic Acid	0.1 g
Seawater	750 ml
Distilled Water	250 ml
Sodium Metabisulfite	0.003 g

Table 3 Typical Produced Water Composition

Component	Concentration (mg/l)
Chloride	13,100
Sulfate	91
Bicarbonate	128
Ammonical Nitrogen	0.2
Total VFAs	380
Sodium	3100
Calcium	350
Potassium	82
Magnesium	56
Iron	5.2
Boron	5.6
Barium	17
Strontium	41
Lithium	1.8
Manganese	0.51
Aluminum	0.41
Zinc	0.7

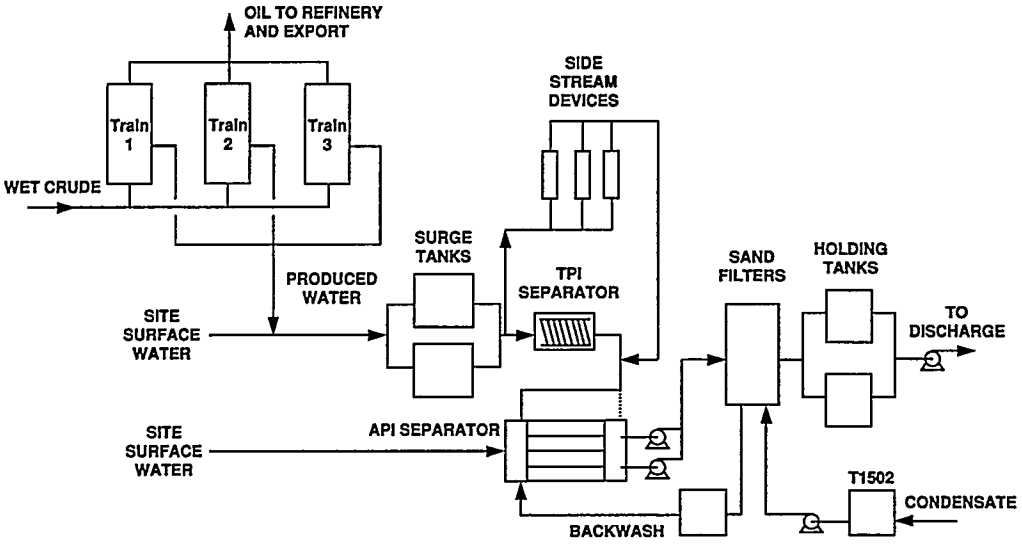

Figure 1 Schematic Diagram of Process Facilities within the Onshore Oil Terminal

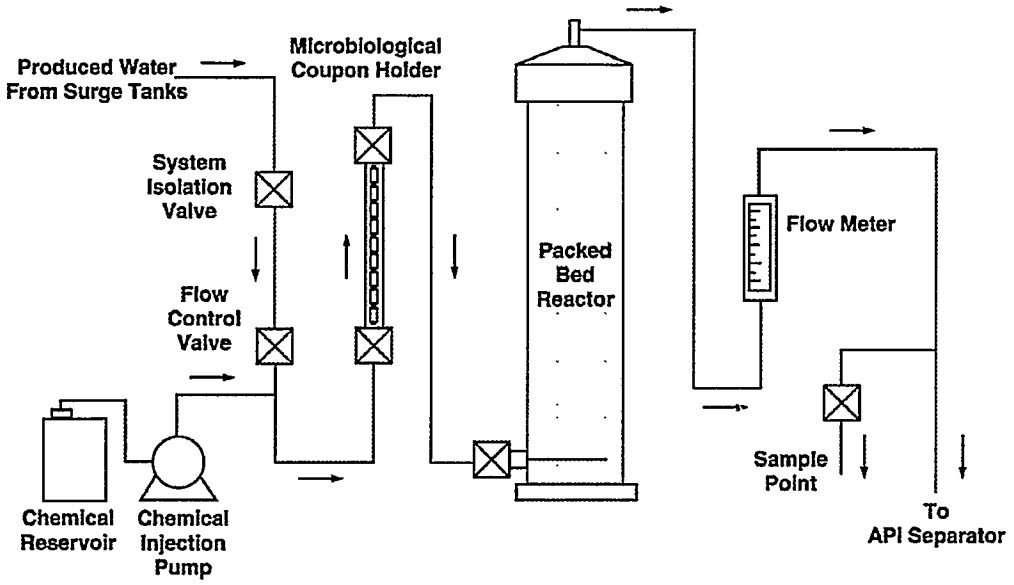

Figure 2 Side Stream Device Components

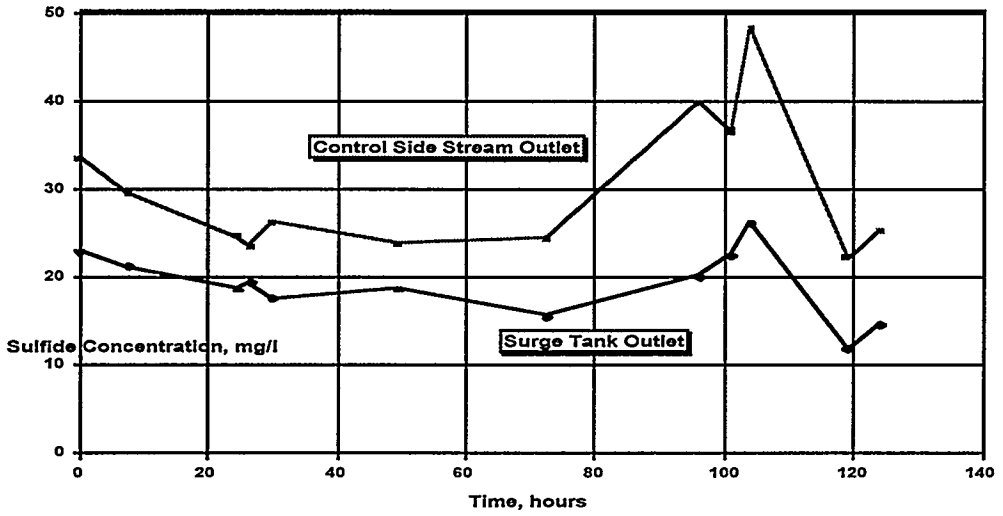

Figure 3 Sulfide Levels in Effluent Waters from Surge Tank and Control Side Stream during Trial 1 Test. The Surge Tank Effluent Is the Influent Water to the Side Stream Devices.

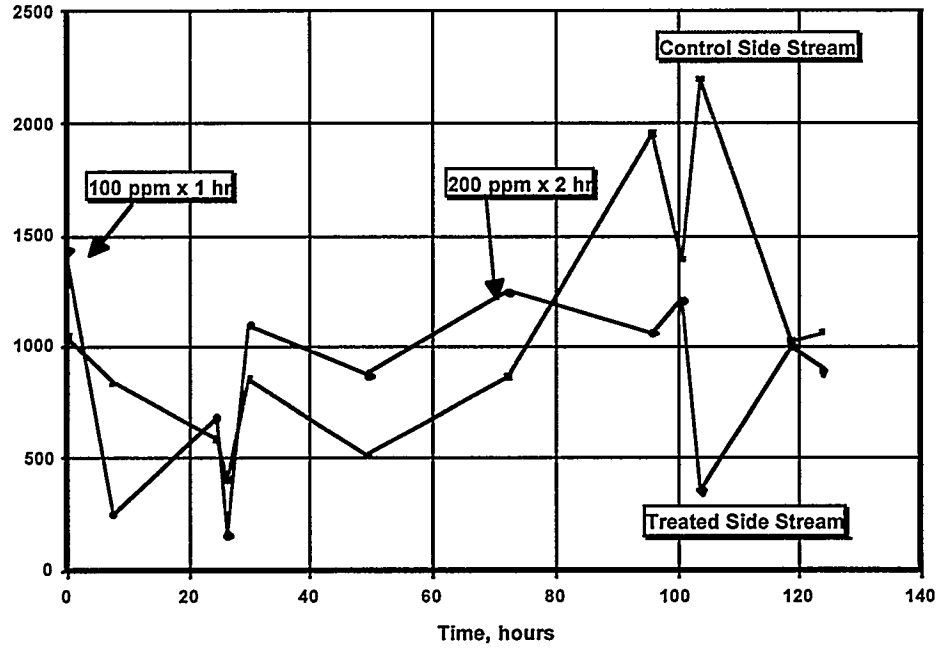

Figure 4 Sulfide Productivity for Control and Treated Side Stream Devices during the Trial 1 Test Period. Both Batch Treatments at Times Indicated by Arrows Were with Super Sulf Control®, the Water-Soluble Form of Anthraquinone. Concentrations Indicated Are for Active Anthraquinone.

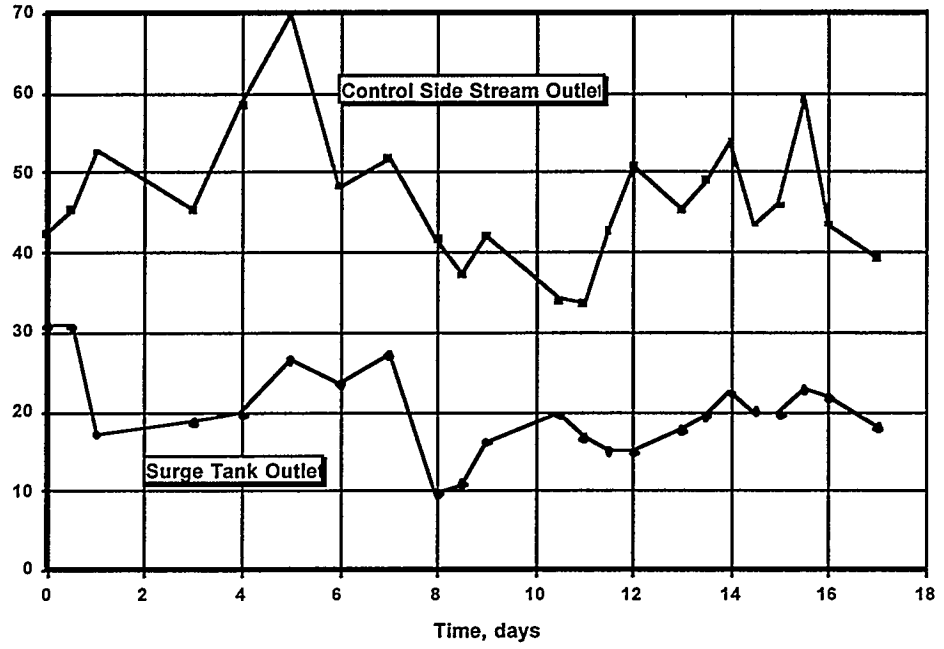

Figure 5 Sulfide Levels in Effluent Waters from Surge Tank and Control Side Stream during Trial 2 Test. The Surge Tank Effluent Is the Influent Water to the Side Stream Devices.

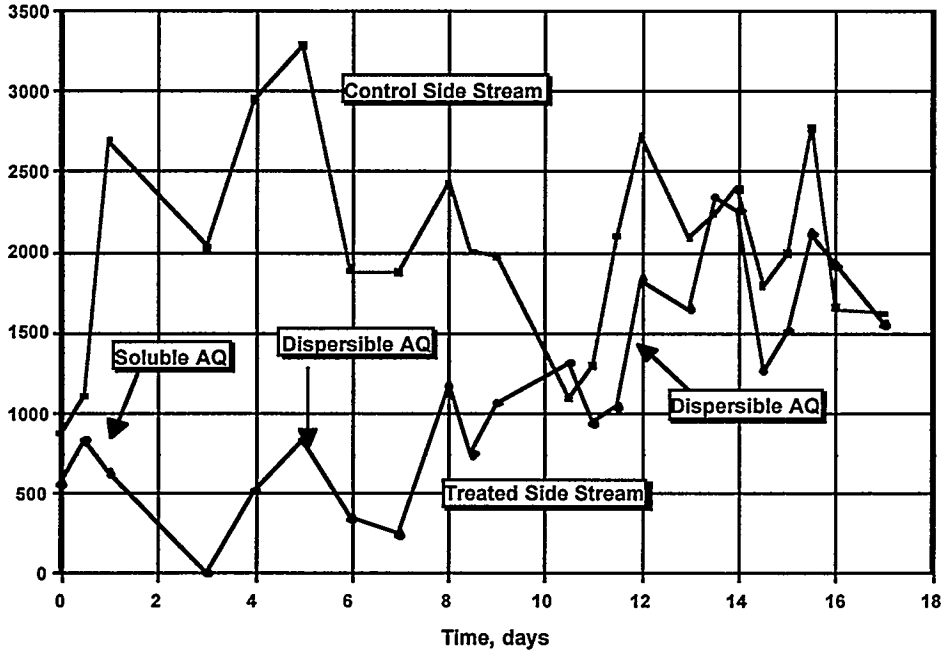

Figure 6 **Sulfide Productivity for Control and Treated Side Stream Devices during the Trial 2 Test Period. All Treatments at Times Indicated by Arrows Were with 400 ppm Active Anthraquinone for 1 Hour.**

Monitoring Sulfide and Sulfate-Reducing Bacteria

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Abstract

Simple yet precise and accurate methods for monitoring sulfate-reducing bacteria (SRB) and sulfide remain useful for the study of bacterial souring and corrosion. Test kits are available to measure sulfide in field samples. A more precise methylene blue sulfide assay for both field and laboratory studies is described here. Improved media, compared to that in API RP-38, for enumeration of SRB have been formulated. One of these, API-RST, contained cysteine (1.1 mM) as a reducing agent, which may be a confounding source of sulfide. While cysteine was required for rapid enumeration of SRB from environmental samples, the concentration of cysteine in medium could be reduced to 0.4 mM. It was also determined that elevated levels of yeast extract (>1 g/liter) could interfere with enumeration of SRB from environmental samples. The API-RST medium was modified to a RST-II medium. Other changes in medium composition, in addition to reduction of cysteine, included reduction of the concentration of phosphate from 3.4 mM to 2.2 mM, reduction of the concentration of ferrous iron from 0.8 mM to 0.5 mM and preparation of a stock mineral solution to ease medium preparation. SRB from environmental samples could be enumerated in a week in this medium.

Introduction

Field and laboratory monitoring of SRB and their important end-product of metabolism, sulfide, is part of any program to control or study these almost ubiquitous and often troublesome bacteria. The methylene blue assay for sulfide is relatively easy to perform, and test kits for sulfide have been developed based on this assay.[1] A quantitative variation of this assay will be described that is suitable for both laboratory and field work. Most probable number (MPN) technique is still used to enumerate SRB from environmental samples.[2-3] Perceived limitations of this technique, especially the required incubation times (up to four weeks), have led to the development of enzyme activity- or antigen-based test kits for SRB.[4] Indeed, in a recent book on SRB there was little mention of general or specific culture methods given for these microorganisms.[5] It may often still be desirable to enumerate or recover SRB from samples, and a medium which permits the determination of MPNs in a two week period has been described.[6] This medium was used to enumerate SRB from an unusual (for SRB) environment: oxygenated soils (unpublished data). The results from these studies (10^5–10^6 SRB recovered/g soil) led us to consider the possibility that degradation of cysteine in the medium was contributing to false positive MPN results. Cysteine and other components of this SRB medium were evaluated, leading to the formulation of a medium described below which could yield MPNs for SRB from some environmental samples after one week of incubation.

Sulfide Assay

It is probably more important to monitor and control sulfide and sulfidogenesis rather than SRB in most fossil fuel operations (control can include direct inhibition or reduction in numbers of SRB with the application of a biocide or other measures). Precise measurement of sulfide concentrations may not be required in many situations, and a sulfide test kit could be used. Test kits are available to determine sulfide at very low concentrations (0–1 mg/liter [kit T-9503]; 0–10 mg/liter [kit K-9510]) or high concentrations (0–10,000 mg/liter [kit K-9510C]) (CHEMetrics, Inc., Calverton, Virginia). These test kits were used to obtain the sulfide data presented below, and the results obtained were essentially the same as those from the more precise field and laboratory sulfide assay described below.

The more precise methylene blue sulfide assay requires preparation of a DMPD (4,5-dimethyl-1,2-phenylenediamene) reagent, a ferric reagent, and a sulfide standard. The DMPD reagent is prepared by dissolving 50 ml sulfuric acid, 1 g $Zn(CH_3COO)_2 \cdot 2H_2O$, and 1 g N, N-dimethyl-*p*-phenylenediamine·HCl (D 5129, Sigma Chemical Co., St. Louis, Missouri) in a liter of distilled water. The DMPD

reagent is dispensed (4.9 ml/tube) into 16 × 125 mm tubes with rubber-lined (14-959-25C) or polypropylene (14-962-26G, Fisher Scientific, Pittsburgh, Pennsylvania) caps which can be closed air-tight. This reagent can be stored at room temperature in the dark for at least a year. The DMPD reagent is water-white; reagent which has a pink tinge should not be used for critical assays. The ferric reagent is prepared by dissolving 5 g $FeCl_3 \cdot 6H_2O$ in water to a final volume of 20 ml. Crystals of ACS reagent-grade $Na_2S \cdot 9H_2O$ (#20,804-3, Aldrich Chemical Co., Milwaukee, Wisconsin) are rinsed with distilled water, dried with lint-free tissue, weighed, and taken into an anaerobic chamber. A 1,000 mg/liter sulfide standard is prepared by adding anoxic 0.01 N NaOH to give a solution containing 7.492 g $Na_2S \cdot 9H_2O$/liter. This solution can be stored in sealed, clean glassware in an anaerobic chamber at room temperature and is stable for at least two years. Some investigators have successfully stored the sulfide standard out on the bench as long as bottles were sealed with thick butyl rubber stoppers (2048-11800, Bellco Glass, Inc., Vineland, New Jersey).

A sample with a final volume of 5 ml (sample plus makeup water) is added to a tube of DMPD reagent, and the methylene blue color developed by the immediate addition of 0.1 ml of the ferric reagent. Assays are incubated at room temperature for at least 10 minutes. The range of the assay if read using a Spectronic 20 spectrophotometer with a 13 mm or 19 mm cuvette is 0–10 or 0–5 μg sulfide, respectively. A sulfide concentration of 3 nM/ml (0.1 mg/l) can be measured accurately by this assay.

Field samples may be processed in the field by the above assay, including color development with the ferric reagent, and the tubes transported back to a laboratory to read absorbances. The developed color is stable for at least 24 hours. The y-intercept of an assay may be shifted slightly upwards (the slope of the standard curve will remain the same) when low concentrations of sulfide are determined in highly saline brines. This may be corrected by standard addition technique or preparing standards in a brine, if required.

Regardless of how sulfide is measured in field or laboratory samples, it is important that the determination be done immediately after a sample is collected.

Effect of Reducing Agents on Enumeration of SRB

Cysteine had been shown to be important for rapid enumeration of SRB from environmental samples.[6] However, concern was raised that the concentration of cysteine was high enough (1.1 mM) to yield false positive results (from the desulfurylation of cysteine) after the medium was used to enumerate SRB from a nontraditional environment: aerated soils. The effect of cysteine and two other

reducing agents used in SRB medium, ascorbic acid and Fe^0, on enumeration of SRB from field samples and on sulfidogenesis in MPN tubes was examined to resolve this possible concern.

The basal medium for the study contained (per liter): NaCl, 5 g; $(NH_4)_2SO_4$, 0.5 g; KH_2PO_4, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.04 g; sodium lactate, 2.4 g; yeast extract, 1 g; TES (2-[tris(hydroxymethyl)methyl]amino-1-ethane-sulfonic acid), 2 g; vitamin solution, 10 ml; trace metal solution, 5 ml; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 0.2 g.[6] Ascorbic acid (0.1 g/l), cysteine (0.05 g/l) and a clean, degreased 1.7-cm iron brad were added to tubes of MPN medium as required as reducing agents. Medium was prepared as described previously in Hungate type anaerobic culture tubes (2047-16125, Bellco Glass, Inc., Vineland, New Jersey) under strictly anoxic conditions.[6] SRB from duck pond sediment (Norman, Oklahoma) and garden soil were enumerated in media containing different combinations of the three reducing agents using a three-point MPN assay carried out in duplicate. Tubes were incubated at 30°C for 28 days.

The results of these MPN determinations are given in Tables 1 and 2. The MPNs determined after 14 days of incubation, and in some cases after four days of incubation, were the same as those determined after 28 days. The results show that cysteine was the reducing agent required for rapid and complete (in this assay) enumeration of SRB from these samples. Ascorbic acid was eliminated from the SRB medium based on these results.

The concentration of cysteine added to medium in the above experiment could still yield 15 mg/l sulfide if completely desulfurylated, and this would be enough sulfide for a false positive result in an MPN tube scored on the basis of a black (ferrous sulfide) precipitate. Sulfide was measured in several of the MPN tubes for each reducing agent condition at several different dilutions of original inoculum using a test kit (0–250 mg/l, K-9510D, CHEMetrics, Calverton, Virginia). The results are given in Tables 3 and 4. Sulfide was produced in MPN tubes free of cysteine, and in most cases the concentration of sulfide measured in culture tubes was above that which could have originated from the degradation of cysteine alone. The results support the conclusion that false positive results were not a major problem in this experiment. The distribution of the results also indicate that an accurate assessment of the sulfidogenic potential of an environmental sample cannot be made on the basis of one or two measurements from an MPN tube set.

Effect of Yeast Extract on Enumeration of SRB

The effect of yeast extract on the enumeration of SRB from environmental samples was determined as part of the project to evaluate the efficacy of biocides against

SRB (biocides may be inactivated by high concentrations of yeast extract). The basal MPN medium described above with cysteine and Fe^0 as the reducing agents was used. The concentration of yeast extract was varied from 0–5 g/l. SRB were enumerated from the duck pond sediment and the garden soil used above.

The MPN results from this experiment are given in Tables 5 and 6. Again, there was no significant change in MPN determinations made after 14 days of incubation compared to 28 days, and in some instances the MPN determined after four days was essentially the same as that made after 28 days. Yeast extract was required for rapid and complete (in this assay) enumeration of SRB from these environmental samples, although the amount required for an observable response was small (0.1 g/l). This was an anticipated result. However, an unexpected result was observed in that enumeration of SRB from the soil sample was inhibited by the presence of 5 g/l yeast extract. This was unexpected because many media used to culture anaerobic bacteria contain 5–20 g/l yeast extract. A possible explanation for this observation is that at elevated concentrations of yeast extract sufficient other compounds could be present in medium to support the recovery of bacteria with other physiologies, such as amino acid fermenters. The amount of yeast extract in the SRB medium was reduced to 0.5 g/l based on these results.

Sulfide was measured in several tubes from each condition at different dilutions in these MPN tubes using a test kit. These results are given in Tables 7 and 8. Again, in most conditions and dilutions the amount of sulfide measured was greater than that which could be accounted for by the complete degradation of the added cysteine, and there was a somewhat random distribution of sulfide concentrations in the MPN tube sets. The sulfide results from the MPN set for the soil sample support the conclusion that elevated concentrations of yeast extract interfered with enumeration of SRB. The results of all of the sulfide data presented here support the conclusion that the SRB enumeration results are not due to false positive scoring of MPN tubes.

SRB Medium

The medium we now use to enumerate SRB is given in Table 9. The amount of phosphate and ferrous iron are reduced compared to the medium used previously.[6–7] However, their concentrations are still sufficient to yield a precipitate in the medium which may enhance recovery of SRB from environmental samples. The change from dibasic to monobasic potassium phosphate and to the chloride salt of calcium were made to facilitate preparation of a stock minerals solution for SRB, which is given in Table 10. This mineral solution may be stored at room temperature for at least a year. The final concentration of NaCl in medium prepared from this stock solution is 5 g/l for enumeration of SRB from aquatic and

terrestrial sources. As described, this medium is relatively easy to prepare and is used in both our research and teaching laboratories.

Acknowledgments

The excellent assistance of D. J. Ferguson and Michelle Payne, who carried out much of the work in the laboratory reported here, is gratefully acknowledged.

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Table 1 Effect of Reducing Agents on Enumeration of SRB from Duck Pond Sediment

Reducing Agent[a]			MPN after Days Incubation, 30°C				
Fe°	Asc	Cys	1	4	7	14	28
+	+	+	2.4×10^3	7.8×10^6	7.8×10^6	7.8×10^6	1.1×10^7
-	+	+	4.6×10^4	6.0×10^6	6.7×10^6	6.7×10^6	7.8×10^6
+	-	+	2.4×10^3	1.1×10^7	1.1×10^7	1.1×10^7	1.8×10^7
+	+	-	$<3.6 \times 10^0$	7.8×10^5	1.5×10^6	4.6×10^6	6.0×10^6
-	-	-	$<3.6 \times 10^0$	4.6×10^2	4.6×10^4	4.6×10^4	6.7×10^5

[a]Metallic iron, ascorbic acid, and cysteine were examined as reducing agents at concentrations given in the text.

Table 2 Effect of Reducing Agents on Enumeration of SRB from Garden Soil

Reducing Agent[a]			MPN after Days Incubation, 30°C				
Fe°	Asc	Cys	1	4	7	14	28
+	+	+	1.4×10^5	6.0×10^5	6.0×10^5	9.8×10^5	2.5×10^6
-	+	+	6.0×10^5	6.7×10^5	7.8×10^5	7.8×10^5	7.8×10^5
+	-	+	4.6×10^5	6.8×10^5	6.8×10^5	6.8×10^5	6.8×10^5
+	+	-	$<3.6 \times 10^0$	6.0×10^4	6.0×10^4	6.7×10^4	6.7×10^4
-	-	-	$<3.6 \times 10^0$	1.1×10^4	1.1×10^4	1.1×10^4	3.4×10^4

[a]Metallic iron, ascorbic acid, and cysteine were examined as reducing agents at concentrations given in the text.

Table 3 Effect of Reducing Agents on Sulfide (mg/l) Produced in MPN Tubes of Duck Pond Sediment

Reducing Agent[a]			Dilution Factor				
Fe°	Asc	Cys	10^1	10^2	10^3	10^4	10^5
+	+	+	50	15–25	25–150	5–25	50
–	+	+	30–75	15–75	25–75	50–80	15–75
+	–	+	10–30	20–25	25–50	15–50	5–50
+	+	–	6–25	6–75	30–35	25–35	3–30
–	–	–	50–100	50–6	50–60	5–60	60–75

[a]Metallic iron, ascorbic acid, and cysteine were examined as reducing agents at concentrations given in the text.

Table 4 Effect of Reducing Agents on Sulfide (mg/l) Produced in MPN Tubes of Garden Soil

Reducing Agent[a]			Dilution Factor				
Fe°	Asc	Cys	10^1	10^2	10^3	10^4	10^5
+	+	+	2–25	15	25–50	5–15	2–50
–	+	+	20–30	15–18	5–10	4–15	1–15
+	–	+	4–15	10–15	10–50	8–10	10–20
+	+	–	2–25	10	8	10–35	5–10
–	–	–	50–100	6–15	3–25	8–10	25–50

[a]Metallic iron, ascorbic acid, and cysteine were examined as reducing agents at concentrations given in the text.

Table 5 Effect of Yeast Extract (g/l) on Enumeration of SRB from Duck Pond Sediment

	MPN after Days Incubation, 30°C					
Yeast Extract	1	2	4	7	14	28
0	$<3.6 \times 10^0$	4.6×10^2	1.1×10^3	2.4×10^4	4.6×10^4	1.1×10^5
0.1	2.4×10^3	2.4×10^4	2.4×10^5	4.6×10^5	4.6×10^5	1.1×10^6
0.2	2.4×10^4	4.6×10^4	1.1×10^5	1.5×10^5	4.6×10^5	4.6×10^5
0.5	1.1×10^4	4.6×10^4	1.1×10^5	4.6×10^5	4.6×10^5	4.6×10^5
1	1.1×10^5	1.1×10^5	4.6×10^5	4.6×10^5	1.1×10^6	1.1×10^6
5	2.4×10^4	2.4×10^4	2.4×10^4	2.4×10^4	2.4×10^4	4.6×10^5

Table 6 Effect of Yeast Extract (g/l) on Enumeration of SRB from Garden Soil

	MPN after Days Incubation, 30°C					
Yeast Extract	1	2	4	7	14	28
0	$<3.6 \times 10^0$	4.6×10^2	2.4×10^3	4.6×10^4	4.6×10^4	4.6×10^4
0.1	2.4×10^2	4.6×10^3	1.1×10^6	2.4×10^6	2.4×10^6	2.4×10^6
0.2	1.1×10^4	4.6×10^4	4.6×10^5	1.1×10^6	1.1×10^6	1.1×10^6
0.5	2.4×10^4	4.6×10^5	4.6×10^5	4.6×10^5	4.6×10^5	4.6×10^5
1	1.1×10^5	1.1×10^5	4.6×10^5	4.6×10^5	4.6×10^5	4.6×10^5
5	1.1×10^4	1.1×10^4	1.1×10^4	1.1×10^4	1.1×10^4	1.1×10^4

Table 7 Effect of Yeast Extract (g/l) on Sulfide (mg/l) Produced in MPN Tubes of Duck Pond Sediment

	Dilution Factor			
Yeast Extract	10^1	10^2	10^3	10^4
0	40–50	25–75	40–60	75
0.1	25–75	25–40	50–75	30–75
0.2	8–25	5–35	5–60	2–60
0.5	2–25	20–60	20–50	30–75
1	25–50	25	30–40	50–60
5	30–35	2–30	8–50	50

Table 8 Effect of Yeast Extract (g/l) on Sulfide (mg/l) Produced in MPN Tubes of Garden Soil

	Dilution Factor			
Yeast Extract	10^1	10^2	10^3	10^4
0	60–70	30–40	5–30	10–75
0.1	35–50	10	10–15	25–80
0.2	50–60	5–10	8–15	2–5
0.5	15–25	8–30	5–55	5
1	2–20	25–30	2–30	30–45
5	5–10	6–20	0	0–2

Table 9 Preparation of SRB Medium

Into anaerobic chamber:	Hungate-style culture tubes, each with a clean, degreased iron brad
	0.05 g cysteine
In boiling flask:	50 ml SRB mineral solution[a]
	10 ml vitamin solution
	5 ml trace metal solution
	4 ml sodium lactate syrup (2.4 g sodium lactate)
	2 g TES
	0.5 g yeast extract
	Adjust pH to 7.5 with KOH
	0.2 g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ a ppt will form
	Boil and degas, transfer into anaerobic chamber, add cysteine, dispense, seal, and sterilize 5 minutes

[a]SRB mineral solution, vitamin solution, and trace metal solution are given in Tables 10, 11, and 12.

Table 10 SRB Mineral Solution (20X)

	g/l[a]
NaCl	100
$(NH_4)_2SO_4$	10
$MgSO_4 \cdot 7H_2O$	4
KH_2PO_4	6
$CaCl_2 \cdot 2H_2O$	0.8

[a]Final concentration of NaCl 0.5% for enumeration from aquatic and terrestrial samples.

Table 11 Vitamin Solution[6–7]

	mg/l		
Pyridoxine·HCl	10	Thiamine·HCl	5
Riboflavin	5	Calcium pantothenate	5
p-Aminobenzoic acid	5	Nicotinic acid	5
Vitamin B_{12}	5	Biotin	2
Folic acid	2	MESA[a]	10
store at 4°C			

[a]Mercaptoethanesulfonic acid.

Table 12 Trace Metal Solution[6–7]

	g/l		
Nitrilotriacetic acid		2.0	
		adjust pH to 6 with KOH	
$MnSO_4 \cdot H_2O$	1.0	$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.8
$CoCl_2 \cdot 6H_2O$	0.2	$ZnSO_4 \cdot 7H_2O$	0.2
$CuCl_2 \cdot 2H_2O$	0.02	$NiCl_2 \cdot 6H_2O$	0.02
$Na_2MoO_4 \cdot 2H_2O$	0.02	Na_2SeO_4	0.02
Na_2WO_4	0.02		
store at 4°C			

[a]Mercaptoethanesulfonic acid

From the Lab to the Field

Chemical and Biological Monitoring of MIOR on the Pilot Area of Vyngapour Oil Field, West Siberia, Russia

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Abstract

The pilot area of the Vyngapour oil field allotted for MIOR tests contains three injection and three producing wells. These wells were treated in summer 1993 and 1994. Before, during, and after MIOR treatments on the pilot area the chemical compounds of injected and formation waters were studied, as well as the amount and species of microorganisms entering the stratum with the injected water and indigenous bacteria presented in bottomhole zones of the wells. The results of monitoring showed that the bottomhole zone of the injection well already had biocenosis of heterotrophic, hydrocarbon-oxidizing, methanogenic, and sulfate-reducing bacteria, which were besides permanently introduced into the reservoir during the usual waterflooding. The nutritious composition activated vital functions of all bacterial species presented in the bottomhole zone of the injection well. The formation waters from producing wells showed the increase of the content of nitrate, sulfate, phosphate, and bicarbonate ions by the end of MIOR. The amount of hydrocarbon-oxidizing bacteria in formation waters of producing wells increased by one order. The chemical and biological monitoring revealed the activation of the formation microorganisms, but no transport of food industry waste bacteria through the formation from injection to producing wells was found.

Introduction

The pilot field test (PFT) of microbial improved oil recovery (MIOR) was carried out in the experimental area of the oil field Vyngapour, West Siberia, Dept "Zapolyarneft," the territory of Yamalo-Nenetsky autonomous area and, partially, Nizhne-Vartovsky region of Khanty-Mansiysky autonomous area. The area of cluster No 30 is 276 ha; it contains 4 injection and 13 producing wells. The average density of the well-net is 12.5 ha/well. The pilot area is 32.5 ha. It contains 9 wells. But only three injection (726, 695, and 1859) and 3 producing (725, 754, 755) wells were operated in 1993 and 1994. Well 726 was microbially treated with a nutritious composition containing nitrogen and phosphorus sources and also the local milk food industry waste. The distances between injection well 726 and producing wells 725, 754, and 755 were 600, 1,150, and 700 m, respectively. Wells 695 and 1859 worked under common conditions, as was described by Murygina.[1] The 272.5 m^3 of the nutritious composition was injected into the reservoir twice with a break between the procedures in 1993, and 450 m^3 of the nutritious composition was injected into reservoir in 1994 without break.

The goal of this work was microbiological and chemical monitoring of formation and injection waters during MIOR.

Materials and Methods

The work of producing wells was under regular control. Liquid samples for microbiological and chemical studies were taken every 10 days during the injection of the nutritious composition. After the injection was over, liquid and gas samples were taken twice a month for four months and afterward once a month. The content of ions CO_3^{2-} , HCO_3^-, Ca^{2+}, Mg^{2+}, Cl^-, K^+ and Na^+ in the formation and injected waters were analyzed titrametrically; pH was determined potentiometrically; density was measured by areometer. The content of phosphates (PO_4^{3-}), sulfates (SO_4^{2-}), and nitrates (NO_3^-) in water samples was determined colorimetrically.

Oil composition was analyzed by methods of column and gas chromatography. Milk food industry waste was analyzed on amino acid analyzer T 339 Mikrotechna (Czechia); protein was determined by Lowry, sugars were measured on a carbohydrate analyzer LC 2000 Biotronik.

Samples of formation and injected waters, as well as food industry waste, were studied by the method of limit dilutions for the presence of microorganisms of various physiological groups by inoculation of the samples on elective liquid nutrient media.[2]

Results

Baseline Monitoring. Preliminary investigation of the formation and injected waters of the pilot area showed that they contained heterotrophic and hydrocarbon-oxidizing (HCO) bacteria. Single cells of sulfate-reducing and methanogenic bacteria were found also (see Fig. 1).

Chemical analyses revealed significant differences in compositions of formation and injected waters, first of all, in the content of ions HCO_3^-, Cl^-, Ca^{2+}, Mg^{2+}, K^+, Na^+, and PO_4^{3-}, SO_4^{2-}, NO_3^-, and general mineralization and pH values that are significantly higher in formation waters (see Fig. 2).

The study of the oil taken from the collector of cluster 30 showed that in the gasoline-free reduced oil, paraffino-naphthenes make up 58.5%, light and mediate aromatic hydrocarbons—17.6%, heavy aromatic hydrocarbons—1%, benzene resins—17.6%, alcohol-benzene resins—5.3%, asphaltenes—0.8%. The oil from the pilot area belongs to the chemical type of A1: $\sum$n-alk/$\sum$i-alk = 6.67; $K_i = 0.45$; i-C_{19}/i-C_{20} = 2.02 (see Tables 1 and 2).

Microbial investigation of the local milk food industry waste showed that the common quantity of lactobacteria makes 10^8 –10^9 cell/ml.

Biochemical composition of the waste appeared to be very rich:

- sugars (lactose, galactose, glucose) made the total of 35.6 to 105.7 g/l
- free protein—4.76 to 6.75 g/l
- bound protein—12.06 to 14.88 g/l
- amino acids before and after hydrolysis—46.1–146.8 mg/l and 2,042.6–3,055.9 mg/l, respectively
- pH is 4.5–5.0

The nutritious composition of PFT-93 contained nitrogen and phosphorus salts in concentrations of 0.5–1.5 and 1.0–1.5 g/l, respectively, and the nutritious composition of PFT-94 contained KCl in the amount of 0.001% instead of KH_2PO_4. Salt $(NH_4)_2HPO_4$ (0.4%) was used for buffering of the nutritious composition. Sometimes dry milk (0.04%) and industrial inoculum of lactobacteria Streptococcus and Lactobacillus (3 liters per 10 m^3) were used for the nutritious composition instead of food industry waste.

Pilot Field Test. In 1993 two cycles of the nutritious composition injection into the formation were completed during the PFT. At the first cycle 158.5 m^3 of nutritious composition was pumped into injection well 726. After that the well was included into the system of usual flooding for 40 days. During the second PFT

cycle the same injection well was pumped with 114 m^3 of nutritious composition with the subsequent inclusion into the flooding system. Thus, the total of 272.5 m^3 of nutritious composition was pumped into one injection well of the pilot area. In 1994 the 450 m^3 of the nutritional composition was pumped during 41 days into the same well.

Microbiological investigations showed that before, during, and after the PFT heterotrophic bacteria in the amount of 10^2–10^5 cell/ml were revealed in the injected waters continuously. Their maximal quantity (10^5 cell/ml) was found in the middle of June 1993 and in September 1994. The quantity of HCO-bacteria varied from 10 to 10^4 cell/ml with the maximum in September 1994. Methanogenic and sulfate-reducing bacteria were found at the amount from 0 to 10^4 cell/ml (see Fig.1). Thus, various microorganisms, including bacteria able to grow on oil, are continuously introduced into oil formation by waterflooding.

Producing wells were also investigated before, during, and after microbial treatment. Inoculations of formation waters from these wells incubated at 45°C revealed no bacteria, or up to 10 cell/ml heterotrophic bacteria (see Fig. 1). During PFT, neither heterotrophic nor HCO-bacteria were isolated from the formation waters of well 755. At the beginning of PFT-93 methanogenic and sulfate-reducing bacteria were not isolated from other two producing wells. But during PFT-94 heterotrophic, HCO-, methanogenic, and sulfate-reducing bacteria were isolated from formation waters of producing wells (see Fig. 1). Lactobacteria transport through the formation was observed neither in PFT-93 nor in PFT-94. Microbial treatment in 1993 and 1994 did not result in oil deterioration.

Chemical analysis showed a similarity in compositions of formation waters from wells 725 and 754. Well 755 differs from the first two by a higher initial level of general mineralization and the content of ions HCO_3^-, Cl^-, K^+, and Na^+. Increase of common mineralization and bicarbonates, phosphates, sulfates, and nitrates concentration was observed during PFT-93 and PFT-94.

Acidity of the formation waters in wells 725 and 755 actually did not change during PFT-93 and PFT-94 and kept a pH level of 8. But well 754 revealed the decrease of formation waters' pH to 7.0–7.5 (see Fig. 2).

Microbial metabolites (volatile fatty acids, alcohols, ketones) were found in formation waters of the producing wells after PFT-93 and PFT-94. Back flooding from the injection well 726 revealed biologically active processes that were controlled by the quantity of microorganisms, by the amount of microbial metabolites, and by the increase of HCO_3^-, PO_4^{3-}, SO_4^{2-}, and NO_3^- ion concentrations. Concentration of microbial metabolites (volatile fatty acids,

methanol, ethanol, and acetone) varied from trace quantities to 20–50 mg/l (see Table 3).

Discussion

It is established that injected waters from cluster 30 of the Vyngapour oil field contain microorganisms, the quantity of which is by 2–3 orders higher than quantity of the same bacteria isolated from the formation waters of producing wells. However, their quantity is not large. So far microbial control has revealed no transport of bacteria presented in the food industry waste through the formation from the injection to producing wells. Evidently, more prolonged microbiological studies are necessary. On the other hand, development of this technology did not presuppose that such transport of bacteria and the nutritious composition itself would take place. It was assumed that the bacteria presented in the waste, with a certain additive effect of indigenous bacteria, would produce oil-displacing metabolites that would be moved by the injected waters through the formation and improve oil recovery.

The nutritious composition injection into the well 726 increased the general mineralization and contents of bicarbonates, sulfates, phosphates, nitrates, and other ions in formation waters from producing wells. Microbial metabolites (volatile fatty acids, alcohols, ketone) were found in the formation waters of the producing wells after PFT-93 and PFT-94, which indicate that biogenic processes occur in the formation after microbial treatment. Microbiological and chemical analyses of back flooding waters from injection well 726 also support this fact.

Conclusions

Pilot test of the MIOR in the pilot area of the Vyngapour oil field showed that:

1. The nutritional flooding increased mineralization of formation waters in producing wells, mostly due to the increase of concentration of bicarbonate ions 1.2–1.7 fold, chlor ions—1.9–1.8 fold, phosphates—2–4 fold, sulfates—1.5–4 fold, and nitrates—14–20 fold.

2. Microbial treatment increased the quantity of heterotrophic and HCO- bacteria in producing wells 725 and 754 by the end of PFT-93 and PFT-94 by 1–2 orders of magnitude. No microorganisms were found in formation waters of producing well 755.

3. Microbiological and biochemical studies of the formation waters and back flooding waters revealed an activation of biogenic processes in the formation after MIOR.

References

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Table 1 Group Composition Analysis of Initial Vyngapour Oil

Group	Composition, %
Paraffino-naphthenes	58.50
Light aromatic hydrocarbons	11.86
Mediate aromatic hydrocarbons 1	2.07
Mediate aromatic hydrocarbons 2	3.69
Heavy aromatic hydrocarbons	0.10
Benzene resins	17.60
Alcohol-benzene resins	5.27
Asphaltenes	0.79
Total	99.88

Table 2 Gas-Chromatographic Analysis of Composition of Initial Vyngapour Oil and after Microbial Treatment

Composition, %			Composition, %		
Paraffins	Initial oil	Oil after microbial treatment	Paraffins	Initial oil	Oil after microbial treatment
n-C_{12}	11.65	11.19	n-C_{24}	3.05	2.95
n-C_{13}	11.31	10.82	n-C_{25}	2.44	2.98
n-C_{14}	12.95	10.87	n-C_{26}	1.73	1.83
n-C_{15}	9.26	8.91	n-C_{27}	1.32	1.24
n-C_{16}	8.17	8.15	n-C_{28}	0.84	0.81
n-C_{17}	8.12	7.69	n-C_{29}	0.61	0.77
n-C_{18}	5.93	6.98	n-C_{30}	0.31	0.41
n-C_{19}	5.90	7.16	K_i	0.45	0.56
n-C_{20}	4.96	5.37	n-C_{18}/i-C_{20}	2.86	2.09
n-C_{21}	4.28	4.48	i-C_{19}/i-C_{20}	2.02	1.45
n-C_{22}	3.77	4.00	$\sum$n-alk./$\sum$i-alk.	6.67	-
n-C_{23}	3.40	3.43			

Table 3 Microbial Metabolites in Formation Waters from Producing Well 754 and Back Flooding Waters from Injection Well 726

Microbial Metabolite		Well 726 Depth of Backflooding, m		
mg/l	Well 754	2.47	3.63	4.53
Acetic acid	trace	72.1	120.2	144.2
Propynoic acid	trace	trace	49.9	61.7
i-Butyric acid	trace	trace	-	-
n-Butyric acid	trace	trace	13.9	23.2
i-Valeric acid	trace	trace	trace	trace
n-Valeric acid	trace	trace	trace	trace
i-Caproic acid	trace	trace	trace	trace
Heptanoic acid	trace	trace	trace	trace
Methanol	20	20	20	20
Ethanol	10	50	4	20
Acetone	30	40	30	40

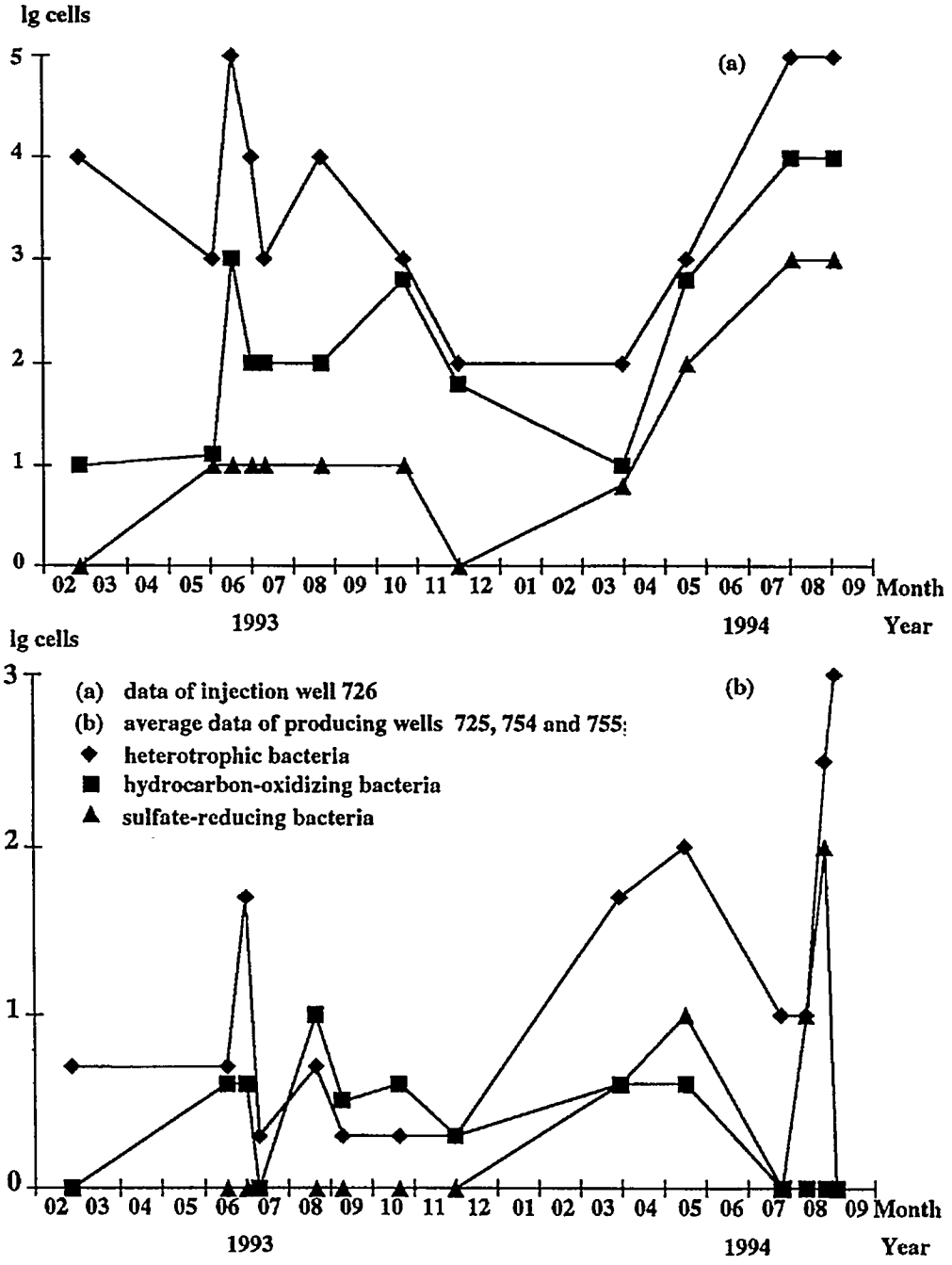

Figure 1 Microbiological Monitoring of Injection and Formation Waters before, during, and after Microbial Treatment in 1993 and 1994 on Pilot Area of the Vyngapour Oil Field

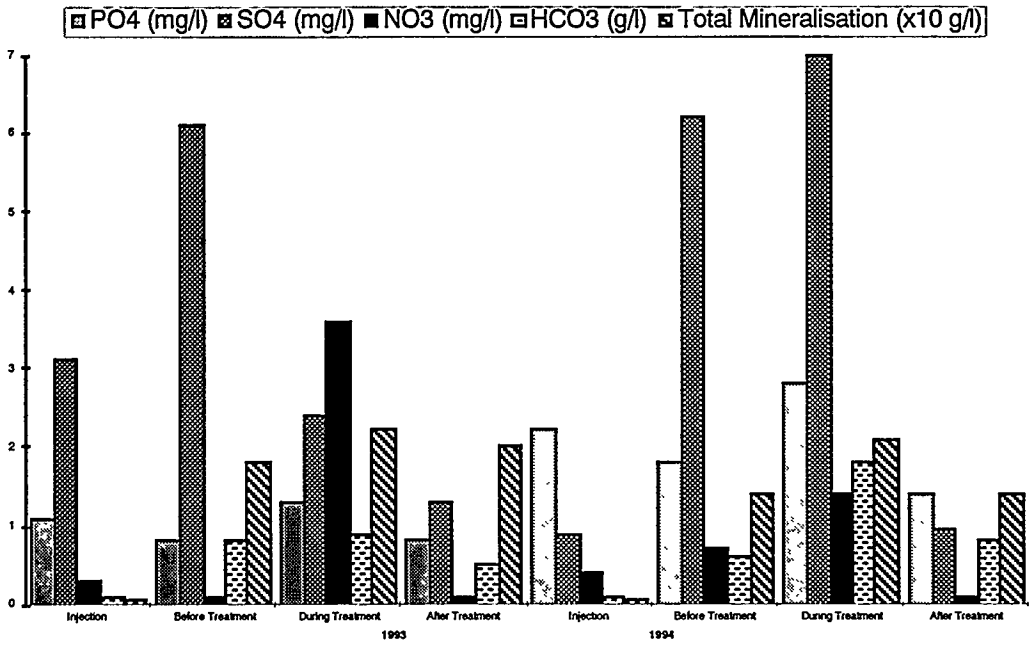

Figure 2 Content of Ions in Injected and Formation Waters

A Study of Microbial Profile Modification

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Abstract

A microbial profile modification method using spores was investigated. A halotolerant, spore-forming, biopolymer-producing mesophile was used in Berea cores with a specifically formulated nutrient package to reduce the permeability of the rock. The degree of permeability reduction varied widely depending on the stimulation protocols used. The incubation period had a significant impact on permeability reduction, and there appeared to be an optimum incubation time for maximum permeability reduction. The reduction persisted for many PV of brine injection and appeared very stable. For our microbes used in this study, the permeability reduction was about the same when the NaCl concentration was above 2 wt% in the range from 0 wt% to 10 wt%.

Introduction

Enhanced oil recovery conventionally refers to chemical, miscible, and thermal recovery processes in the oil industry. Other field operations that may result in improved oil recovery have specific names such as profile modification, wellbore cleanup, etc. We are adding the adjective microbial to this conventional terminology to indicate that these operations involve microbes.

It has been known for many years that microbes can produce most of the agents used in enhanced oil recovery, hence the idea of the microbial enhanced oil recovery (MEOR).[1] These include surfactants, biopolymers, solvents such as ethanol and acetone, acids, and gases such as CO_2 and methane. Thus, it has been purported that these chemicals and gases produced in situ, alone or in combination, improve the oil displacement efficiency. This is certainly true in a qualitative sense. However, it should be clearly understood that mere production of chemicals and/or gases will not effect improved oil recovery.

As for surfactants, the oil-water interfacial tension should be reduced by three orders of magnitude to have any significant improvement in oil recovery in a typical waterflood. Even with all the commercial surfactants, it is difficult to formulate a system to produce such ultralow interfacial tensions for a given reservoir condition. What is, then, the chance of producing the right surfactants in situ by indigenous bacteria in the right quantity for this purpose? Also, can we produce sufficient biopolymers or gas to emulate polymer flooding or gas flooding in situ? Ineffective amounts of these chemicals and gas cannot produce more oil by synergism. It appears that microbial enhanced oil recovery in the conventional sense is difficult to achieve, and quantitative mechanistic evidence is needed to support this claim of enhanced oil recovery.

From the beginning, we felt that microbial profile modification (MPM) is much easier to achieve. This process depends on in-situ formation of biofilm, the multilayer growth of cells and support material on solid surfaces, consisting of biopolymer and biomass produced by the microorganisms, to reduce the permeabilities of watered-out thief zones or high-permeability streaks. Thus, this process does not depend on delicate chemical systems produced by the microbes. One immediate question was whether indigenous bacteria would have the desired properties for MPM. We decided that we could not rely on them. Our process thus involves the injection of biopolymer-producing bacteria in the form of spores and nutrient package. Also, this process could be used for in-depth treatments of high-permeability or thief zones to overcomes the shortcomings of the conventional treatments such as polymer gels, squeeze cementing, and selective perforation,

whose effects are negated by crossflow in the reservoir. Thus, MPM appeared to have the best chance of technical and economic success.

In our previous paper,[2] we reported some results of our microbial profile modification process, mostly microbiological test results. This paper is an extension of that study with an exclusive emphasis on spore transport and permeability reduction in Berea cores.

Characteristics of Salton-1

A culture was isolated from a saline sediment by researchers at former Gulf Research[3] and was named Salton-1. Salton-1 is a gram-positive, rod-shaped bacterium with a width of about 0.2–0.3 μm and a length of 0.5–1.0 μm. It closely resembles the species *Bacillus licheniformis*, as described in *Bergey's Manual.*[4] The cells of Salton-1 are somewhat smaller than the dimensions given in Bergey. These bacteria have the desired properties for MPM as detailed below. They are facultative anaerobes and can grow either in the presence or absence of atmospheric oxygen, thus facilitating ease of handling during storage and injection operations. They are halotolerant or moderately halophilic, being able to grow and produce the biofilm in moderately saline reservoir environments. They are mesophiles, suitable for the temperature range of 20°C to 55°C, a range of temperatures expected in the reservoir.

They are single endospore-forming bacteria, a very important trait for MEOR. Because the microbes should be able to penetrate far in the reservoir, they must be small in size. Bacterial spores are small, and their wall is inert and thus easier to propagate in reservoir rocks than vegetative cells. Earlier, the use of spores in MEOR was mentioned in the literature.[5] When conditions are right, spores germinate into vegetative cells, which will produce biofilm. Salton-1 spores easily germinate and grow when stimulated with a nutrient. They produce an extracellular water-soluble polymer, primarily polyglutamic acid of molecular weights from 2.1 to 2.8 million, during the early stationary phase of their growth cycle. The production of polymers facilitates biofilm formation and enhances the stability of the biofilm. They are also nonpathogenic and produce no animal or plant toxins. Details of characterization results are reported in our previous paper.[2]

Experimental

The core test procedures are standard in the industry and are not describe here. Berea cores of 2.54 cm (diameter) × 30 cm (length) were fired at 850°F for 24 hours. Berea cores harbor indigenous bacteria, and they can be resuscitated by

nutrient injection. This procedure eliminated any permeability reduction due to indigenous bacteria in the core. We used a backpressure of 1,500 psi to remove any spurious effect of gas produced by microbial activities on permeability reduction.

Most tests were done at a constant flow rate of 10 ml/hr and 100°F, and we had two pressure taps along the core at one-third of the core length. Effluent was collected in increments of one-tenth of a PV in most cases. Spore count was made using the plate-count method. Properties of cores used are summarized in Table 1.

A nutrient medium formulation optimized for rapid spore germination and growth and maximum polymer production[6] was used in all experiments. The medium included 100 g NaCl, 1 g $NaNO_3$, 0.7 g NH_4NO_3, 0.25 g $MgSO_4$, 5 g KH_2PO_4, 10 g sucrose, 1.3 g citric acid, 0.5 g yeast extract per liter of water, adjusted to pH 8. The brine used contained 1 wt% NaCl.

In permeability reduction tests, a one-PV spore suspension was followed by a one-PV nutrient. The test was continued either with brine injection or shut in for a period of time, followed by brine injection. When experimental conditions differed from those previously listed, they are specified.

Results

Spore Transport

Several core tests were run to investigate the transport of spores in Berea cores. Figure 1 shows the effluent spore concentration profile in a 860-md Berea core. A one-PV spore suspension of 2.7×10^{11} cfu/ml was injected into the core, which was followed by another PV of chase brine at an injection rate of 10 ml/hr. The maximum effluent concentration was about 10^5 cfu/ml. This is higher than that of 10^4 cfu/ml reported previously with 10^9 cfu/ml in a 650-md core. With a higher injected spore concentration, a higher effluent concentration was attained.

In the next series of experiments, the effect of slug size on the spore transport was investigated. The cores had permeabilities in excess of 1.3 darcies, and spore suspension of 10^8 cfu/ml was driven with brine at a rate of 100 ml/hr. This is the highest rate we used in our tests. The slug size varied from one to one-half PV. In these tests, effluent was collected in an increment of one PV for analysis. The results are shown in Figure 2. The maximum effluent concentration reached 5×10^5 cfu/ml. The actual peak concentration would have been much higher than the maximum shown here because the maximum is an average of a whole PV where the effluent peak is normally expected, as shown in the previous figure. The effluent

spore concentration is an order of magnitude lower with a 0.75 PV slug, and with a 0.5 PV slug, only tracer amounts of spores were detected.

Permeability Reduction

A series of runs were conducted to study the effects of several variables on permeability reduction in Berea cores. The spore concentration used was 10^8 cfu/ml. The permeability reduction in the first section during the nutrient injection is shown in Figure 3, where the permeability ratio is the final permeability divided by the original permeability. At the end of nutrient injection, this section was almost completely plugged. However, the permeability reduction was about 30% and 10% for the second and third sections, respectively, as shown in Figure 4, where the permeability ratio is shown as a function of brine PV injected. There was no incubation period in this run. With the injection of brine, the reduction in permeability for both sections reached a plateau value of about 60%, or a permeability ratio of 0.4. This is quite reasonable in terms of nutrient propagation in the core. At the end of one PV nutrient injection, the throughput in the first section was three PV of nutrient while that of the last section only one PV of nutrient. Furthermore, because of nutrient consumption, the last section would not see the full strength of the nutrient. As the brine injection begins, the nutrient in the first section will propagate into the second and third sections, thus reducing the permeability even further.

Figures 5 through 7 show the permeability reduction during brine injection for different shut-in or incubation periods. After the nutrient injection, the core was shut in for a specific time period. Brine injection resumed after the shut-in period, and the sectional permeability reductions are shown in these figures. At the injection rate of 10 ml/hr, it takes about 3.5 hours to inject one PV of fluid. At the end of nutrient injection, normally we observed a permeability reduction of about 10% or so in these tests, which was mainly due to spore injection. We do not expect any substantial microbiological activities during this time in view of the lag time of 10 hours at this experimental conditions. With a shut-in period of 17 hours, permeability reduction continued during the brine injection in all sections of the core as shown in Figure 5.

What is surprising is the behavior shown in Figure 6. After a one-day incubation, the third section exhibits initially the largest amount of permeability reduction, and eventually the first section reaches the same degree of reduction as that of the third section. On the other hand, with the shut-in time of 1.75 days, the reduction in permeability is pretty much uniform throughout the core, and it remains the same for seven PV of brine injection, as shown in Figure 7. This trend was generally the same for longer shut-in periods.

Figure 8 summarizes the permeability reduction for different shut-in periods obtained after a seven-PV brine injection. The variation in permeability reduction is very significant, ranging from 20% to 80%. In general, after a day, the permeability reduction decreases with increasing shut-in period. The maximum permeability reduction occurs at an incubation time of about one day.

The unusual first sectional permeability change is also shown in Figure 9. This core is slightly less permeable, and the spore concentration somewhat higher than that of previous tests. The shut-in period was 11 days. The permeability reduction in the first section remained constant for a while, and then it increased significantly over the next several PV of brine injection, eventually leveling off at about 12 PV. Injection rate was 10 ml/hr.

Figure 10 shows the effect of NaCl on permeability reduction. In these tests, the NaCl concentration varied while all the other components of the nutrient remained the same. When the NaCl concentration is above about 2 wt%, the permeability reduction is not much affected by NaCl.

Discussion

In our previous paper, we reported that spore breakthrough was prominent in 710- and 1350-md cores, but no spores were detected in the effluent in 100-, 300-, or 380-md cores at an injection rate of 10 ml/hr for a range of spore suspension concentrations. With a spore concentration of 10^9 cfu/ml, we had a significant spore breakthrough in the effluent in a 650-md core. The present data clearly show that, with one PV spore suspension, spore can be transported effectively in cores of reasonable permeability at high injection rates. Thus, spores can be transported in depth in thief zones, depending on the thief zone permeability. The current data also show that the transport of spores in Berea cores depends on the amount of spores injected and the permeability of the rock, indicating that the depth of penetration can be controlled by adjusting these variables.

In this work, we wanted to test further the permeability reduction behavior in porous media in view of the microbiological studies reported previously. In the bioreactor, we observed a lag time of about 10 hr and the maximum biological activities at about 25 hr. Biopolymer production, however, continued for a couple of days. At 3 ml/hr, it takes a little more than 10 hours to inject a PV of fluid. Thus, the permeability reduction observed in Figure 4, without a shut-in period, appears quite reasonable in view of these factors.

With the experiments involving incubation, the duration for nutrient injection was about 3.5 hours, less than the lag time. Thus, we did not observe any significant

permeability reduction before the cores were shut in. With a 17-hr shut-in time, permeability continued to decline as brine injection resumed. With a one-day shut-in period, the sectional permeability reduction behavior is much more complex. The permeability reduction in the first section was the least, while that of the third section the greatest. As the brine injection continued, the first section permeability was reduced to the same level as that of the third section. This type of behavior for the first section was sometimes observed, as shown in Figure 9. At this point, we do not have a clear explanation for this.

With longer incubation periods, the permeability reduction is quite uniform along the core, as shown in Figure 7. Furthermore, the degree of reduction is generally lower than that we observed with shorter incubation times. It is interesting that the permeability did not change with a large volume of brine injection, indicating the stability of biofilm. Thus, the wash-out of the biofilm appears to be minimal. In these tests, brine was forced into the cores, but, in a real situation, water will be diverted, and the plugged zones might not experience a large volume of water.

It is clear that the process of biofilm formation and core plugging is highly complex. To begin with, there will be a distribution of spores along the core. Qualitatively, the spore concentration will decrease downstream of the core, and the nutrient concentration and composition will also undergo changes as it moves along the core. This will affect the kinetics of growth and also affect the biopolymer production as it may very well be affected by an excess or deficiency of carbon or nitrogen sources. With incubation, the situation is even more complicated as the bacteria slowly produce depolymerase, thus degrading the biofilm. At this point, we do not have a clear understanding of the relationship between biopolymer production and core plugging efficiency.

Conclusions

1. With a one-PV slug size, spores were detected in the effluent in Berea cores in significant quantities. The spore concentration in the effluent depended on variables such as core permeability, injected spore concentration, slug size, and flow rate.

2. Permeability of cores was reduced effectively by this process, ranging from 20% to almost 100%. The degree of permeability reduction depended on many variables.

3. Incubation or shut-in period had significant impact on overall permeability reduction, as well as on sectional permeability reduction behavior. There

appeared to be an optimum incubation time for the given set of experimental conditions used.

4. Permeability reduction persisted for many PV of brine injection, indicating the stability of biofilm formed.

5. For Salton-1, the permeability reduction is not much affected when the NaCl concentration is above about 2 wt% for the given experimental conditions.

Acknowledgment

We would like to thank L. E. Henry, who did the microbiological work.

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Table 1 Core Test Conditions

Run	Ka, md	Porosity	PV, ml	Flow Rate, ml/hr	Comment
1	860	0.27	41.1	10	Fig. 1
2	1310	0.26	40.2	100	Fig. 2, 1 PV
3	1610	0.26	39.3	100	Fig. 2, 0.75 PV
4	1340	0.25	39.3	100	Fig. 2, 0.5 PV
5	1820	0.26	40.3	3	Fig. 3
6	1820	0.26	40.3	10	Fig. 4
7	1150	0.23	34.5	10	Fig. 5
8	1280	0.23	34.5	10	Fig. 6
9	1350	0.23	34.5	10	Fig. 7
10	1154	0.23	34.5	10	Fig. 8, 0.75 day
11	1275	0.23	34.5	10	Fig. 8, 1 day
12	1346	0.23	34.5	10	Fig. 8, 1.75 days
13	1211	0.23	34.5	10	Fig. 8, 3 days
14	1346	0.23	34.5	10	Fig. 8, 7 days
15	1275	0.23	34.5	10	Fig. 8, 33 days
16	1280	0.26	39.8	10	Fig. 9
17	1350	0.23	34.5	10	Fig. 10, 0% NaCl
18	1350	0.23	34.5	10	Fig. 10, 1% NaCl
19	1350	0.23	34.5	10	Fig. 10, 2.5% NaCl
20	1350	0.23	34.5	10	Fig. 10, 5% NaCl
21	1350	0.23	34.5	10	Fig. 10, 7.5% NaCl
22	1350	0.23	34.5	10	Fig. 10, 10% NaCl

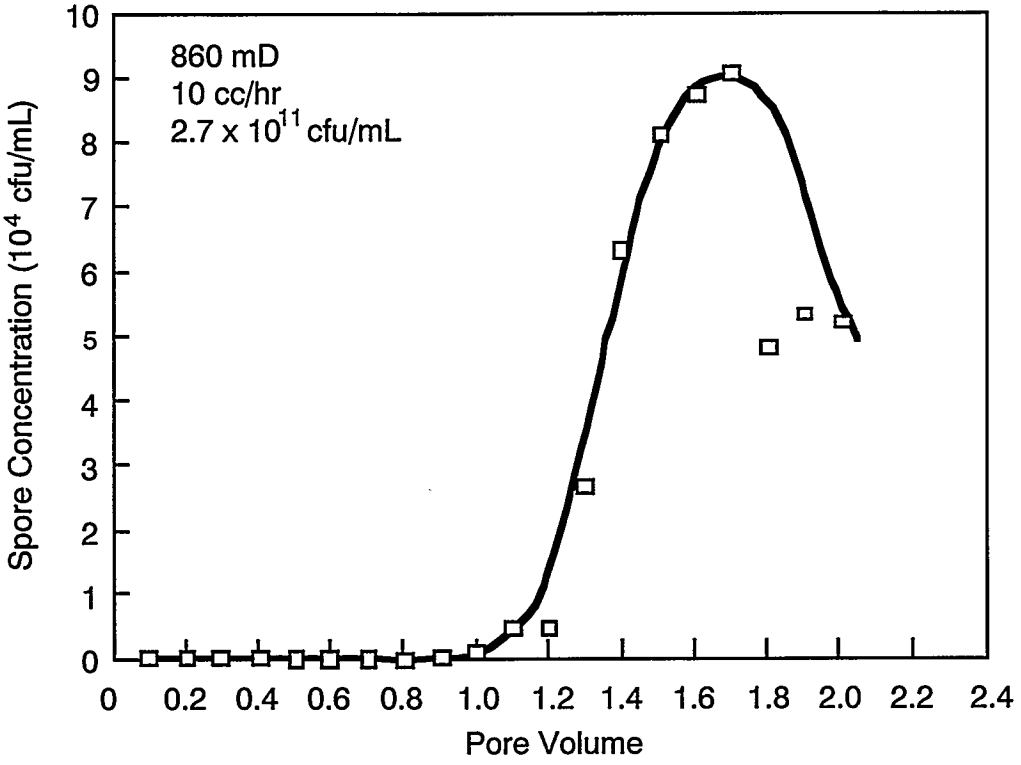

Figure 1 Spore Transport in Berea Core

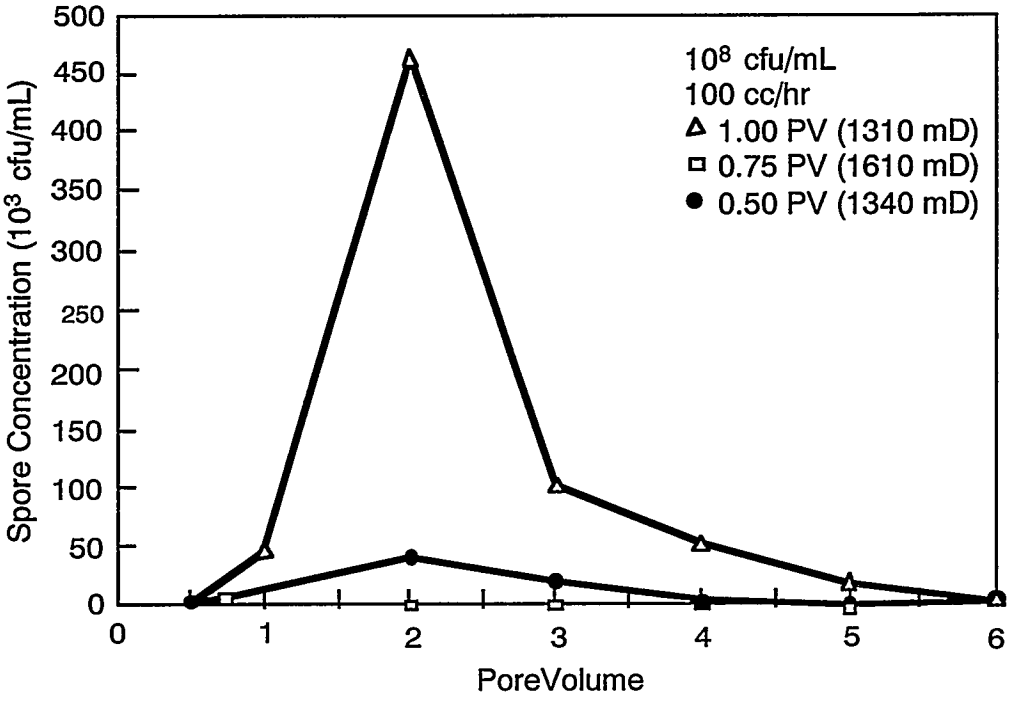

Figure 2 Effect of Slug Size on Spore Transport

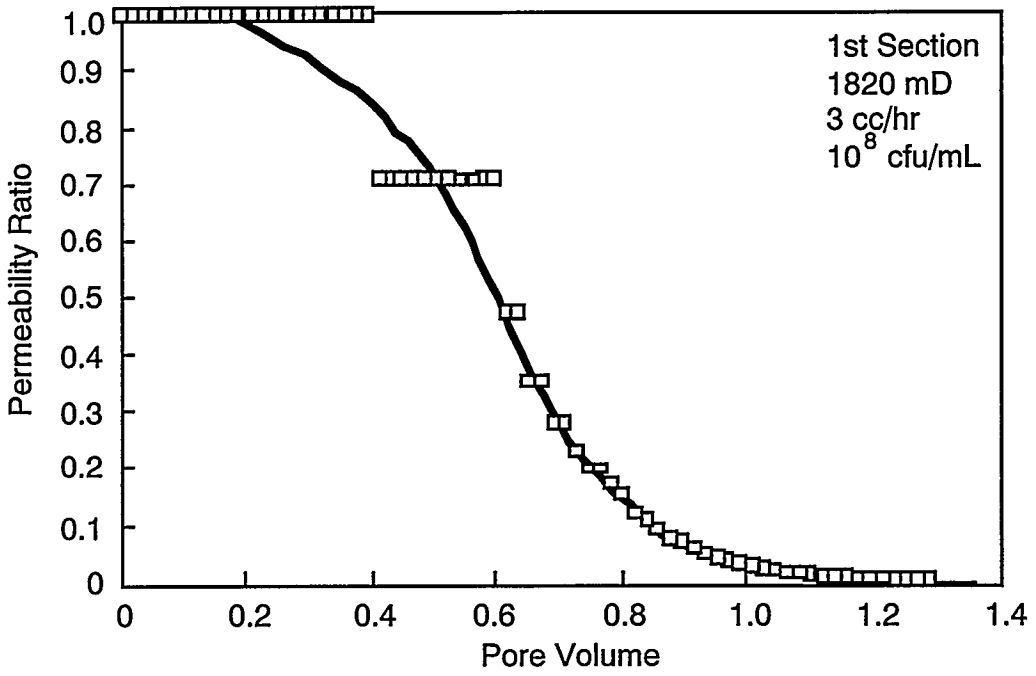

Figure 3 Permeability Reduction during Nutrient Injection

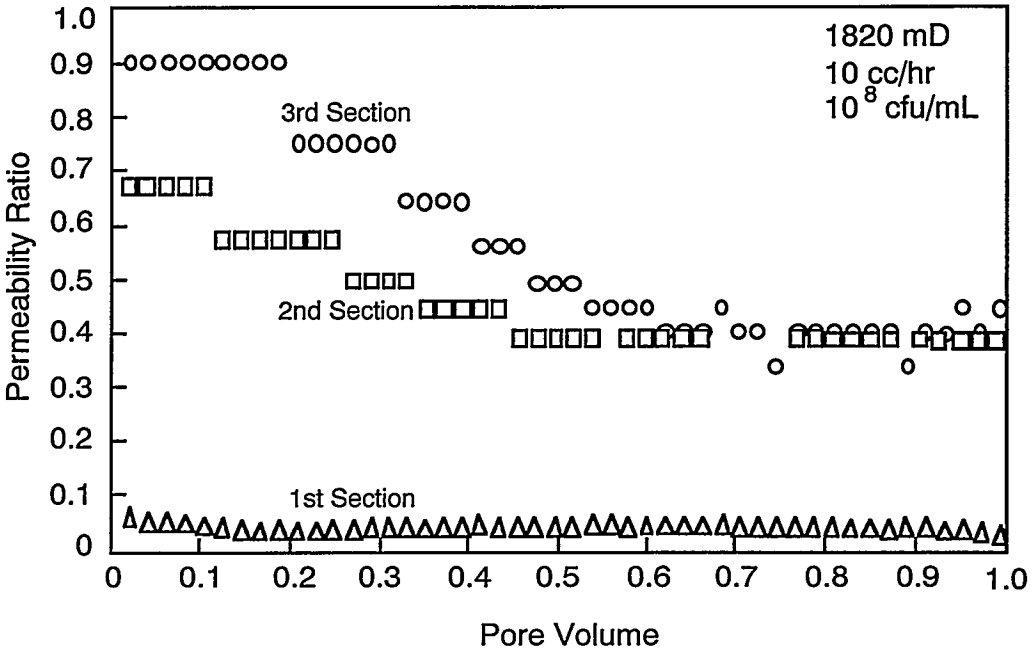

Figure 4 Permeability Reduction during Postbrine Flush

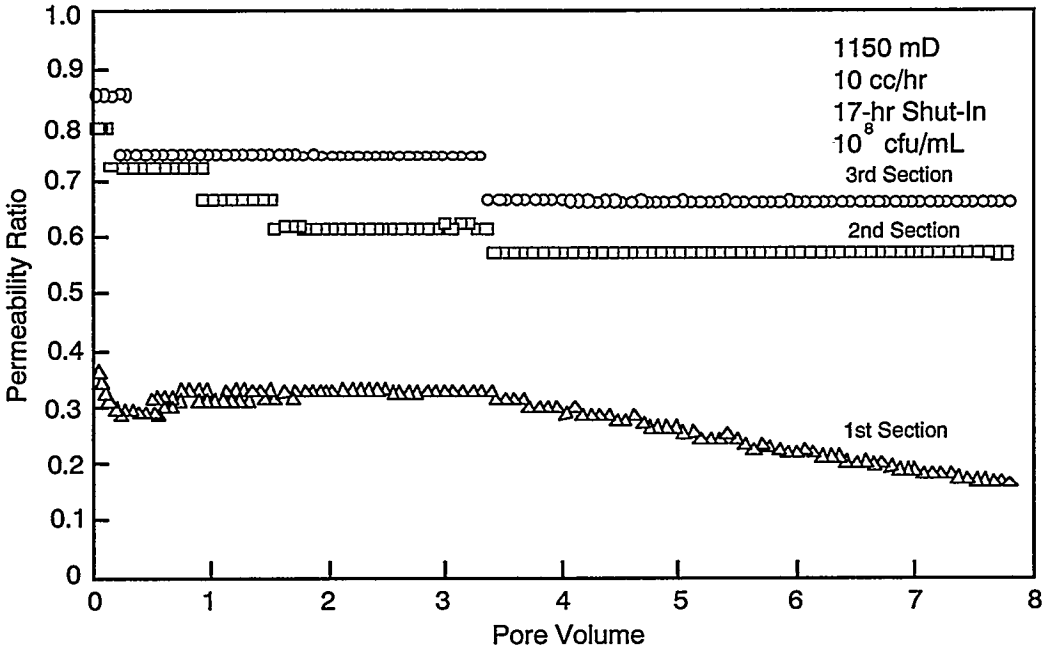

Figure 5 Permeability Reduction during Postbrine Flush

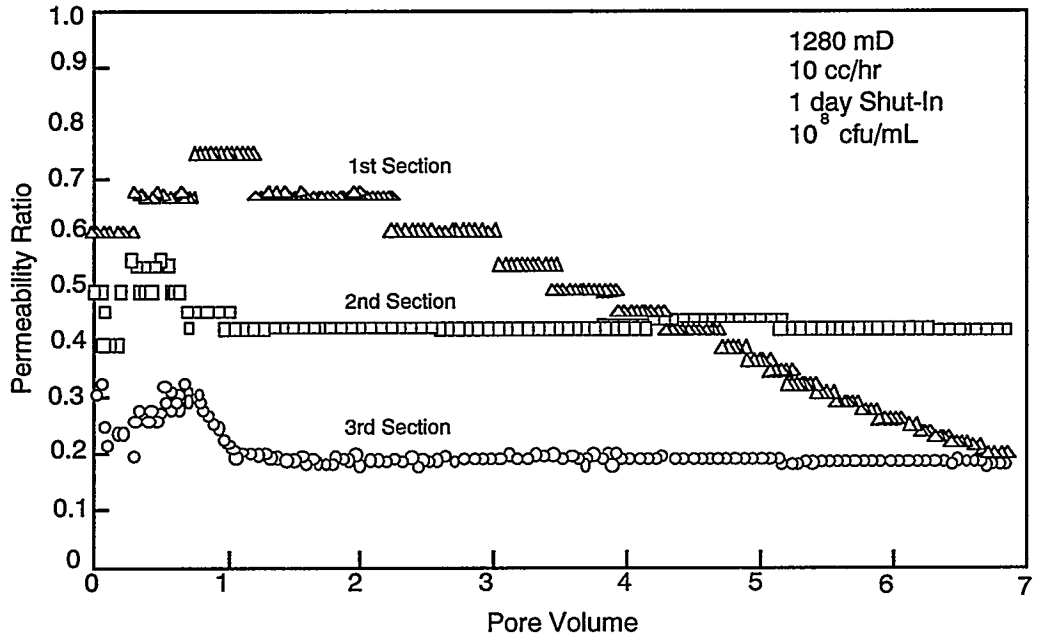

Figure 6 Permeability Reduction during Postbrine Flush

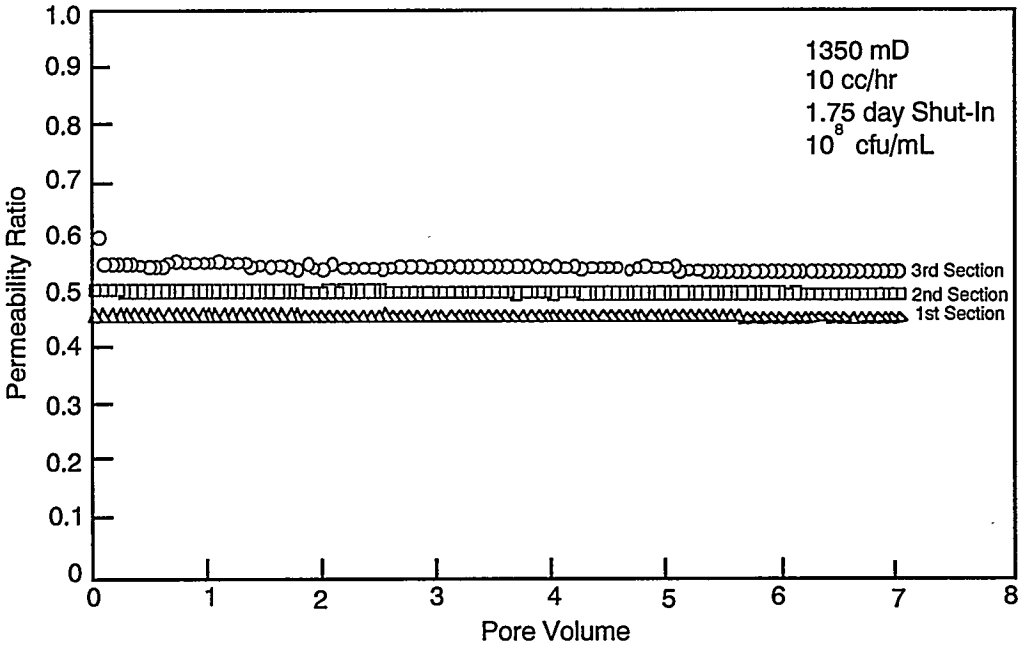

Figure 7 Permeability Reduction during Postbrine Flush

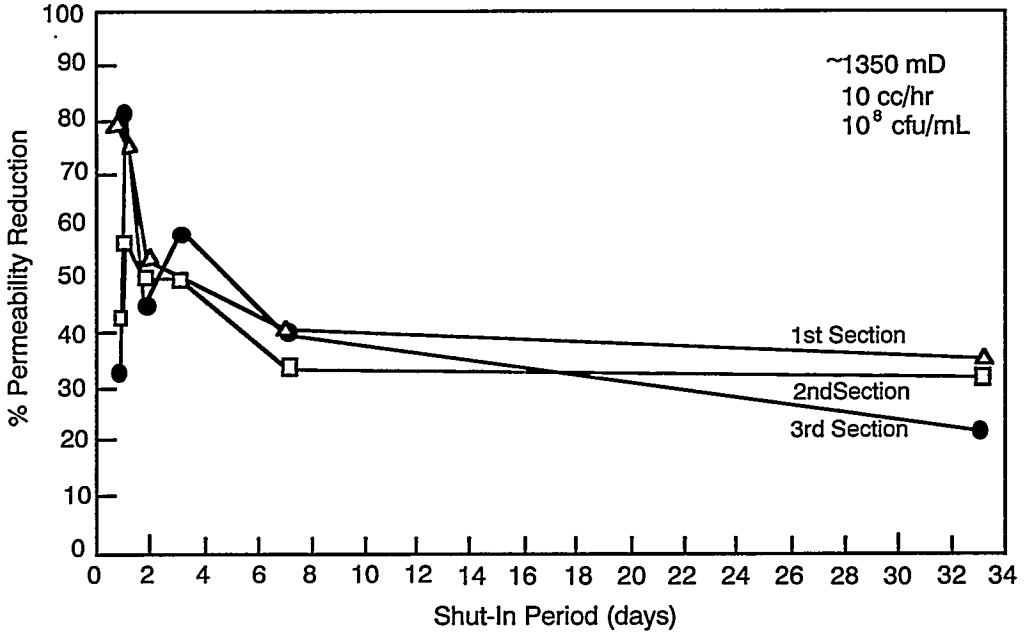

Figure 8 Permeability Reduction during Postbrine Flush after Shut-in

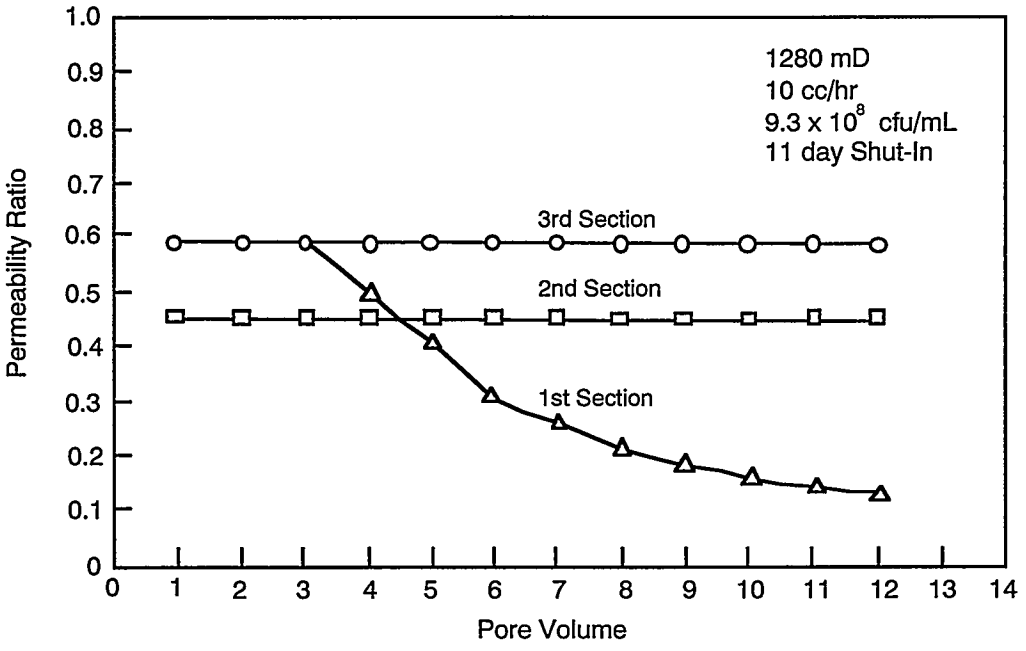

Figure 9 Permeability Reduction during Postbrine Flush

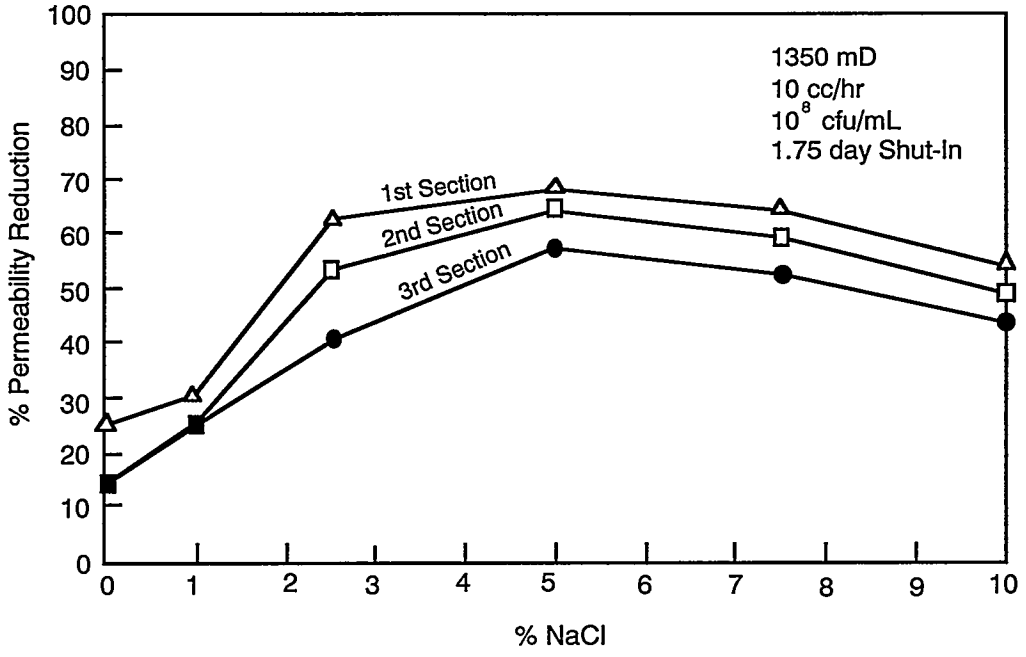

Figure 10 Permeability Reduction during Postbrine Flush

Biopolymer System for Permeability Modification in Porous Media

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Abstract

New technologies are needed to reduce the current high rate of well abandonment. Improved sweep efficiency, reservoir conformance, and permeability modification can have a significant impact on oil recovery processes. Microorganisms can be used to selectively plug high-permeability zones to improve sweep efficiency and impart conformance control. Studies of a promising microbial system for polymer production were conducted to evaluate reservoir conditions in which this system would be effective. Factors which can affect microbial growth and polymer production include salinity, pH, temperature, divalent ions, presence of residual oil, and rock matrix. Flask tests and coreflooding experiments were conducted to optimize and evaluate the effectiveness of this system. Nuclear magnetic resonance imaging (NMRI) was used to visualize microbial polymer production in porous media. Changes in fluid distribution within the pore system of the core were detected.

Introduction

Efforts to develop methods for sweep improvement stem from the need to improve the efficiency of applied recovery methods. Inherent reservoir properties and characteristics can significantly impact field production performance. Effective modification of reservoir sweep can improve the economics of an oil recovery process. These reservoir conformance treatments can extend the productive lives of active recovery projects, curtailing the prospect of premature abandonment. Current state-of-the-art technology in this area uses crosslinked polymer technology to alleviate problems associated with reservoir heterogeneity. Treatments in both injection and production wells have been applied, with about a 50% chance of success. Limitations of technology are being addressed by continued research efforts in the area, but many of the current applications still rely on the use of crosslinking agents that may pose an environmental hazard.

This paper addresses the development of alternative methods for sweep improvement of oil recovery processes. Experimental studies were conducted to evaluate the effectiveness of these methods for permeability modification. This included screening studies to identify conditions suitable for the application of these treatments.

Experimental Apparatus and Procedures

Microorganisms

A polymer-producing microorganism, designated as NIPER 11, which is most probably a strain of *Leuconostoc mesenteroides*, was isolated from frozen peas.[1] This microorganism was tested with various nutrients to find the best polymer-producing system. Many experiments were conducted to define conditions in which this system would be effective for permeability modification. This strain of microbe ferments sucrose and produces lactic acid, acetic acid, ethanol, and a polymer, dextran. This polymer can be used to plug high-permeability zones in petroleum reservoirs, diverting fluids to oil-bearing low-permeability zones. Another polymer-producing microorganism, designated as TG_2-32, which is a strain of *Bacillus licheniformis*, was isolated from desert conditions in Arizona.

Flask Tests

Solutions were prepared on a weight/volume basis. Microbial counts were determined using the Standard Plate Count Method. An Orion model 301 pH meter was used for all pH measurements. The standard nutrient used was 10% sucrose and 1% peptone in deionized water, unless otherwise stated.

Experiments were conducted to evaluate the effects of salinity, pH, temperature, and nutrient concentration on the growth of microorganisms and polymer production.

Corefloods

Unfired Berea sandstone cores were used in these studies. Cores were drilled 3.8 cm (1.5 in.) in diameter and 26.5 cm (10.5 in.) in length. Coreflooding experiments were conducted to determine the feasibility of the application of this system for pore plugging.

CT Imaging

A multilayer sandpack model was used for computed tomography (CT) experiments to determine the feasibility of applying this system to pore plugging. The model was packed with finely ground crushed and sieved Berea sandstone of various grain sizes. The vibration technique was employed to help pack the model as tightly and as uniformly as possible. Aluminum was used in the construction of the model to allow for CT monitoring of the progress of the experimental run.[2]

MRI

Magnetic resonance imaging has been developed at NIPER during the past few years into a useful tool for the visualization of fluid distributions within the pore spaces of rock at pore scale.[3] Coreflooding experiments were conducted to visualize the effects of this biopolymer system for pore plugging.

Results

Salinity Tolerance

An experiment was conducted with NIPER 11 in standard nutrient using salinity concentrations ranging from 0% NaCl to 4% NaCl. The test tubes were incubated aerobically at 28°C. In the range from 0% to 4%, good growth was achieved, although it took longer to reach maximum growth at the higher salt concentrations (see Fig. 1). Polymer production was good at 0% and 0.5% NaCl, but no polymer was visible in the tubes with higher salt concentrations up to the end of the experiment (103 hours). The tubes were checked again at 552 hours, and polymer was observed in the salinity range up to 2% NaCl.

A second salinity gradient experiment was conducted over a longer time period to determine if growth would eventually increase at the higher salinity levels. NaCl concentrations ranged from 4% to 6% (see Fig. 2). Microbial counts decreased in all concentrations of NaCl, but increased after 100 hours for the 4% NaCl. Growth could be delayed by the NaCl. Small amounts of polymer were produced in 4% NaCl after 300 hours incubation at room temperature (approximately 25°C, which is optimum for polymer production according to the literature). The upper limit for microbial growth appears to be 4% NaCl. Sample size seems to affect polymer production and/or consistency of the gel. When 10 mL test tubes are used, polymer is easily visible as a solid mass. When larger flasks are used, polymer is not visible as a solid mass, but is observed as more particulate. This may be due to adhesion of cells to surfaces, which would occur more readily in smaller volumes such as test tubes.

TG_2-32 was screened for growth, acid production, and biomass production at different salinity concentrations. Tryptic soy broth was used instead of sucrose peptone broth since previous tests showed poor growth in sucrose peptone broth with NaCl added. Concentrations of 0%, 10%, 20%, and 30% NaCl were used. The bottles were incubated anaerobically at 30°C. Microbial counts and pH were measured daily, as shown in Figure 3. Bottles with no NaCl and 10% NaCl developed a lot of biomass, but no evidence of polymer. This would be expected since there was no sucrose in the broth. Growth was delayed in 10% NaCl, but eventually reached the same level as those without NaCl. The bacteria did not grow in 20% and 30% NaCl. Another experiment was conducted at salinity levels between 10% and 20% to determine the upper limit. The bacteria grew well in 10%, 12%, and 14% NaCl, although there was a lag at 14%, as shown in Figure 4. The upper salinity limit for this strain of bacteria appears to be 14%.

TG_2-32 was screened for growth, acid production, and polymer production at five salinity concentrations. Nutrient broth consisting of 10% sucrose and 1% peptone in deionized water was used to obtain the most polymer production. Concentrations of 0%, 2.5%, 5%, 7.5%, and 10% NaCl in square milk dilution bottles were used, each containing 200 ml broth. The cultures were incubated anaerobically at 30°C. Microbial counts and pH were measured daily for 10 days. Results shown in Figure 5 indicate growth is inhibited in the presence of NaCl, even at a concentration of 2.5%. The broth with no NaCl turned a milky white after 72 hours incubation, indicating polymer production. The broth containing 2.5% NaCl did show slight visual evidence of polymer production after 144 hours incubation.

pH Experiments

An experiment to evaluate growth and polymer production of NIPER 11 in standard nutrient with a pH range of 3 to 6.45 was conducted. The flasks were incubated aerobically at 28°C. Acid production by NIPER 11 reduced the pH to around 4 (see Fig. 6). Growth was very good in the pH range of 4.5 to 6.45. Polymer was produced only in the pH range of 5.5 to 6.45. An experiment was conducted using buffered solutions to keep the pH constant. The standard nutrient solution was buffered with soluble buffers ranging from pH 3 to pH 6.45 (see Fig. 7). The flasks were incubated aerobically at 28°C. Small amounts of polymer were produced in nutrient with pH 5, 6, and 6.45. No polymer was produced at pH 3 and 4. The pH had to be adjusted daily in the pH 6 and 6.45 flasks. Further experiments studied the effect of using insoluble buffers in the nutrient solutions on overall solution properties. According to the literature, the pH of media used for growing bacteria that produce large amounts of acid cannot be controlled by soluble buffers. Experiments were conducted to test growth and polymer production of NIPER 11 in nutrient with a high pH since the coreflood effluent had a pH between 8 and 8.4. An experiment to evaluate growth and polymer production of NIPER 11 in standard nutrient buffered with 0.3% calcium carbonate was conducted. Each of the nutrients was adjusted to pH 5, 6, 7, 8, and 9. Flasks were incubated aerobically at 30°C. Polymer production was apparent in the pH 5 nutrient only. Growth was considerably less in the higher pH range (see Fig. 8).

TG_2-32 was screened for growth, acid production, and polymer production at four pH levels. Nutrient broth consisting of 10% sucrose and 1% peptone was mixed with pH 3, 5, 7, and 9 buffer solutions in square milk dilution bottles were used, each containing 200 ml broth. The cultures were incubated anaerobically at 30°C. Microbial counts and pH were measured daily for 10 days. Results are shown in Figure 9. This strain of *Bacillus* did not grow at pH 3 and 5, so it was then tested at pH 6 to find out its lower limit. Best growth and polymer production was at pH 9,

which might be expected since this strain was isolated from a desert environment. This strain was then tested at pH 10 to find the upper limit.

Temperature Effects

NIPER 11 was incubated at 35°C to test the effects of this temperature on bacterial growth and polymer production. Standard nutrient was used, and the bacteria were incubated aerobically (see Fig. 10). A small amount of polymer was produced.

TG_2-32 was evaluated for growth, acid production, and polymer production at various temperatures from 30° to 55°C. The microbes were grown in nutrient broth consisting of 10% sucrose and 1% peptone in deionized water. The bottles were incubated aerobically. Microbial counts and pH were measured daily. Figure 11 shows good growth at 30°, 35°, and 45°C, but no growth at 55°C. The broth turned white, indicating polymer production, in all cases except at 55°C. The temperature limit for this strain is between 45° and 55°C.

Nutrient Concentration Experiments

A nutrient concentration experiment was conducted with NIPER 11 (see Fig. 12). The media contained 1% peptone, 0.5% NaCl, and sucrose ranging from 2.5% to 30%. The flasks were incubated aerobically at 28°C. A small amount of polymer was produced in all concentrations of sucrose. The pH was reduced from around 6.4 to 4.2 in all concentrations. A sucrose concentration of 10% is enough to give good growth and polymer production, so higher concentrations are not necessary.

Corefloods

A brine-saturated high-permeability brown Berea core was injected with NIPER 11 microbes grown in standard nutrient to determine if microbial polymer production would cause plugging. The microbes were injected at 0.22 ml/min. The pressure drop across the core increased from 0.25 psi to 39.5 psi after injecting 6 PV of microbes. The permeability was reduced from 3 darcies to 19 md (see Table 1).

A 467 md Berea core was saturated with brine, followed by oil, then waterflooded to residual oil saturation. The permeability after waterflooding was 54 md. The core was then injected with 0.5 PV NIPER 11 in nutrient broth, followed by 0.5 PV nutrient broth. The core was shut in for five days, then waterflooded at one ft/day. The additional oil recovery was low, 4.9% recovery efficiency, but the permeability

was reduced to 3 md (see Table 1). The pressure drop during waterflooding rose to 35 psi.

A 483 md Berea core was saturated with brine, followed by oil, then waterflooded to residual oil saturation. The permeability after waterflooding was 26 md. The core was then injected with 0.2 PV NIPER 11 in nutrient broth, followed by 0.3 PV nutrient broth. The core was shut in for five days, then waterflooded at one ft/day. Recovery efficiency was 10.8%. Permeability was reduced to 5 md (see Table 1). Pressure drop during waterflooding rose to 33.5 psi.

Another coreflood was completed using NIPER 11. A Berea core was saturated with 0.5% NaCl brine, then injected with one PV of standard nutrient that had just been inoculated with NIPER 11 microorganisms. The injection was done at one ft/D, then the core was shut in and incubated for six days at room temperature. The permeability of the core was reduced from 500 md to 30 md (see Table 1). NIPER 11 also produces gas, so the reduction in permeability may be due to both gas and polymer production.

Residual Resistance Corefloods

A coreflood was completed to provide data on residual resistance factors for the microbial simulator. A Berea sandstone core was saturated with 0.5% NaCl brine and then injected with one pore volume of sucrose peptone broth that was inoculated with NIPER 11 bacteria just prior to injection. The core was then shut in for six days, and the pressure built up to 37.5 psi. The core was then flooded with brine, and flow rates and pressures were measured. The residual resistance factors were then calculated. A coreflood was also conducted with TG_2-32 to evaluate its effect on pore plugging (see Fig. 13).

CT/Imaging Experiments

A fluid flow experiment using a sandpacked/layered model to evaluate the effectiveness of microbial polymer treatments for sweep improvement was conducted. This experiment was conducted in a three-layer model, yielding a permeability ratio of 1:10:20. The highest permeable layer was at the top of the model, the second highest permeable layer was at the bottom of the model. CT scans were done during all phases of the coreflood to monitor fluid flow. The model was injected with tagged brine to show the path of fluid flow, which was mainly through the most permeable layer. The top layer was then injected with one PV of sucrose peptone broth inoculated with NIPER 11 just prior to injection. The model was shut in for one week. The shut-in pressure increased to 38 psi. A back

pressure regulator was installed on the model to prevent the gas from blowing down, and a second tracer test was conducted. This test showed the high-permeability zone was blocked by biopolymer, and the fluid was diverted into the bottom layer, as shown in Figure 14. A videotape was made of this coreflood to demonstrate the effectiveness of microbial permeability modification.

MRI Experiments

Microorganisms can be retained in porous rock by several possible mechanisms. High-resolution NMRI can image the fluids in the pore space at pore scale resolution. By comparing before and after images of the pore space after microbial growth and activity, it may be possible to identify sites of microbial influence from growth or metabolite production on fluid distribution. A microcoreplug of Bentheim sandstone was used to evaluate the effects of NIPER 11. A previous microcoreplug experiment was conducted with NIPER 7, a *Clostridium* that produces large amounts of carbon dioxide. The purpose of using NIPER 11 was to compare with the previous coreplug experiment. The NMRI results showed that changes in fluid distribution occurred over time in the presence of the NIPER 11 microorganisms (see Fig. 15). Some areas within the pore system of the coreplug were occupied by microbial growth, reducing the pore volume. Other areas revealed some apparent expansion of local pore space. After the imaging experiments were completed, the coreplug was flushed with brine. Very slow flow rates were observed, indicating a significant obstruction of the pore network. This was not observed when the *Clostridium* coreplug experiment was conducted. Surfaces of the pore space also appeared smoother after the NIPER 11 treatment when compared to the brine alone.

Conclusions

If properly applied, these microbial systems can cause blockage of high-permeability zones and fluid diversion. Further screening must be done to develop systems which will work in various reservoir conditions.

Acknowledgments

This work was sponsored by the U.S. Department of Energy under an M&O contract agreement DE-AC22-94PC91008. The authors wish to thank Deanna B. Evans, Idell A. Cook, Sam W. F. Swan, Kathy M. Bertus, and Daryl A. Doughty, of NIPER/BDM-Oklahoma, Inc., Renee Horning, Scott Bailey, and Michele Bruning, DOE/AWU Summer Internees, and Mike Dennis of Geo-Microbial

Technologies for their assistance in all phases of the experimental work performed for this project.

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Table 1 Coreflood Results

Initial Permeability (mD)	Permeability after Treatment (mD)	PV Injected (%)
NIPER 11		
3,000	19	6
467	3	0.5
483	5	0.2
500	30	1
TG_2-32		
424	83	1

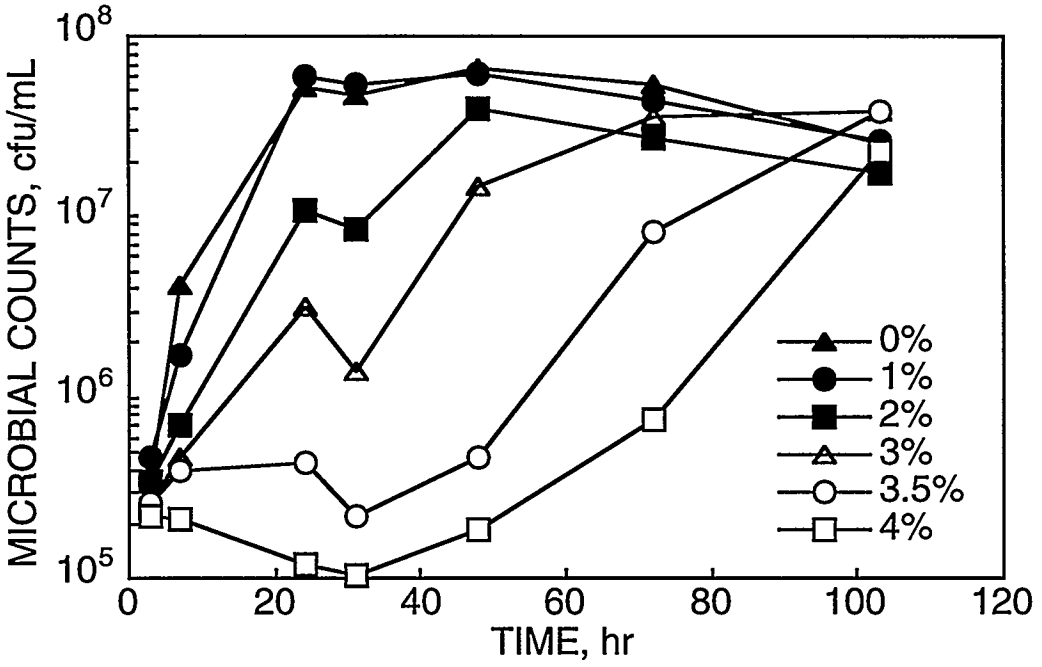

Figure 1 Effect of Salinity (%NaCl) on Growth of NIPER 11 in Sucrose Peptone Broth

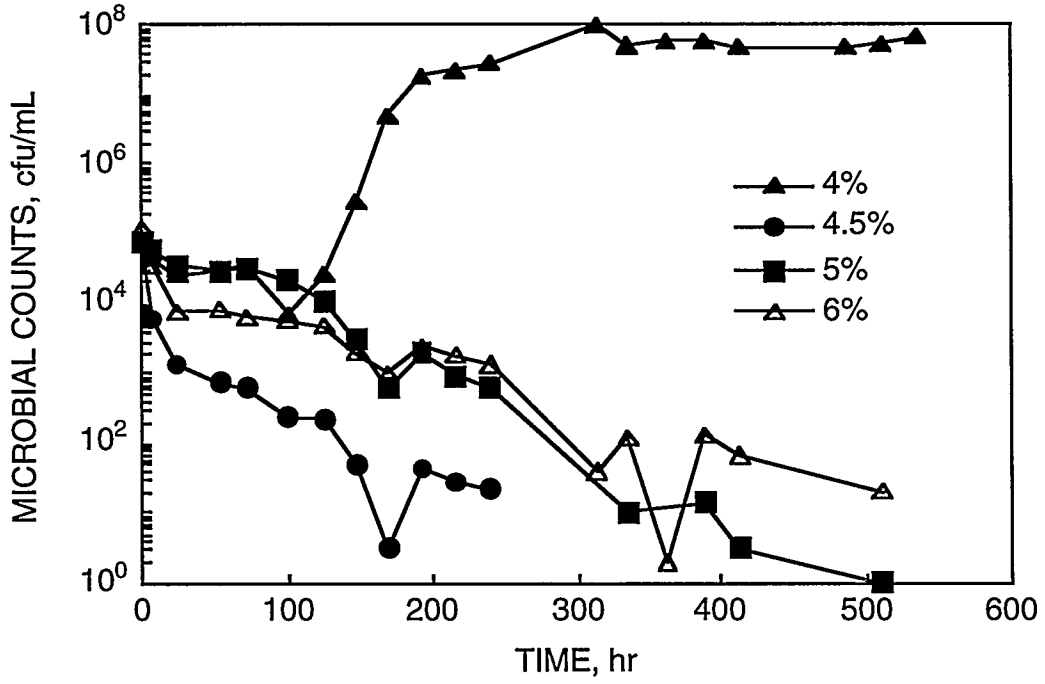

Figure 2 Effect of Salinity (%NaCl) on Growth of NIPER 11 in Sucrose Peptone Broth

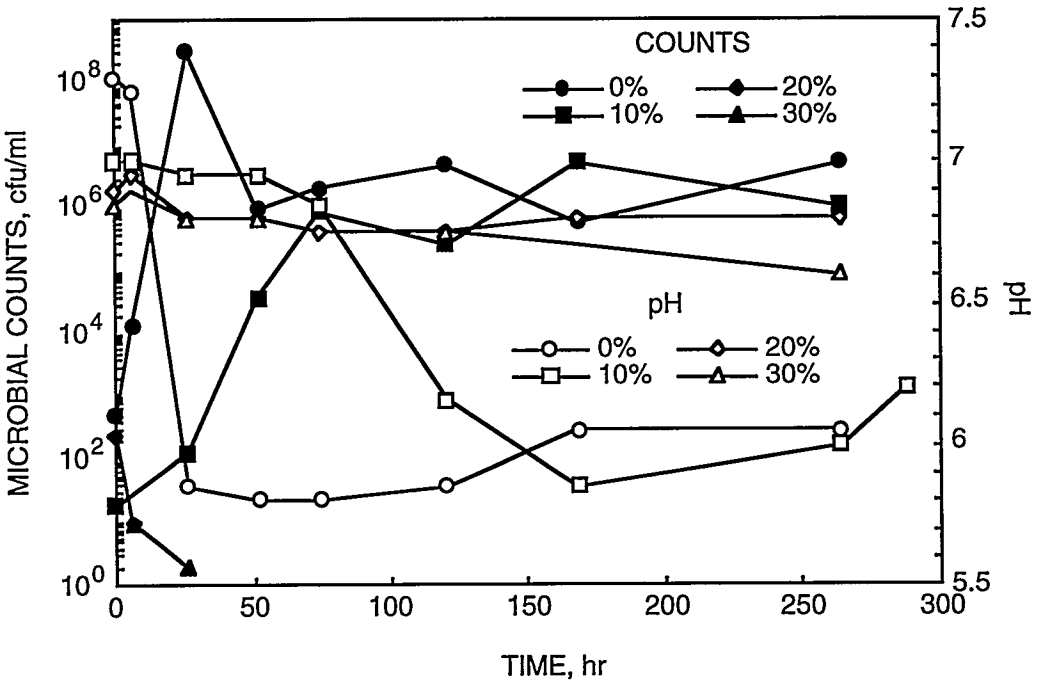

Figure 3 Effect of Salinity (%NaCl) on Growth of TG_2-32 in TSB

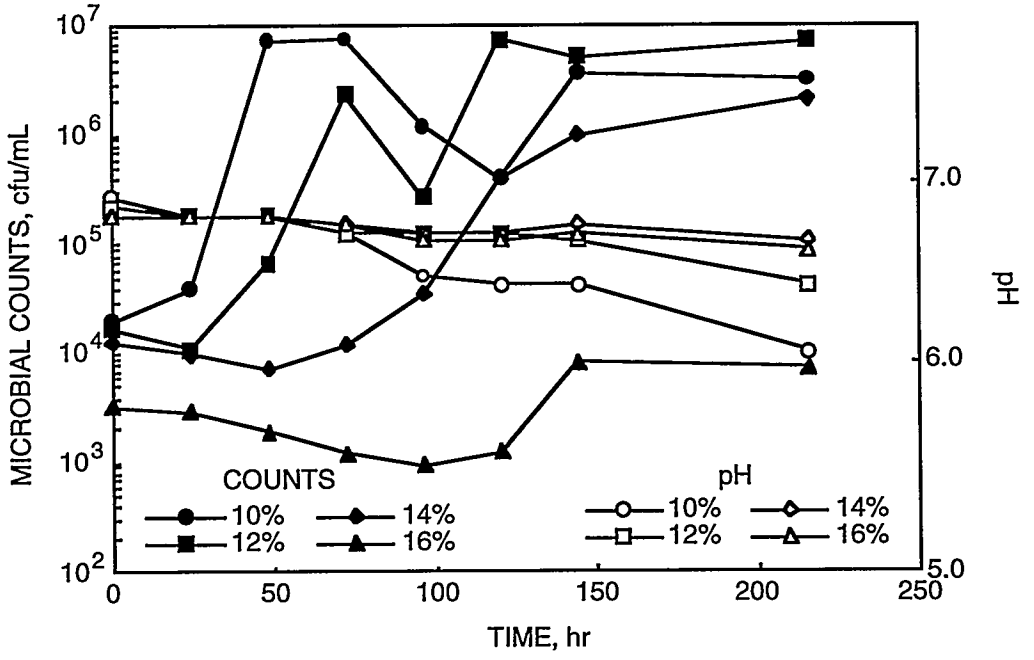

Figure 4 Effect of Salinity (%NaCl) on Growth of TG_2-32 in TSB

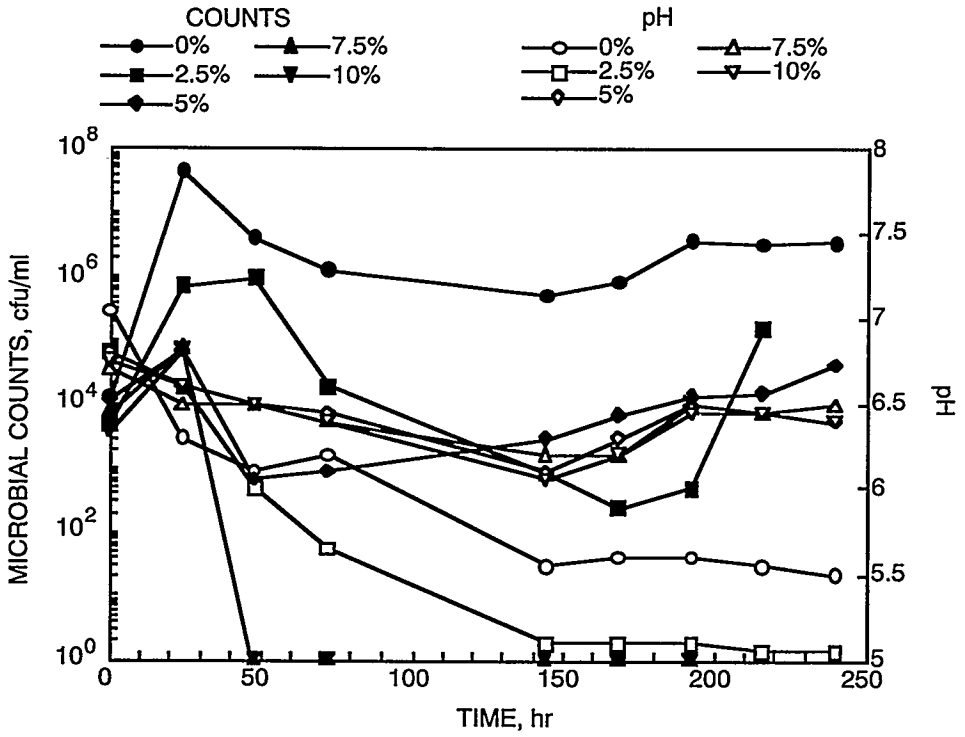

Figure 5 Effect of Salinity (%NaCl) on Growth of TG_2-32 in Sucrose Peptone Broth

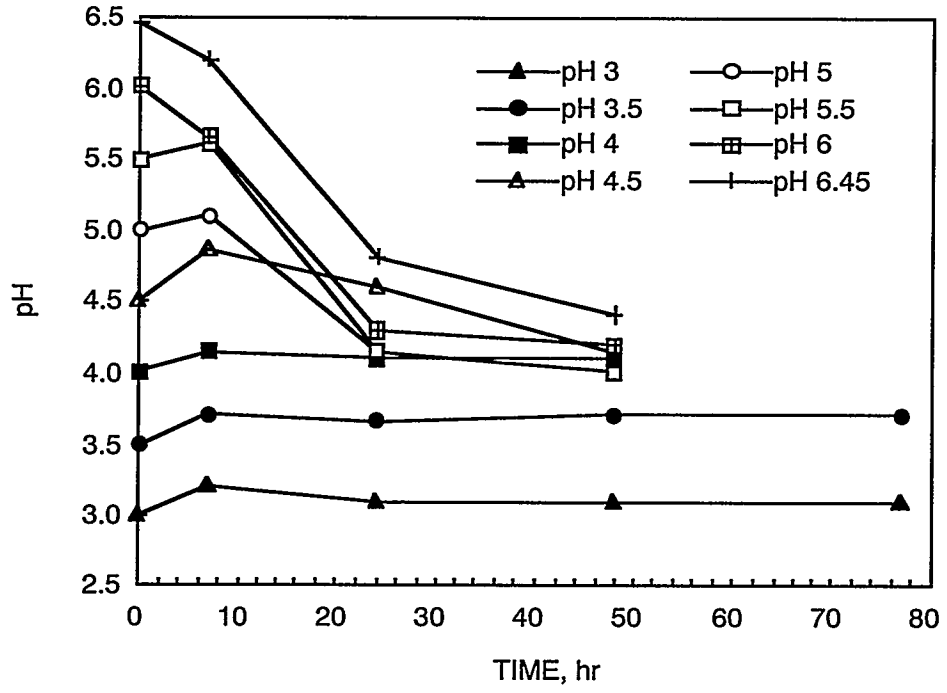

Figure 6 NIPER 11 Acid Production

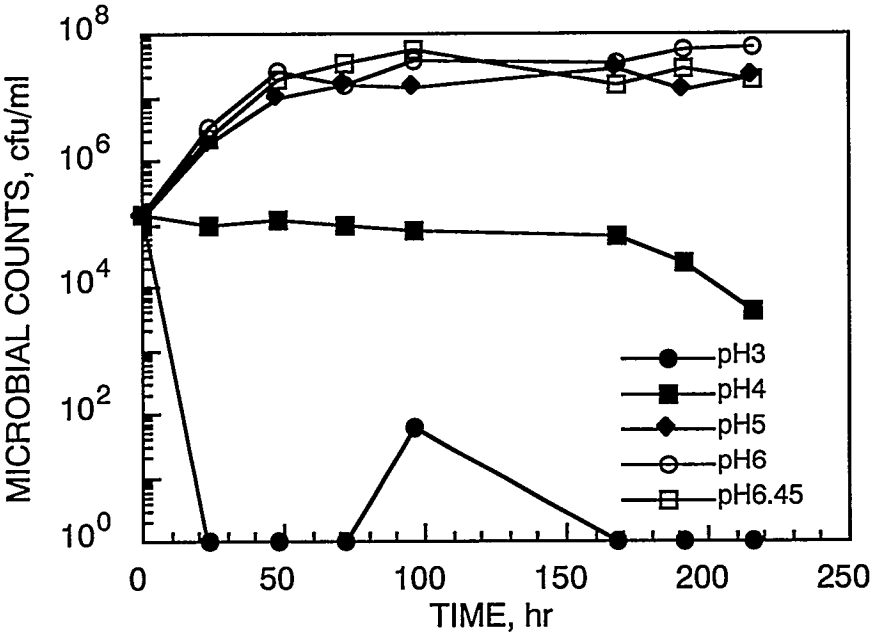

Figure 7 Effect of pH on Growth of NIPER 11

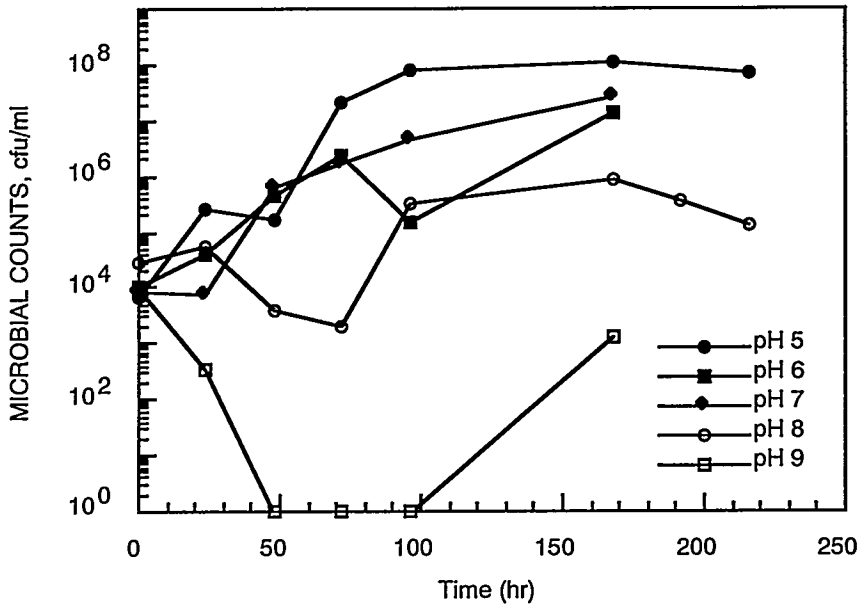

Figure 8 Effect of Buffering with Insoluble Buffer on Growth of NIPER 11

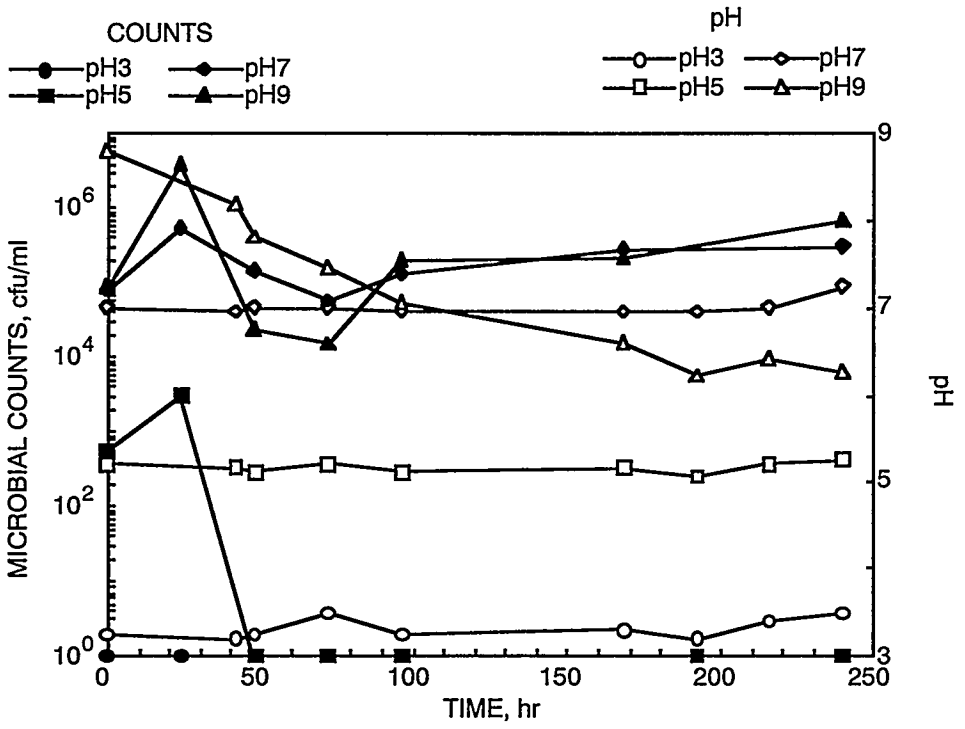

Figure 9 Effect of pH on Growth of TG_2-32

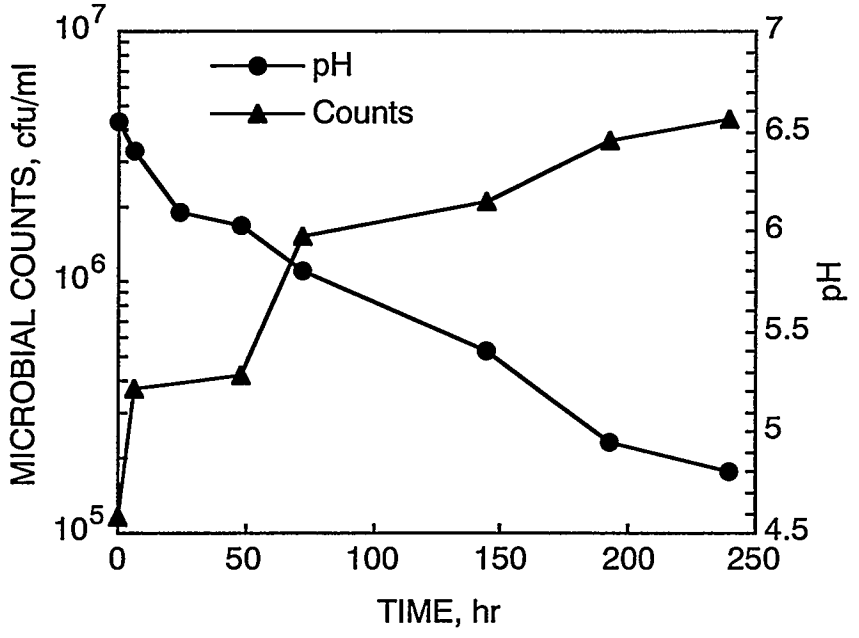

Figure 10 Effect of Temperature (35°C) on Growth of NIPER 11

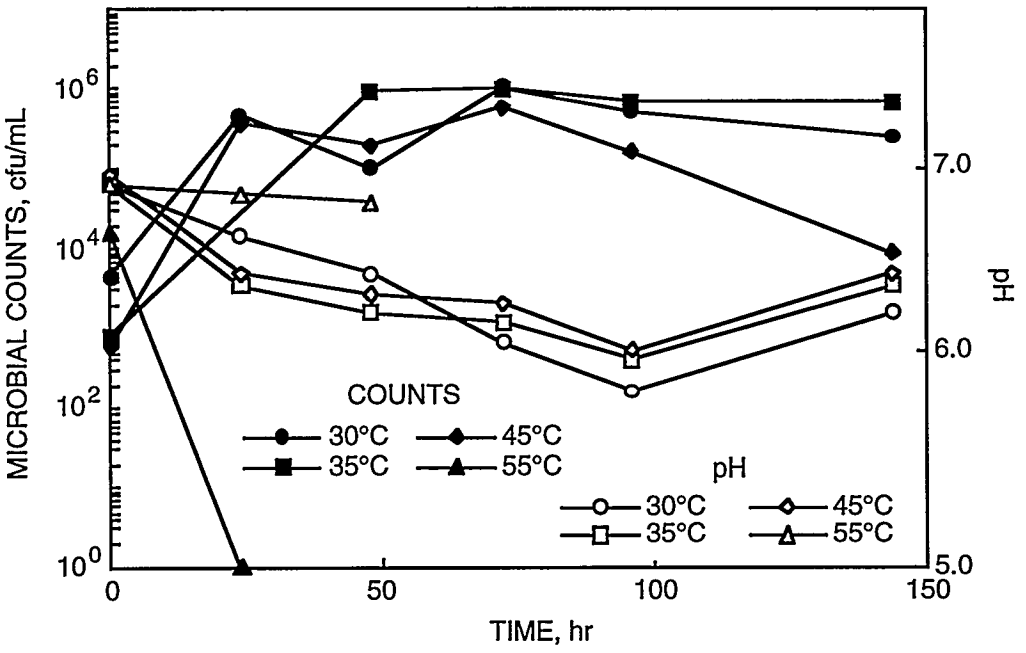

Figure 11 Effect of Temperature on Growth of TG_2-32

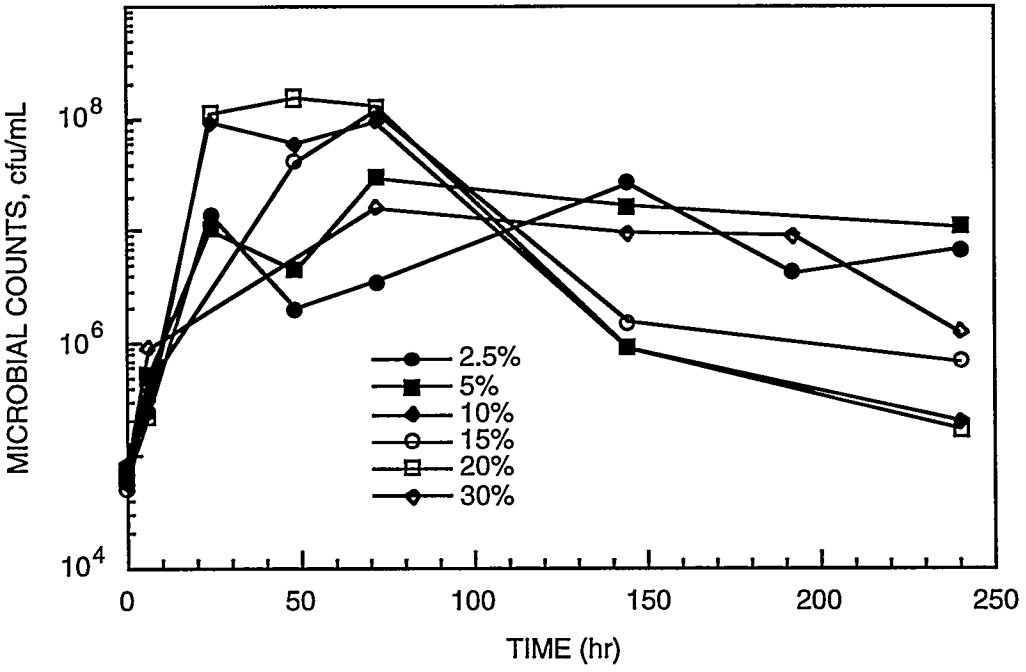

Figure 12 Effect of Sucrose Concentration on Growth of NIPER 11

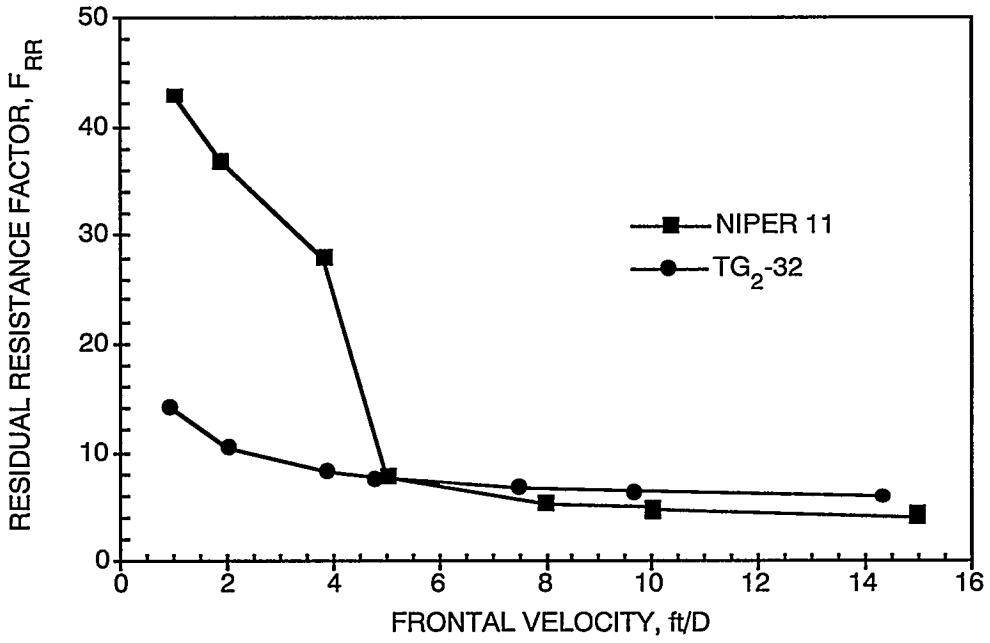

Figure 13 F_{RR}—NIPER 11 vs. TG_2-32

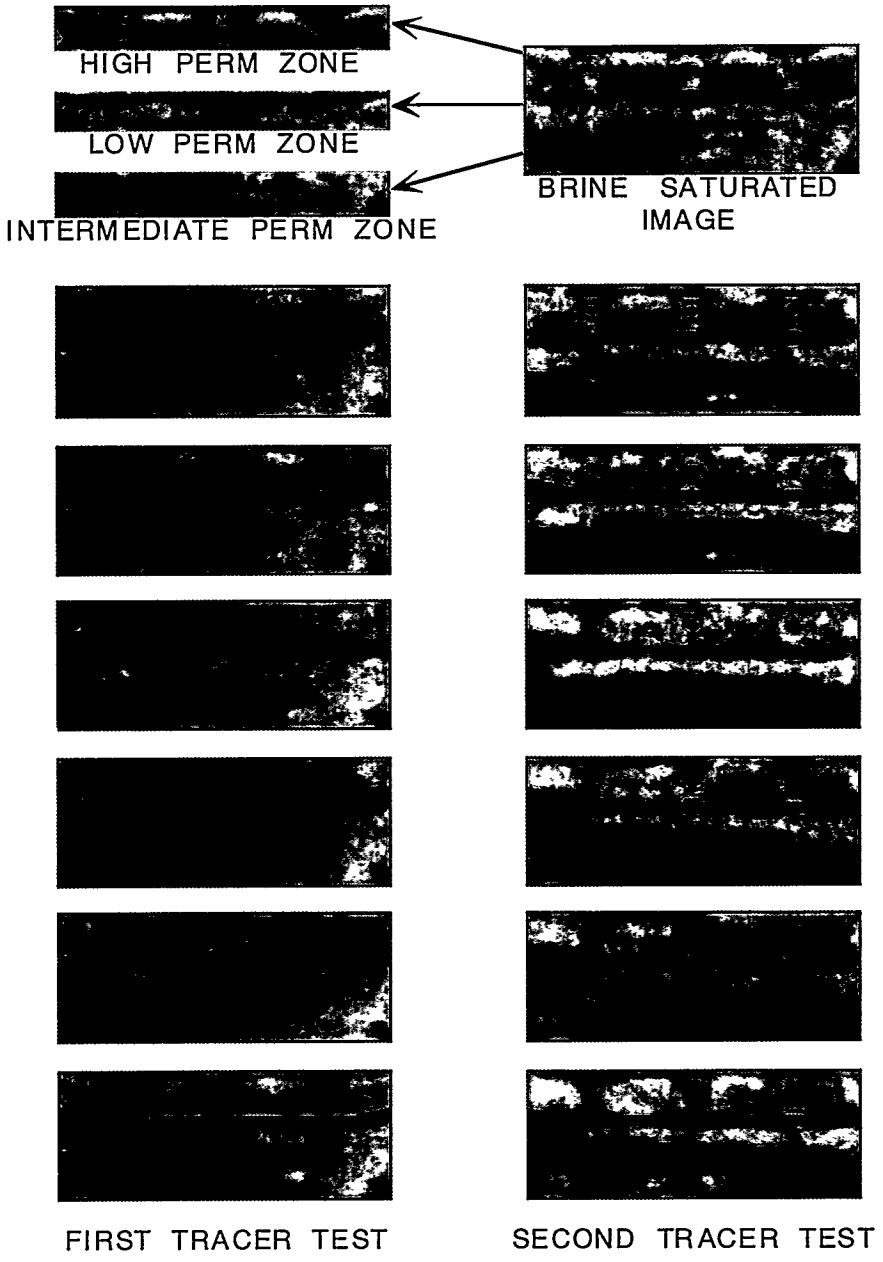

Figure 14 Fluid Diversion Experiment Monitored by CT

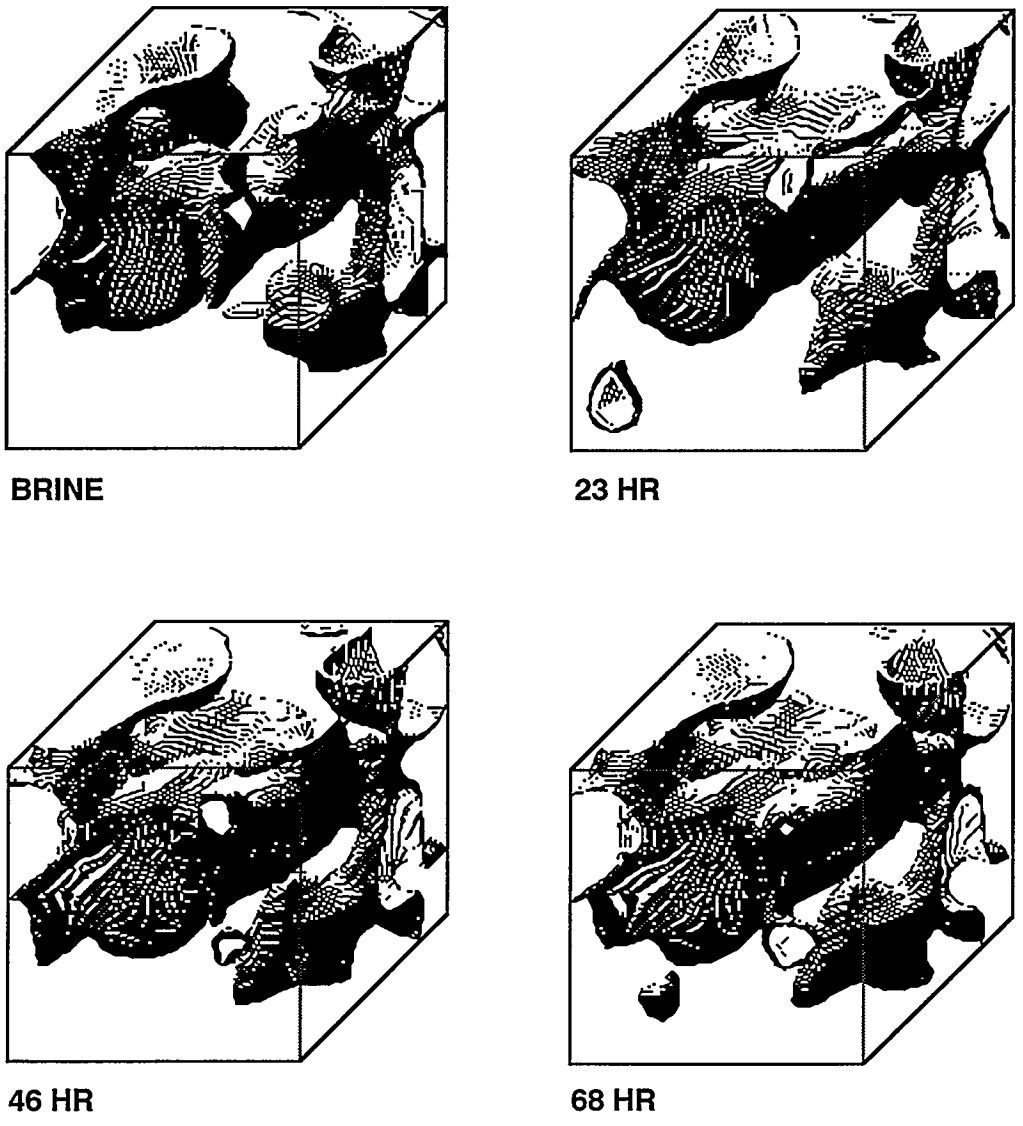

Figure 15 NMR Imaging of Pore Structure before and after Microbial Polymer Production (Pore Crevices Became Filled in with Polymer)

Characteristics of Enriched Cultures for Bio-Huff-'n'-Puff Tests at Jilin Oil Field

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Abstract

Three enriched cultures (48, 15a, and 26a), selected from more than 80 soil and water samples, could grow anaerobically in the presence of crude oil at 30°C and could ferment molasses to gases and organic acids.

Oil recovery by culture 48 in the laboratory model experiment was enhanced by 25.2% over the original reserves and by 53.7% over the residual reserves.

Enriched culture 48 was composed of at least 4 species belonging to the genera *Eubacterium, Fusobacterium, and Bacteroides*. This enriched culture was used as inoculum for MEOR field trials at Jilin oil field with satisfactory results. The importance of the role of these isolates in EOR was confirmed by their presence and behavior in the fluids produced from the microbiologically treated reservoir.

Introduction

The selection and breeding of suitable cultures of bacteria should continue to be key to the real breakthroughs in MEOR. It has been reported that mixed or enriched cultures, including aerobic, facultative, and anaerobic bacteria, had been successfully used for single well bio-huff-'n'-puff technology.[1-6] Our previous work indicated that the basic investigations on obligate anaerobes are very important for in-situ application of MEOR.[7]

This paper describes the characteristics of enriched cultures from environmental samples for the field trials at the Jilin oil field.

Materials and Methods

Sampling and Enrichment Procedures

The methods for sampling and for screening of adapted enrichment cultures were described in our previous paper.[7] Soil and water samples were taken from the environments contaminated with or without oil at different locations. To select for gas- and acid-producing anaerobes adapted to the reservoir conditions, a 0.5 g or 0.5 ml sample was inoculated into tubes with 4% molasses broth (pH 7.0–7.5) and crude oil from Jilin oil field. The inocula were incubated at 30°C. The grown cultures were separately transferred into fresh medium in an anaerobic system (Forma Scientific Co.) and incubated. To remove transient bacteria, this procedure had to be done repeatedly. In this way, only the organisms capable of growing and metabolizing anaerobically in the presence of oil and molasses remained active.

Isolation and Identification

The bacterial suspension made by the serial dilution was inoculated by roll tube cultivation. After incubation for 10–15 days, several colonies were separately picked up from the tubewall and transferred into molasses broth.

Media for identification are described in Holdeman et al.[8] All cultures were incubated anaerobically at 30°C.

Assays

The enumeration of anaerobic bacteria was carried out by the MPN method with five tubes at each dilution.

Analyses of gases, organic acids, and alcohols were performed through gas chromatography using a GC-7AG gas chromatograph (Shimadzu) or SC-3A gas chromatograph (Sichuan). Surface tension was determined with a surface tensionmeter ST-1 (Shimadzu).

Evaluation of Displacement Efficiency

The oil displacement efficiencies by enriched cultures were estimated in a laboratory scale model packed with natural or artificial sandstones. The procedure is presented in Figure 1.

Results

Selection of Enriched Cultures

The characteristics of the reservoir in the area selected for MEOR are shown in Table 1. These conditions are suitable for microbial growth and metabolism.

Three enriched cultures (48,15a, and 26a), which could better and faster ferment molasses to produce gases and organic acids, were obtained from 86 samples on the basis of quantity of gas produced. They were chosen for further study. Figure 2 shows that their gas-producing processes gradually achieved stationary phases after 100–140 days of incubation respectively. Gas-chromatographic analyses (see Fig. 3) indicated that their gas composition mainly was H_2 and CO_2. The total amount of organic acids produced by cultures 48, 15a, and 26a in 4% molasses broth was 7,035, 4,423, and 2,174 mg/l, respectively, after 144 days. The pH of media dropped from 7.5 to 5.8–5.0, and surface tension did not change.

Effects of Some Factors on Metabolite Production

Temperature. Culture 48 was incubated at different temperatures, and gas production was measured. Results showed that the highest gas production was at 30°C (see Fig. 4), which is coincident with the temperature of the test reservoir.

Additives. The bacterial viability in crude oils and oil reservoirs can be stimulated by the addition of suitable additives.[10–13] Table 2 shows that the addition of calcium carbonate, oil sand, and scum into molasses broth had a positive effect on the metabolite production by cultures 48 and 15a. The addition of scum had an obvious effect, but resulted in the production of unfavorable H_2S. It is noteworthy that the H_2S was not produced in the presence of calcium carbonate and oil sand. Calcium carbonate had the best effect on the production of gas and organic acid, which is similar to that described before.[7] It appears that the carbonate content of an oil-bearing formation might be an important factor in MEOR.

Molasses Content. Table 3 indicated that the gas production of culture 48 after 4 days of incubation was increased with molasses content in the medium. This means that the increase of molasses content is favorable for field trials.

Displacement Efficiency of the Enriched Culture

The displacement efficiency of two cultures was evaluated on the laboratory-scale model. Results (see Table 4) indicated that the oil recovery of culture 48 was increased by about 14–25% of original reserves, which was equal to 27–79% of the residual reserves. Culture 15a increased recovery by 5–14% of the original reserves (approximately 11–63% of residual oil).

Isolation and Identification of the Enriched Culture 48

The results mentioned above indicated that culture 48 is a suitable culture for the single-well biostimulation at Jilin oil field. Culture 48 was isolated and purified for characterization and identification. Four bacterial strains (48-1, 48-2, 48-3, 48-4) were obtained under anaerobic condition. Figures 5–8 showed their electron micrographs, in which the strains 48-1B and 48-2B were isolated from post-treatment samples of coproduced water of well 8-27.

Table 5 briefly summarized the characteristics of the strains isolated from culture 48. Based on their major morphological, physiological, and biochemical properties, both of motile strains 48-2 and 48-3 belong to the genus *Eubacterium*, the nonmotile rods 48-1 and 48-4 belong to the genus *Fusobacterium* and *Bacteroides*, respectively.[14] The genera of *Fusobacterium and Eubacterium* have not been previously reported on in MEOR studies.

Oil Field Tests

On the basis of the properties of culture 48 and the evaluation of results from laboratory model experiments, the culture has been successfully applied in 44 wells of Fuyu oil area at Jilin oil field. Detailed information on these tests was described elsewhere.[15]

Microbiological analyses of the coproduced water before MEOR treatment indicated that the anaerobic gas-producing bacteria were absent. After the treatment, the numbers of gas-producing bacteria in the random sample of coproduced water (well 16-18) reached up to 10^2–10^5 (see Table 6). Organic acids in the coproduced water were similar to those produced by the enriched culture 48 (see Table 2). After microbiological treatments, the CO_2 contents of producing wells were increased by 10–32% (see Table 7).

Discussion and Conclusion

It is well known that the success of MEOR trials depends on the predominance and activation of the effective bacteria in the reservoir ecosystem through natural selection processes, especially when exogenous bacteria are injected. It is necessary for MEOR technologies to study the behavior of injected bacteria, not only quantitatively, but also qualitatively, and to understand the complex relationship between the injected culture and the indigenous microflora. However, information about the characteristics of bacteria applied in past MEOR trials is not detailed or unavailable. In the sixties, on the grounds that the exogenous bacteria from various environments were capable of vitality in crude oil, we had inferred that the allochthoneous bacteria would be applicable to MEOR.[13] This inference has been confirmed not only by the successful MEOR tests at Daqing oil field, but also by these tests done in many more wells. A method for monitoring the injected bacteria through reisolation and morphological, as well as physiological, criteria was developed.[7] In this way, the presence and activity of strains 48-1 (see Fig. 5B) and 48-2 (see Fig. 6B) remained in tested wells. Their metabolites are presented in Table 8. These results satisfy the theory that the enriched culture 48 played an important role in EOR of Fuyu area at Jilin oil field.

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Table 1 Reservoir Characteristics

Development time	1965
Waterflooding time	Sept. 1973
Depth (m)	285.0–479.4
Temperature (°C)	30
Porosity (%)	22–26
Residual oil saturation (%)	54
Permeability ($10^3/m^2$)	180
Pressure (mPa)	40–44
Lithologic character	Powdery sand
Carbonate content (%)	1.4
Water cut (%)	about 80
Reservoir water type	$NaHCO_3$
Reservoir water salinity (mg/l)	4,000–6,000
Crude oil properties	
specific gravity	0.868
viscosity (mPa)	19–31
condensation point (°C)	17–21
paraffin content (%)	18–23
sulfur content (%)	0.05–0.17

Table 2 Metabolites of the Enriched cultures (inocubation 144 days)

Culture Medium*	Gaseous phase Amount (ml)	Gaseous phase Content H_2	Gaseous phase Content CO_2	Gaseous phase Content H_2S	Liquid phase Organic acid (mg/l) Amount	C_2	C_3	C_4	Pyruvic	pH	Surface tension** (mN/M)
48 Molasses	92	34.6	21.3	-	7035	265	129	6416	225	5.0	56
Molasses+$CaCO_3$	225	35.8	30.0	-	13560	796	260	12265	238	5.8	48
Molasses+scum	145	29.6	23.2	+	13751	617	150	12603	381	5.8	50
Molasses+oil sand	93	33.6	20.4	-	6396	644	241	5269	242	5.0	56
15a Molasses	67	17.9	31.3	-	4423	1981		2313	97	5.1	56
Molasses+$CaCO_3$	220	8.5	31.1	-	8907	2253		6423	231	6.2	55
Molasses+scum	166	35.0	23.1	+	2759			2614	145	5.5	55
Molasses+oil sand	105	16.8	23.8	-	2260			2135	125	5.1	56

*medium 15 ml

**surface tension for the molasses 4%: 56 mN/m

Table 3 Effect of Concentrations of Molasses

Molasses (%)	Gas (ml)
2	26
4	40
6	62
10	100

Table 4 MEOR Efficiency of Enriched Cultures in Laboratory Model Tests

Model No.	Core-1	Core-2	Core-3	Core-4	Core-5
Core volume (cm^3)	55.77	40.30	38.93	41.49	40.30
Porosity (%)	29.10	24.90	33.30	26.00	26.00
Water permeability (md)	666.40	2260.30	1117.20	810.00	2601.50
Saturated oil (ml)	10.40	7.90	9.50	8.42	8.28
Oil saturation (%)	70.50	70.00	73.00	77.90	78.80
Water drive efficiency					
Oil production (ml)	4.80	4.00	7.35	6.50	4.70
Oil recovery	46.20	53.00	77.40	77.20	56.80
Residual oil (ml)	5.60	3.54	2.15	1.92	3.68
MEOR efficiency					
enriched culture no.	48	48	48	15a	15a
Viable cells/ml	1.2×10^7	1.2×10^7	1.2×10^7		
Incubation period (d)	5	10	10	10	10
Gas production (ml)	2.60	4.90	9.80	5.70	2.80
Oil production (ml)	3.20	1.1	7.90	14.30	4.80
Oil recovery (%)	14.40	25.20	17.90	14.30	4.80
Residual oil recovery (%)	27.30	53.70	79.00	62.50	10.90

Table 5 Characteristics of the Strains from Enriched Cultured 48

Characteristics	48-1	48-2	48-3	48-4
Morphology	rod	rod	rod	rod
Cell size (μm)	1.2–1.5 × 5–9	1.0 × 4–8	1.5 × 3–7	0.7 × 4–9
Gram stain	–	+	+	–
Mobility	–	+	+	–
Flagellate	–	single	polar peritrichous	–
Sporulating	–	–	–	–
Capsulate	+	–	–	–
Oxygen requirement	–	–	–	–
Optimum temperature(℃)	35	35	30	35
Glucose fermented	+,gas,acid	+,gas,acid	+,gas,acid	+,gas,acid
Catalase	–	–	–	–
Organic acids from glucose (mg/l)				
Acetic acid	669	771	957	1787
Propanoic	38	16	–	18
Butanoic	1517	1812	1864	85
Isobutanoic	–	–	–	100
Isopentanoic	–	609	10	1267
Belongs to	*Fusobacterium*	*Eubacterium*	*Eubacterium*	*Bacteriodes*

Table 6 Content of the Gas- and Acid-Producing Anaerobes and Their Metabolites in the Co-Produced Water

Date	Gas- and Acid-Producing Anaerobe (cells/ml)	Gas Volume (ml)	Organic Acid (mg/l) C2	C3	C4
Before treatment	0	0	0	0	0
After treatment					
93, 8, 17	1.1×10^5	20	1320	1332	528
93, 8, 27	0.4×10^4	24	2070	791.8	1856.8
93, 10, 6	1.1×10^3	12	1782	162.8	404.8
93, 10, 16	2.5×10^2	13	1608	347.8	853.6
93, 10, 28	1.1×10^3	12	1536	199.8	1214.4

Table 7 Changes of CO_2 Content (%) from Wells Treated by MEOR

Well	8-01	42-7	52-6	4-24	40-17	40-24	18-17	18-18	72-13	Average
Before treatment	2.95	1.36	6.41	0.93	4.67	3.56	3.08	0.63	0.1	2.64
After treatment	13.09	23.58	24.24	32.52	40.86	14.82	14.56	26.47	31.13	25.25
Balance	+10.14	+22.22	+17.83	+31.59	+36.19	+11.26	+11.48	+26.30	+31.03	+22.61

Table 8 Metabolites of the Strains 48-1 and 48-2 Isolated from Co-Produced Water after Treatment

Strains	Gaseous phase Amount (ml)	H_2	Liquid phase—Organic acid (mg/l) CO_2	C_2	C_4
48-1	32	34.42	10.8	452.8	4255.7
48-2	16	53.76	12.2	427.6	2998.4

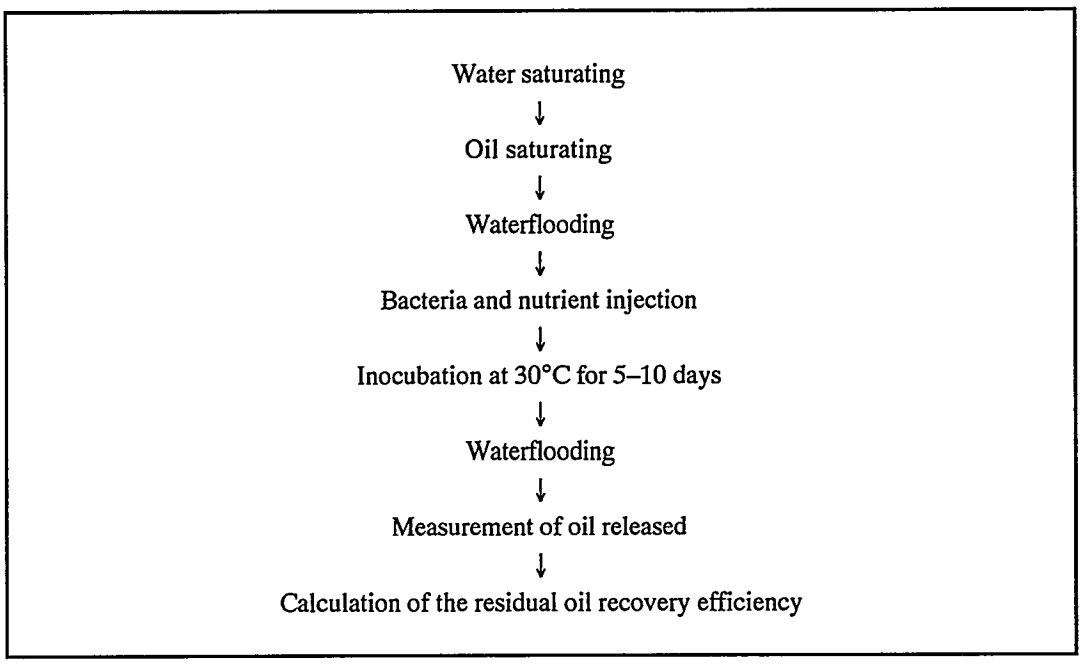

Figure 1 Protocol of the Laboratory Model Experiment

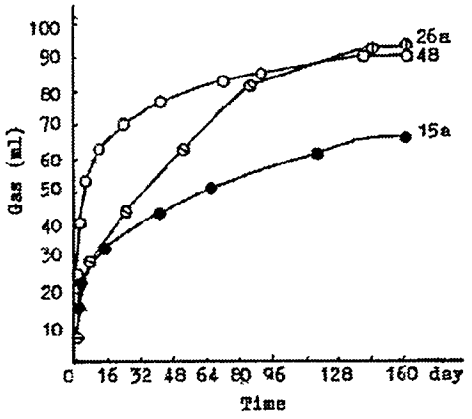

Figure 2 Time Course of Gas Production: 48—from Methane-Producing Enriched Culture; 26a—from Oil-Contaminated Soil of Daqing Petroleum Refinery; 15a—from Aeration Tank of Zibo Petroleum Chemical Industry

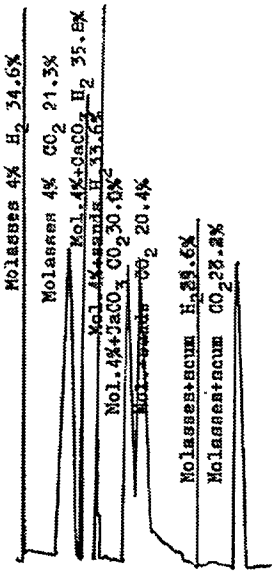

Figure 3 Gas Chromatogram of Gases Produced by Enriched Culture 48 in Several Media

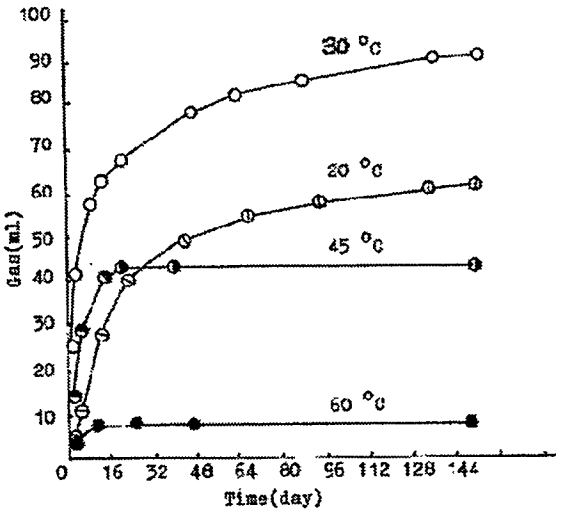

Figure 4 Effect of Temperature on Gas Production of Culture 48

Figure 5 Electron Micrograph of the Strain 48-1: (A) X12,000, (B) X10,000 from Well 8-27

Figure 6 Electron Micrograph of the Strain 48-2: (A) X10,000, (B) X10,000 from Well 8-27

Figure 7 Electron Micrograph of the Strain 48-3 X10,000

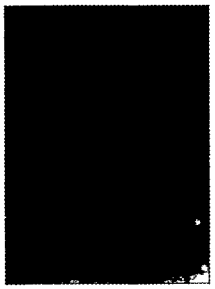

Figure 8 Electron Micrograph of the Strain 48-4 X10,000

Microbial Water Diversion Technique—Designed for Near Well Treatment in Low Temperature Sandstone Reservoirs in the North Sea

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Abstract

A Norwegian Research Program on Improved Oil Recovery (IOR) in North Sea reservoirs was launched in 1992. Microbial methods, applied in this context, is a part of this program. The scope, the methodological approach, and results from the three first years are presented. Water profile control, using biomass to block high permeable zones of a reservoir, has been investigated using nitrate-reducing bacteria in the injected seawater as plugging agents. Emphasis has been put on developing a process that does not have disadvantages secondary to the process itself, such as souring and impairment of the overall injectivity of the field. Data from continuous culture studies indicate that souring may successfully be mitigated by adding nitrate to the injected seawater. The morphology and size of generic nitrate-reducing seawater bacteria have been investigated. Screening of growth-promoting nutrients has been carried out, and some sources were detected as favorable. Transport and penetration of bacteria in porous media have been given special attention. Investigations with sand packs, core models, and pore micromodels have been carried out. The inherent problems connected with permeability contrasts and flow patterns, versus bacterial behavior, are believed to be critical for the success of this technology. Data from the transport and blocking experiments with the porous matrices confirm this concern. The technology is primarily being developed for temperatures less than 40°C.

Introduction

In 1992 a 4-year Norwegian Research Program on IOR (RUTH) was established. One subprogram focuses on Microbial Methods to enhance the oil recovery of a field. The objective of this subprogram was to examine microbial techniques applicable in North Sea reservoirs, and to investigate their potential in laboratory scale experiments.

General features characterizing a *generic* North Sea reservoir: sandstone petrology, stratified with high permeability contrasts between different zones of the reservoir, high temperature (60–160°C) and pressure (170–760 bar), long well spacing (1–2 km), pH of formation waters reported to vary between 5–7.9 (personal communication, Norwegian Petroleum Directorate), and salinity (TDS) varying 50 –100,000 ppm. For deep wells the salinity increases.[1] Chemically the pore water is in a reducing state, and seawater employed in water injection schemes is stripped for oxygen before injection.

Water injection is by far the dominant secondary recovery method in North Sea reservoirs and will remain so in the future according to the Norwegian Petroleum Directorate.[2] The high content of sulfate ions in the intruding seawater (average: 2,700 mg/l) is recognized to cause problems associated with microbial sulfate reduction. As water injection history evolves, increasing problems with souring is expected.

Microbial methods comprise a variety of concepts that generally share a lack of acceptance as viable methods in a field. This is definitely true in the context of North Sea reservoirs. At an early stage of the subprogram, water diversion was selected as a technique that was considered both applicable and having a future in North Sea production technology. The most obvious reason for this choice was the rapid response in terms of incremental oil recovery and reduced water cut that may be expected when water is diverted from thief zones to unswept low permeable zones. Opposed to other in-situ microbial processes that are usually slow, the benefit of a rapid production improvement is desirable.

Bacteria in open seawater are predominantly active by aerobic metabolism. A large fraction of these bacteria are able to sustain life after oxygen is depleted from the environment using alternative electron acceptors or fermentative metabolism. Numerous publications report adding nitrate salt to anoxic marine environments to avoid and mitigate the evolution of sulfidogenic consortia [3–6] and resulting souring of an oil field. The nitrate concept is incorporated in our microbial water diversion process. The indigenous nitrate-reducing seawater (= injection water) bacteria serve the purpose as plugging agents in a plugging process.

The size, morphology, and mode of living of bacteria are crucial for their transport to the zones where the desired process preferentially should take place.[7,8] To get a better understanding of the mechanisms involved and the relation to reservoir characteristics, special methods and equipment were designed.[9] Continuous surveillance of transparent pore micromodels with realistic pore size and geometry, using a video system, enabled us to learn about the phenomena on the pore level. The challenge is to demonstrate that a microbial plugging process is actually most pronounced in the swept zones of a reservoir, and is not impairing overall injectivity. The biology of the technology is undoubtedly important, but solid knowledge about reservoir technology and flow pattern in a porous matrix is required for the development of a successful injection protocol.

The nature of problems connected with the water diversion technique and various methodological approaches involved in the *Microbial Methods* project are briefly presented, along with some of the results from the first three years of research.

Experimental Approach and Strategy

It is generally recognized that the chemo-physical state of a given habitat selects for the most fit organism to be expressed. A waterflooded zone of an oil reservoir may conceptually be regarded as an environment that transiently moves from a state of equilibrium to an unsteady state as cold seawater invades the formation, dislodges oil, and changes the composition of the brine. Alien bacteria and limiting nutrients are carried in by the seawater, and some of the bacteria will thrive and proliferate in the new environment. This fact is critical in our strategy, where we intentionally bias the development of a bacterial population that for a given period of time is intended to carry out a predicted job. The strategy is thus dependent on the bacteria that are naturally occurring in North Sea water, at 50–70 meter depth (typical depth of riser intake of injection water).

Bacteria in North Sea Waters (NSW)

Numbers of bacteria in NSW able to use oxidized nitrogen compounds in dissimilative metabolism and grow at different temperatures were determined. Seawater samples, collected at 50 m depth, were brought to the laboratory in sterile bottles. Most Probable Numbers[10] of bacteria were determined using two media: medium for general heterotrophic bacteria (GHB) and that for nitrate-reducing bacteria (NBN-medium). GHB was determined after incubation in aerobic atmosphere, and the number of nitrate-reducers was determined after incubation in $H_2/CO_2/N_2$ atmosphere Series with 3 parallels of both GHB and NBN-media were inoculated and incubated at 4°, 12°, 20°, 28°, 37°, 42°, and 50°C for 3 weeks

maximum. Determination of nitrate reducers was carried out according to standard procedure.[11]

A number of nitrate-reducing bacteria were isolated and enriched for elaborate screening studies. They were further characterized using the BIOLOG™ system. Samples were incubated in aerobic and anaerobic atmosphere. Samples incubated in anaerobic atmosphere were divided in plates to which nitrate (1%) had been added, and plates without added nitrate. Incubation time was 4 days maximum at 35°C before analysis in a multiwell photometer. In addition to the taxonomic classification, the data were used to discriminate among suitable growth-supporting organic compounds.

Utilization of polymer-producing bacteria in microbial water diversion technique is well known.[12,13] Carbohydrates are often used to trigger the production of polymeric material. Extracellular polymers improve the strength of bacterial aggregates and biofilm attachment in porous matrices. The possibility to induce polymer production in our seawater-based concept was investigated. Initially, the isolated strains were studied with respect to polymer-producing ability when different carbohydrate-based compounds (1%) were added to solidified (agar) seawater medium. Different blends of carbohydrate and protein extracts were also tested. Polymer production was determined as an increase in volume of glycocalyx material, by light microscopy after negative staining.[14] Production of polymeric material in seawater cultures was also investigated. Different combinations of peptone, yeast extract, sucrose, nitrate, and orthophosphate were tested. The test cultures were incubated anaerobically at 28°C. At different time intervals the cultures were examined for exopolymer production, as recommended by Morgan et al.[15]

Penetration of bacteria into a formation matrix may be improved by selecting for small-sized bacteria. Use of ultramicrobacteria (UMB) and spores have been proposed[16,17] to optimize the depth of penetration. Seawater bacteria may be considered as being in a starved mode. The size and morphology of seawater bacteria have been studied, both before and after adding nutrients. Fresh seawater samples were amended with 0.5% of peptone, yeast extract, sucrose, 0.1% nitrate, and 0.01% phosphate. Incubation was performed under reduced atmosphere, and slow stirring at 22°C. One sample, without a carbon source, was included as a reference. Samples were then collected at different time intervals and examined by direct epifluorescence technique, after staining with DAPI.[18] Size and morphology were determined from photomicrographs.

After a North Sea field has been waterflooded, residual oil saturation (S_{or}) is low and problems connected to sulfide producing bacteria may be expected.[19,20] A continuous culture system (see Fig. 1) was arranged to study the long-term impact

of adding nitrate to a reduced sulfidogenic system. An automatically controlled Chemap-Fermenter system (3 liter) was used as a model reservoir. Inside the fermenter 3 pieces of a Berea sandstone core (length = 10 cm, diameter = 3.75 cm) presaturated with stock tank oil (from the Gullfaks field) were immersed to allow establishment of a large sessile population. Oil was released slowly during the experimental periods, lasting 30 days at 30°C and 32 days at 40°C. The cultures were run anaerobically, in the dark, by keeping an overburden pressure of 0.5 atm pure nitrogen gas. The experiments started with fresh untreated seawater (collected at 70 m depth). The fermenter was then sealed for 8 days in the 30°C experiment before a continuous supply of nutrients was started. A small inoculum consisting of enriched mixed SRB, originating from open seawater, was added after a few days in both cases.

The nitrate supply was started after 10 days, and the system was now fed ($D = Q \times V^{-1} = 0.00625\ t^{-1}$) with two sources of seawater, fresh untreated seawater, and autoclaved seawater, added organic acids, yeast extract, and nitrate-salt. The corresponding continuous supply at 40°C commenced day 5 for the nutrients and day 7 for the nitrate. Both seawater sources were kept cold (4°C) and in darkness. Oxygen was not removed from the solutions. Precautions were taken to avoid light penetration. The pH, temperature, and E_h (measured with a combined Platinum and a Calomel reference-electrode) were automatically recorded. The following analyses were carried out: enumeration of sulfate-reducing, nitrate-reducing, and general heterotrophic bacteria. Chemical analyses included sulfides[21] and nitrate (NS 4745, Merckoquant® 1020, and photometrically by Dr. Langes Instrument-analyses).

Bacterial Transport and Behavior in Porous Matrices—Penetration

In our context, biomass includes prokaryotes directly adhering to surfaces, or embedded in biofilm, and those colonizing mucous excreted by other organisms. Sessile bacteria may be in a different physiological state compared to planktonic organisms;[22,23] and it is conceivable that they exhibit a physiological response to contact with a surface. This aspect is important in understanding the behavior and response of bacteria inhabiting oil reservoirs. The shear stress imposed by injected fluids is high in the near well zone and decreases as the distance from the injection point increases, and gradients of nutrient concentrations will occur. The characteristics of the outer membrane of the bacteria are expressed as a response to external influences from the habitat. These characteristics are critical to the success of a microbial process, as they constrain or facilitate transport of the bacteria into a reservoir. Experiments with model cores have been conducted to illuminate these questions. The microbial technique pursued in this case comprises a number of different bacteria and respective modes of growth. Every field situation is somewhat different from the other, and a prediction model of a plugging process is

dependent on a number of parameters that varies from case to case. The following porous media experiments have been carried out:

1. C-Source Influence on Biomass Blocking of Sand Matrices. Steel cylinders measuring 5 cm (PV = 15.5–18.7 ml, K = 230–280 md) and 30 cm (PV = 40 ml, K = 130 md) were packed with quartz sand. Backpressure was applied, and the differential pressures (ΔP) across inlet and outlet were recorded continuously (Honeywell Smart transmitter). The 30-cm core was equipped with ports to measure the ΔP in the middle region. The temperature was controlled to 40°C. De-aerated seawater was injected continuously, and the indigenous bacteria were allowed to develop without additives. A nutrient package with different carbohydrates (seawater, 0.1% nitrate, 1% carbohydrate, and 5 ml/l of a vitamin mixture) for each sand pack was injected (flow = 1 ft/D) in the small sand packs. Effluents were analyzed chemically to determine residual nitrate/nitrite, sugars, and pH. Numbers of nitrate- and sulfate-reducing bacteria were also determined. Changes in the measured permeability were used to determine plugging efficiency.

2. Critical Parameters Involved in Bacterial Transport. Pore micromodels of transparent, and transparent-reflective material were connected to an automatic observation-reaction system[9,24] developed for optimum data collection. Pore geometry, bacterial size, and mode of growth—mechanisms that are secondary to flowrate—were thus subjects for visual interpretation. Injection of dead cells, starved cells, and actively exopolymer-producing bacteria were examined using *Leuconostoc mesenteroides* (DSM 20187). The systems were run anaerobically at 20–28°C and low backpressure (30 mbar) for 7 days maximum. The microscopic views were collected as time-lapsed and live videos, as well as selected images that were digitized and processed with an image-analyzing program.[9] Injection pressure versus time was recorded.

3. Deep Plug Placement. Sand packs 180-cm long with 10–15 Darcy permeability were employed for the purpose of examining how plugging may be controlled in time and space. Differential pressure was measured at 5 ports downstream from the injection point. Bacteria were injected and allowed to acclimatize to the sessile mode of growth before nutrients were injected at different time intervals and according to different schemes.

Results and Discussion

The North Sea water samples in our experiments contained 2.5×10^5 bacteria/ml, determined by direct epifluorescence technique (DAPI). The numbers of GHB and nitrate-reducers determined at different incubation temperatures are shown in Table 1. It is noteworthy that even at temperatures allowing high numbers of bacteria

(plate counts), nitrate reducers still constitute less than 10% of the total number of bacteria detected by the DAPI method. This is a common observation and is generally explained by unfavorable growth conditions and that some of the bacteria may be dead. The "Great Plate Count Anomaly" is discussed in detail by Amman et al.[25] At temperatures between 10° and 37°C, the number of bacteria detected is fairly stable. Above 40°C very few bacteria sustain vegetative life. These numbers do not take into account spores, carried by the water, that may be revived and proliferate at higher temperatures. Seawater bacteria detected in our experiments are typically members of the genera *Vibrio* (17%), *Acinetobacter* (8%), and *Shewanella* (33%). Some 46% of the total population was not identified. Ninety-five different carbon sources (mainly organic acids, alcohols, carbohydrates, esters, amines, and polymers) were tested on the isolated nitrate-reducing bacteria. The majority of the isolated bacteria were identified as common marine genera (e.g., *Vibrio*) or species. Some 33% affiliate to the species *Shewanella putrefaciens*, among the most common identified sulfide-producing organisms in seawater that do not use sulfate as an electron acceptor. Other sulfur oxyanions like thiosulfate are utilized.[5] Members of the species have frequently been found in produced waters from oil fields undergoing waterflooding.[26] The *S. putrefaciens* species were isolated from seawater at anaerobic conditions. We therefore infer that it may be expected that members of this species play a detrimental part during waterflooding, as they may contribute to a souring process. The very same isolates were confirmed to be able to use nitrate as an alternative electron acceptor. It is therefore reasoned that nitrate salt may intelligently be applied in injection waters with the objective of reducing the risk of souring in an oil field.

Evaluation of the raw data from the screening studies with nutrients revealed that no single carbon source protrudes as the preferred one by all the pure cultures. The different bacteria do, however, utilize a range of carbon sources including carbohydrates (polymers and monomers), amino acids, sugar alcohols, and organic acids (see Table 4). It is interesting that inosine appears among the most preferred carbon sources by marine bacteria. Inexpensive inosine-containing nutrients (e.g., meat extracts and molasses) may thus be used in a plugging scheme. The utilization of organic carbon by the entire microbial seawater community under different energy conservation conditions is given in Table 4. Carbon sources supporting at least a 50% increase in respiration activity are registered. The number of carbon sources utilized at this level were 2, 13, and 38 for anaerobic, anaerobic and nitrate, and aerobic conditions respectively. A wider range of carbon sources is utilized under aerobic compared to anaerobic conditions.

Addition of nitrate during anaerobic incubation was observed to positively influence utilization of a wider range of carbon sources. It is suspected that nitrate is used as an electron acceptor and thus has a positive influence on the growth. Sulfate reduction metabolism was not evident, the redox potential probably being too high

to support sulfidogenic reactions. The incubation period was only 48 hours, which is important to have in mind when interpreting the data; as adaptation periods vary from specie to specie. The use of acetic acid and arabinose may still have been due to sulfate reduction or fermentation respectively. Glucose-6-phosphate was readily utilized at anaerobic conditions with nitrate salt. This is intriguing because bacteria in seawater do not respond easily to glucose. Phosphate limitation is a possible explanation for this phenomenon. At anaerobic conditions with nitrate present, several types of carbon sources were utilized; such as carbohydrates, organic acids, and organic nitrogen-compounds. This correlates with the results from the pure culture studies.

The amount of cell-associated exopolymers produced in different seawater enrichment cultures is given in Table 2. A larger production of cell-associated exopolymers seems to coincide with a rich medium, compared to a sucrose medium. Bacterial numbers reached approximately 2×10^9 per ml for both the rich media, while 5×10^7 per ml was detected in the sucrose culture, indicating growth-limiting conditions when sucrose was added as the sole carbon and energy source. Other workers have reported higher values of extracellular polymers using pure cultures.[27,28] Further studies regarding suitable carbon sources for exopolymer production in marine bacteria are needed, and the production should perhaps be monitored while the bacteria is in a sessile mode.

Vegetative bacteria are usually associated with exopolysaccharides that aid the bacteria in adhering to rock surfaces and forming skin-plugs.[29] Using ultramicrobacteria (UMB) may help to obtain a deeper penetration. UMB may be found in deep ocean waters and is formed as a starvation response in vegetative bacteria.[30] The UMB have a size of about 0.2–0.3 μm, while vegetative bacteria are about 1–4 μm.[29] Table 3 shows that in seawater samples collected at a depth of 50 m the single bacterial cells seem to be somewhat larger than UMB. The majority of the measured cells were still found to be small. No significant increase in the size of single cells was observed after adding nutrients, but the microscopic views clearly demonstrated cell division and formation of aggregates for some of the species. The diameter of the aggregates was measured to 10–150 μm. The comparably small size of marine bacteria indicates that they may penetrate deeper into reservoir rocks than other vegetative bacteria can.

The continuous culture experiments showed that a low redox potential was established after a short period of time in both cases. Volatile sulfide was measured (data not shown), and it correlated with low E_h values. The addition of nitrate salt had a significant impact on the shift in redox potential at 30°C. At 40°C a shift from negative toward a positive E_h was less expressed (see Fig. 2). Nevertheless, a tendency toward a positive shift is seen at the end of the experimental period. In both cases sulfide, if produced, was below the detection level after nitrate had been

introduced into the systems. The pH was significantly influenced, as seen in Fig. 2, and a better balanced buffer capacity may be required for field applications, as scaling problems may be expected. The observed difference between the 30°C and 40°C experiment is tentatively explained by a qualitative difference in the bacterial population thriving at the respective temperatures.

Table 5 shows the number of bacteria in suspension during the experiments. The high number of SRB detected is intriguing since sulfide production was undetectable. At least two theories may explain this: (1) the bacteria may be able to use both sulfate and nitrate in metabolism and (2) the bacteria ferment the yeast extract in the selective medium, produce H_2S, and precipitation of FeS is mistakingly read as positive for SRB. This needs to be examined further. SRB capable of using nitrate in dissimilative metabolism has been known for a long time.[31] Dissolved nitrate concentration was reduced from 226 ppm nitrogen to 61 ppm nitrogen after 30 days in the 30°C experiment. This corresponded to a conversion rate of 0.34 ppm nitrogen per liter per time period. Seawater normally contains <10 ppm nitrogen.

Experiments with small sandpacks showed that within a period of 16 days with continuous injection of de-aerated seawater with different carbohydrates and vitamins, glucose gave the quickest and most significant response in terms of reducing the permeability. A reduction factor (initial permeability divided by the end permeability) of 2,800 was observed, while fructose and lactose showed 384 and 1.35 respectively. The experiments showed that carbohydrates support growth of biomass in a fashion that reduces the permeability. However, the plug formed was probably not penetrating deep into the core. We suspected it to be a shallow plug, or a filter cake. The data from the experiments with the medium-long sandpack (30 cm) confirmed that the biomass plug was a shallow one, although a mixture of carbohydrates, phosphorus, and vitamins were injected. It was not deeper than 2–3 cm according to the ΔP measurements. This experiment was run for 60 days, and a 86% reduction in permeability was measured. This problem has been reported by others,[32] and is the essential problem of a microbial blocking process. Both glucose and fructose conversion were followed by a significant pH drop from 8.1 to 7.3.

The data from the experiments convincingly showed that there is much to learn before the growth of biomass can be controlled and predicted. The micromodel studies showed that there was a clear relation between cell size and the production of extracellular material (see Figs. 3, 4 and 5). Although the study was carried out with a pure strain, *L. mesenteroides* (DSM 20187), a fundamental understanding of flow pattern, growth, and blocking behavior was possible to attain (data to be published). A blocking process has to be designed in a fashion that allows the growth of biomass to occur at a predicted rate. The process is thus dependent on: the pore size, flow rate, cell concentration, *stickiness* of the cell/aggregate, and cell

size. Clearly, the protocol of nutrient-feed is important, and different schemes with continuous/discontinuous flow, alternating between nutrient-free water / water and biocide to delay growth, etc., can be explored, along with shut-in periods. The long core experiments show that a firm plug may be placed with an alternating feeding process (data to be published). Yeast extract and nutrients with a high content of proteins proved to be more efficient in developing biomass for blocking. The experiments with the long sandpacks are still in progress, and the data remain to be reviewed.

The experimental data so far are considered encouraging. The mixed population is responding to nutrients with both an increase in volume and the production of extracellular material. Both factors are important for the overall retention and blocking process. Sulfidogenic conditions seem to be discouraged by the high redox potential that is created when NO_3, or another oxyanion of nitrogen, is utilized as electron acceptors. The process needs to be further investigated, and mass balances worked out. The temperature and pressure window limiting this concept also need to be determined. The impact of competition between indigenous reservoir bacteria and injected bacteria is a matter of discussion. At the moment, we have no experimental data to lean on, but temperature is clearly important for the ecophysiology that persists under reservoir conditions.[33]

Acknowledgments

We thank the Research Council of Norway, Norwegian Petroleum Directorate, Statoil, and Pelican for the financial support. Norsk Hydro and Louis Castanier of Stanford University are thanked for their contributions to the micro models.

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Table 1 Number of Bacteria per ml in Seawater Able to Grow at Various Temperatures

Temperature (°C)	General Heterotrophic Bacteria	Nitrate-Reducing Bacteria
5	0.9×10^2	1.5×10^2
12	4.6×10^3	2.1×10^2
20	1.1×10^4	1.1×10^3
28	1.1×10^4	4.6×10^2
37	4.6×10^3	2.4×10^2
42	<1	2.1×10^2
50	<1	<1

Table 2 Extracellular Polymer (mg/l) Extracted from Seawater, after Incubation with Different Nutrients

	Yeast Extract, Peptone, Sucrose	Yeast Extract, Peptone	Sucrose
Start	0	0	0
15 hr	60	nd	7
22 hr	54	63	5
28 hr	44	64	7
40 hr	44	54	7

Table 3 Size of Single Cells of Seawater Bacteria, after Incubation with and without Nutrients

	Length (μm)		Width (μm)	
	Range	Mean	Range	Mean
Start	0.4 –2.2	1.1	0.4 –0.7	0.5
18 hr w/nutrients	0.6 –2.0	1.1	0.5 –1.0	0.7
120 hr w/nutrients	0.5 –2.9	1.2	0.5 –1.5	0.8
18 hr	0.6 –1.5	0.9	0.4 –0.7	0.6
120 hr	0.7 –2.4	1.5	0.7 –1.3	0.8

Table 4 Relative Utilization of Different Carbon Sources by Marine Microorganisms Compared to Activity without Respective Carbon Sources. Activity without Carbon Source Is Calibrated As 1.

Anaerobic		Anaerobic with nitrate		Aerobic	
Acetic acid	1.7	Arabinose	2.0	Tween 40	3.8
Arabinose	1.5	Propionic acid	1.8	Maltose	2.9
		Fucose	1.7	Succinic acid	2.7
		Glucoronamide	1.6	Trehalose	2.6
		Itaconic acid	1.6	Propionic acid	2.2
		Formic acid	1.6	Methyl pyruvate	2.1
		Psicose	1.5	Psicose	2.0
		Glucose-6-phosphate	1.5	Inosine	1.9
		Mannose	1.5	Glucose-1-phosphate	1.9
		Thymidine	1.5	Melobiose	1.9
				Pyro-glutamic acid	1.9
				Alaninamide	1.9
				Glutamic acid	1.9
				Cellobiose	1.8
				Turanose	1.8
				Fucose	1.7
				Galactose	1.7
				Asparagine	1.7
				Fructose	1.7
				2,3-butanediol	1.7
				Carnitine	1.7
				Malonic acid	1.7
				Sorbitol	1.7
				Keto-glutaric acid	1.7
				Raffinose	1.7
				Phenyl etylamine	1.6
				Formic acid	1.6
				Thymidine	1.6
				Saccharic acid	1.6
				Glucose-6-phosphate	1.6
				Glycogen	1.6
				Glucoronamide	1.6
				Tween 80	1.6
				Alanyl-glycine	1.6
				Quinic acid	1.6
				Mannose	1.6
				Galacturonic acid	1.5
				Leucine	1.5

Table 5 Enumeration of Bacteria in the Continuous Culture Experiment. Numbers Are Given As MPN. SRB = Sulfate-Reducing Bacteria, NRB = Nitrate-Reducing Bacteria, GHB = General Heterotrophic Bacteria.

Time (days)	MPN, SRB/ml 30°C	MPN, NRB/ml 30°C	MPN, GHB/ml 30°C
10	9.3×10^4	$>1.1 \times 10^4$	6.5×10^6
13	$>1.1 \times 10^6$	4.3×10^7	4.3×10^8
17	4.3×10^5	2.3×10^8	9.3×10^8
22	4.4×10^4	1.5×10^7	9.3×10^8
30	4.3×10^6	1.5×10^7	4.3×10^9
Time (days)	**40°C**	**40°C**	**40°C**
7	9.3×10^6	4.6×10^7	1.5×10^8
10	7.5×10^6	9.3×10^7	4.3×10^8
20	4.3×10^6	2.4×10^8	1.5×10^9
28	4.3×10^6	7.5×10^7	1.5×10^9

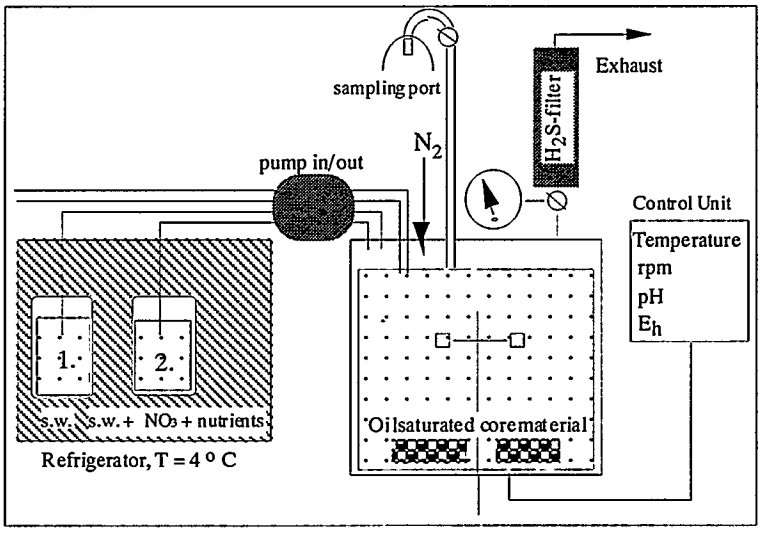

Figure 1 Schematic of Continuous Culture Experiment

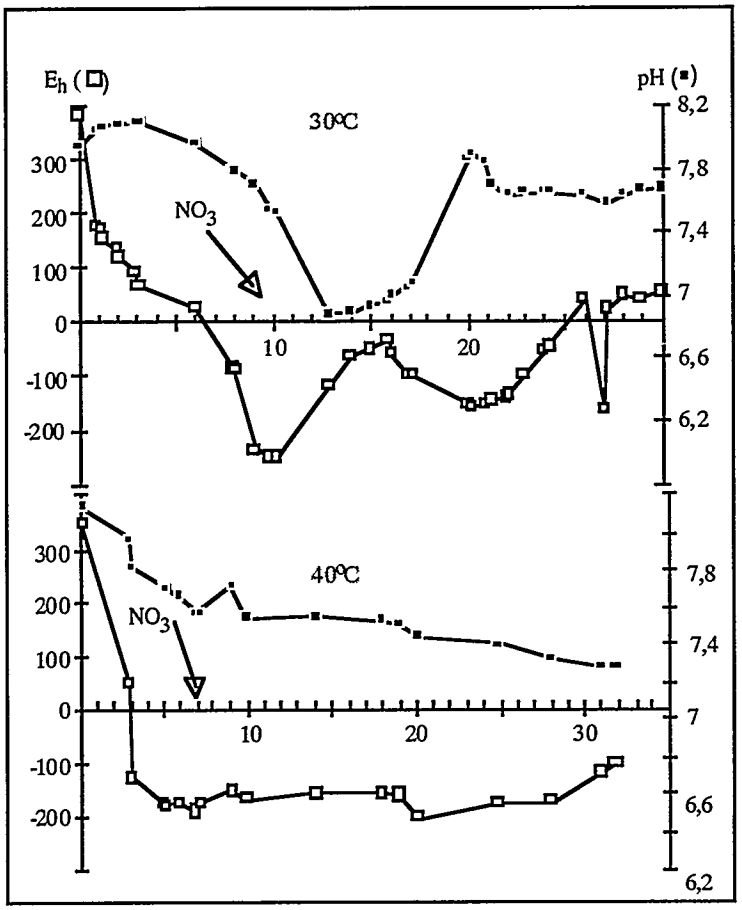

Figure 2 **Redox (SHE) and pH Data from the Continuous Culture Experiments. X-axis Shows Time (Days), Arrow Indicates When Nitrate Was Added. The Graph at the Top Shows Data Recorded during the Experiment Run at 30°C, and the Bottom Graph Shows Data Recorded during the Experiment Run at 40°C.**

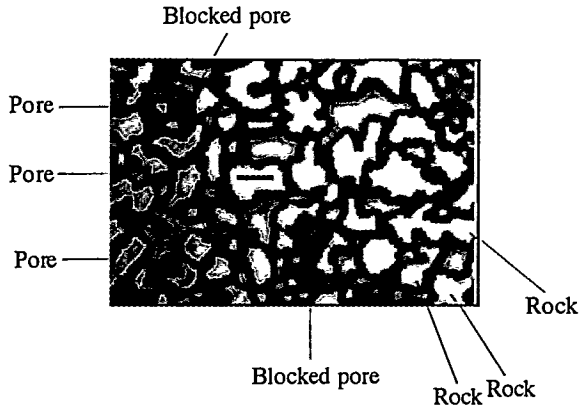

Figure 3 **Segment of a Berea Sandstone Replica Micro Model, Digitized from a Video film. Bar Equals 50 μm. The Dark Areas in the Porespaces Represent Trapped Bacteria (*L. Mesenteroides*).**

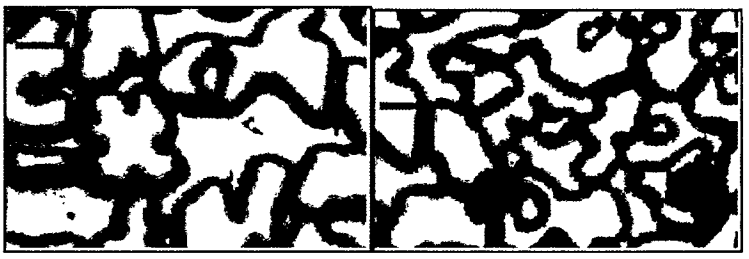

Figure 4 Digitized Images from Video Film Recorded during Injection of *L. Mesenteroides*. Scale Bar Is 35 μm. Left: Section from the Middle of Micro Model Showing Scattered Clusters of Bacteria Traveling from Left to Right. Right: From the Injection Point of the Same Model, at the Same Time, Showing Plugging of Pores.

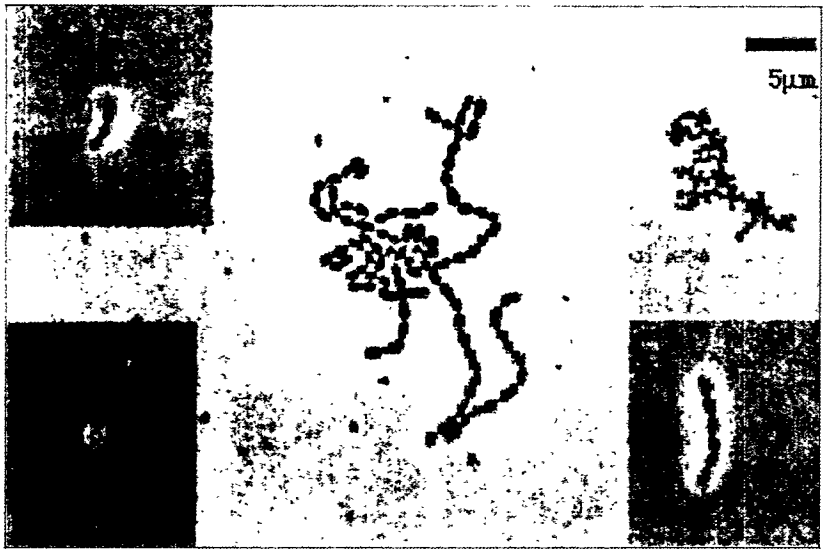

Figure 5 Digitized Images from Microscope Views. *L. Mesenteroides* Showing Different Size and Mode of Growth As a Response to Cultivation Time and Nutrition.

Aerobic Microbial Enhanced Oil Recovery

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Abstract

In aerobic MEOR, the ability of oil-degrading bacteria to mobilize oil is used to increase oil recovery. In this process, oxygen and mineral nutrients are injected into the oil reservoir in order to stimulate growth of aerobic oil-degrading bacteria in the reservoir.

Experiments carried out in a model sandstone with stock tank oil and bacteria isolated from offshore wells showed that residual oil saturation was lowered from 27% to 3%.

The process was time dependent, not pore volume dependent. During MEOR flooding, the relative permeability of water was lowered. Oxygen and active bacteria were needed for the process to take place. Maximum efficiency was reached at low oxygen concentrations, approximately 1 mg O_2/liter.

Introduction

The use of microorganisms to enhance oil recovery has for many years been considered possible, and successful field trials have been reported both from the United States and Eastern Europe.[1] The method used has been to inject a sugar solution (approximately 5%) with nutrients and adapted bacteria into the formation. Because of the huge amounts of sugar needed (3,000 metric tons per day on a typical North Sea platform), the process presents sizable logistical problems. This method is therefore not economically feasible for use on large offshore installations.

Use of residual oil as the carbon source and injection of oxygen and nutrients together with aerobic oil-degrading bacteria can, on the other hand, be a technically and economically feasible both onshore and offshore.[2–5] Using this method, core flooding experiments have been performed in the laboratory. Experiments were carried out in a model sandstone with stock tank oil and bacteria isolated from offshore wells. This paper presents results from these aerobic MEOR experiments.

Experimental Procedures

Mineral Medium

The medium used for flooding is shown in Table 1. For anaerobic flooding, the medium was reduced by flushing with nitrogen. Traces of oxygen were removed with sodium dithionite. For aerobic MEOR flooding, the reduced medium was flushed with air or oxygen gas to reach the desired oxygen concentration. Oxygen was measured according to the method described by Winkler.[6] The NaCl concentration of the medium was chosen to give optimal growth of the bacterial culture used, and ranged from 2% to 3% in different experiments.

Microorganisms

In most experiments, a mixed culture of aerobic oil-degrading bacteria was used.

Bacteria were grown in 50 ml bottles with 25 ml aerobic medium and 0.1 ml crude oil or tetradecane as the carbon source. Oil-degrading bacteria were isolated from various oil-infested areas, including the water treatment plant of an oil refinery and backflooded water-injection wells of an oil platform in the North Sea. Mixed cultures were enriched that could grow on crude oil and n-alkanes. The effect of

microbes on surface and interfacial tension was measured by the microdrop method.

Crude Oil

Crude oil samples were obtained from two North Sea oil fields. Crude oil 1 was used alone. On the other hand, North Sea Model Oil, consisted of crude oil 2 with 18% pentane added. Crude oil 1 has a gravity of 0.830 g/cm^3 and a viscosity of 3.7 cP at 45°C. North Sea Model Oil has a gravity of 0.803 g/cm^3 and a viscosity of 2.3 cP at 45°C.

Flooding Conditions

The coreflooding experiments were performed at 45°C. In early experiments, the cores were embedded in araldite and flooded against atmospheric pressure. In later experiments, the cores were mounted in a core holder. The external pressure was 50 bar, and the cores were flooded against a backpressure of 10 bar.

Coreflooding Apparatus and Procedures

Experiments were carried out in a flow rig, shown in Figure 1. The cores were Hopeman or Bentheimer sandstones with permeability of 250 and 538 md, respectively. Other properties of the cores are shown in Table 2. Cores were air dried at 80°C for 48 hours before they were embedded in araldite or mounted in a core holder. A core was evacuated to 0.1 mbar before saturation with anaerobic brine, and the pore volume was measured. The core was then flooded with anaerobic brine, and the absolute permeability, *Kabs,* was determined. The brine was exchanged with Marcol-82 and finally saturated with crude oil. The core was then flooded with oil, and the effective oil permeability at irreducible water saturation, *Ko(Swi)*, was determined. Finally the core was flooded with anaerobic brine until residual oil saturation was established. The residual oil saturation after anaerobic waterflooding, *Sorl,* and effective permeability to water at residual oil saturation, *Kw(Sorl),* was determined, and flooding with anaerobic brine was continued for several pore volumes to establish a baseline before starting MEOR.

Flow Rate

In each experiment, the flow rate was constant during anaerobic and aerobic flooding. In different experiments, the rate ranged from 0.14 to 0.084 ml/minute,

depending on the pore volume of the core. With continuous flooding, this was equivalent to flooding one PV per day.

MEOR Flooding

MEOR flooding was started by injecting oxygen and a mixed culture of oil-degrading bacteria into the core. Oil production was monitored by collecting the oil in an inverted, water-filled burette. The volume produced was recorded daily, and residual oil saturation (*Sor2*) was calculated. The effective permeability to water, *Kw(Sor2),* was measured after the flooding experiment had been terminated.

Results

Growth of Bacteria on Crude Oil and n-Alkanes

When grown in batch cultures, the bacteria showed a strong tendency to grow in the oil/water interface. There, the microbes grew in a single cell layer on the oil surface (see Fig. 2). In young cultures, there had been little or no growth of bacteria in the water phase. Only in old cultures, where most of the oil was degraded, did the water phase turn turbid from bacterial growth. Bacterial growth led to a 30–40% reduction of the oil/water interfacial tension, and the cultures showed a marked ability to clean oil from glass surfaces. In most cases, bacterial growth had no effect on the surface tension. This indicated that the reduced interfacial tension was caused by microbes adhering directly to the oil surface, not by production of soluble surfactants. The cultures showed little tendency to produce emulsions.

Bacteria originating from the oil refinery treatment plant grew optimally in a medium with 1–2% NaCl. Microbes isolated from the backflooded injection wells grew optimally in a medium with 2–3% NaCl. These bacteria also grew well in a seawater or artificial seawater medium at temperatures between 30°C and 45°C. The bacteria did not grow under anaerobic conditions with oil or tetradecane as the carbon source, but many bacteria survived periods of anaerobic incubation.

Preliminary Flooding Experiments

In these experiments, araldite-embedded cores were flooded against atmospheric pressure.

Results from an experiment are shown in Figure 3. During anaerobic flooding for 2.6 PV, *Sor* was reduced to 0.363, and no oil was produced over the next 2.4 PV. At this point, oxygen was added to the medium at a concentration of 1 mg/l, and 100 ml of a bacterial culture originating from a water treatment plant from an oil refinery, containing 6×10^7 bacteria/ml, were introduced into the core. Oil production started one PV after the start of MEOR flooding and continued at over the next 14 PV. Oil was produced in droplets at irregular intervals. There was no gas production during the experiment. In this process, *Sor* was reduced to 0.33, and 9% of the residual oil was produced (see Table 3).

Bacteria started to be produced out of the core 0.66 PV after inoculation at low concentrations ($1 \times 10^{5)}$ and continued to be produced at this low rate throughout the experiment.

Additional experiments showed that both oxygen and bacteria were needed to ensure oil production. In the absence of oxygen, there was no oil production. Under aerobic conditions in the absence of bacteria or when bacterial growth was inhibited by the addition of antibiotics or in the presence of dead bacteria, there was no oil production. These experiments showed that oil production was caused by active growth of aerobic bacteria.

Temperature

Most experiments were carried out at 45°C. One experiment was carried out at 60°C using a pure culture of a thermophilic oil-degrading bacterium; it showed results similar to those experiments carried out at 45°C. In this case, oil production was slow, probably because the pure strain was less efficient in mobilizing oil than mixed cultures.

Experiments Run with Core Mounted in Core Holder

In these experiments, the cores were mounted in a core holder under a surrounding pressure of 50 bar. The experiments were run with 10 bar backpressure at 45°C. Results from one experiment are shown in Figure 4. The bulk of the oil was produced during the first pore volume. During the first 6 PV of anaerobic flooding, *Sor* was reduced to 0.266 and no oil was produced over the next 8 PV.

The core was inoculated with a mixed bacterial culture of aerobic oil-degrading bacteria isolated from a backflooded water injector. Oxygen gas was added to the medium at a concentration of 5.45 mg/l. The core was flooded with aerobic medium

for 7 PV. In this period, 0.05 ml oil was produced, lowering the *Sor* to 0.260. Oxygen was not consumed in the core, showing that there was no bacterial growth.

Next (after 21.6 PV had been flooded), the core was reinoculated with 115 ml of a bacterial culture containing 7×10^7 bacteria/ml. Oil production started 1.5 PV after the core was reinoculated. In the following MEOR process, *Sor* was lowered from 0.266 to 0.032 (see Table 3), at which point the experiment was terminated.

During the flooding, which took place over 114 PV, several incidents occurred that affected oil production.

1. An accidental abrupt increase in the water pressure from 10 to 50 bars led to an immediate stop in the oil production. After reinoculation of the core, oil production was resumed at the same rate. The interpretation was that the sudden pressure increase had killed the bacteria in the core.

2. The core was flooded with anoxic medium, which led to an immediate stop in oil production. The interpretation was that oxygen was essential for the oil production. After the reintroduction of oxygen, oil production commenced at the same rate as before. This continued for 54 PV. During this period, the flow rate was constant at 0.084 ml/minute, but oxygen concentration was varied with no apparent effect on oil production.

3. After 98 PV the flow was reduced by flooding the core for 8 hours per day. This led to an increase in oil production when plotted as a function of PV flooded. Further reduction of the flow to 4, 2, and 1 hour per day led to a further increase in the production rate.

Effect of Time

When the same data is plotted as a function of time, oil production is seen to be time dependent, not dependent on the volume flooded (see Fig. 5). The MEOR process that resulted in reduction of *Sor* from 0.26 to 0.03 took place over 92 PV and 260 days. The results from this experiment showed that the flow rate could be reduced more than twentyfold without significantly decreasing the oil production rate, reducing the necessary number of PV flooded to less than 7. In North Sea oil reservoirs the time for flooding one PV varies from several months to years, which would allow ample time for the aerobic MEOR process to be effective during the time available for oil production from one field.

Oxygen Concentration

Maximum oil production was observed at low oxygen concentrations, about 1 mg O_2 per liter. Increased oxygen concentrations did not lead to increased oil production (see Fig. 6).

Relative Permeability

The MEOR process led to a decrease in the relative permeability of water (see Table 3), indicating that pore blocking is one possible mechanism to increase oil production.

Conclusions

Results from the laboratory experiments showed that increased oil production under the conditions used was a biological process. In this process oxygen and active bacteria were needed for enhanced oil recovery. The core flooding experiments showed that:

1. Residual oil concentration was lowered from 27% to 3%.
2. The process was time dependent, not pore volume dependent.
3. Relative permeability of water was lowered.
4. Maximum efficiency was reached at low O_2 levels, approximately 1 mg O_2/liter.

These results indicate that the process could be effectively used to enhance oil recovery from North Sea oil reservoirs.

Acknowledgments

The authors wish to thank Statoil for funding the study and granting permission to publish this paper.

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Table 1

Mineral Content of the Medium (Grams of Minerals per Liter)	
NH_4Cl	1.0 g
$NaNO_3$	1.0 g
$MgSO_4 \cdot 7H_2O$	0.2 g
Na_2HPO_4	0.21 g
$NaH_2PO_4 \cdot H_2O$	0.1 g
KCl	0.04 g
$CaCl_2 \cdot 2H_2O$	0.02 g
NaCl	20.0 g
$FeSO_4 \cdot 7H_2O$	1.0 mg
Trace solution	1.0 ml
Distilled water	1,000 ml
pH	7.0
Sterilize by autoclaving	
Mineral Trace Solution: Content of Minerals per Liter	
$CuSO_4 \cdot 5H_2O$	5 μg
H_3BO_3	10 μg
$MnSO_4 \cdot 5H_2O$	10 μg
$ZnSO_4 \cdot 7H_20$	70 μg
MoO_3	10 μg
Distilled water	1,000 ml
Sterile filtered	

Table 2

CORE PROPERTIES		
Experiment	1	2
Sandstone Core	Hopeman	Bentheimer
Length, cm	45	30
Pore Volume, cm^3	166	122
Permeability, md (*Kabs*)	250	538
OIL PROPERTIES	**Model Oil**	**Crude Oil**
% Pentane Added	18	0
Gravity, g/cm^3 at 45°C	0.803	0.830
Viscosity, cP at 45°C	2.3	3.7
EXPERIMENTAL CONDITIONS		
Temperature °C	45	45
Pressure, bar	atmospheric	10

Table 3

ANOXIC FLOODING		
Experiment	1	2
Pore Volumes Flooded	5	13
Residual Oil Saturation, *Sor1*	0.363	0.266
Effective Permeability, *Kw(Sor1)*	–	35.1
MEOR FLOODING		
Pore Volumes Flooded	15	100
Duration, Days	23	270
Residual Oil Saturation, *Sor2*	0.326	0.032
Effective Permeability. *Kw(Sor2)*	–	20.3
O_2 consumed, mg	2.6	133.5

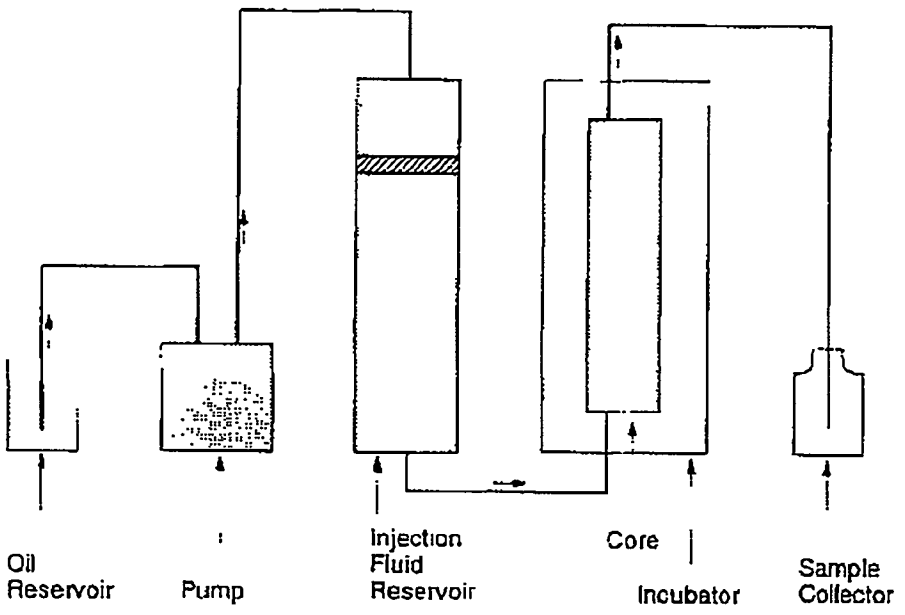

Figure 1 Schematic Drawing of Flow Rig

Figure 2 Bacterial Microcolonies Grow at the Oil/Water Interface. Photo Taken in Phase Contrast Microscopy.

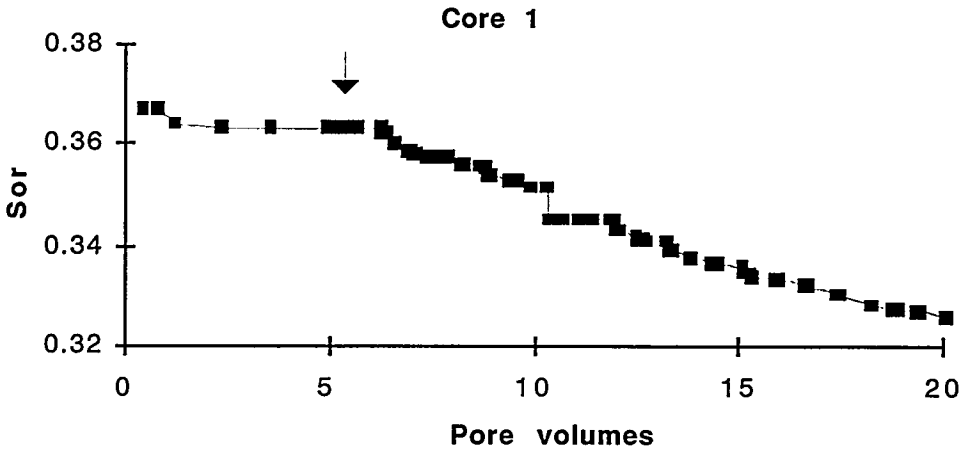

Figure 3 Oil Saturation As a Function of Pore Volumes Flooded. Results for Experiment No 1. Arrow Indicates Addition of Bacteria and Oxygen.

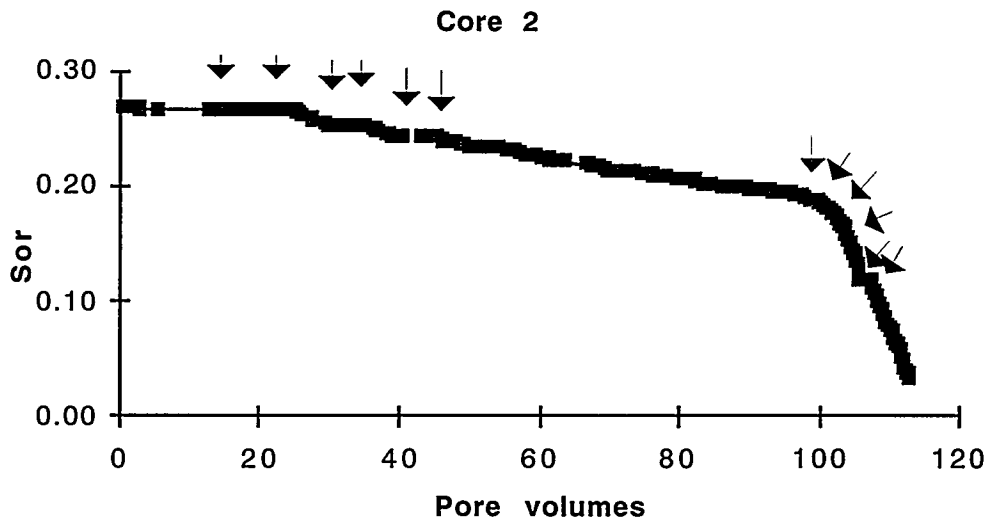

Figure 4 Oil Saturation As a Function of Pore Volumes Flooded. Results for Experiment No 2. Arrows Indicate: (1) Initial Addition of Bacteria and Oxygen. (2) Reinoculation of Core with Bacteria. (3) Intermediate Pressure Increase from 10 to 50 Bars. (4) Reinoculation of Core with Bacteria. (5) Flooding with Anaerobic Medium. (6) Oxygen Added. (7) Core Flooded 8 Hours/D. (8) Core Flooded 4 Hours/D. (9) Core Flooded 2 Hours/D. (10) Core Flooded 1 Hour/D. (11) Flooding Stopped for 12 Days. (12) Flooding Resumed for 2 Hours/D.

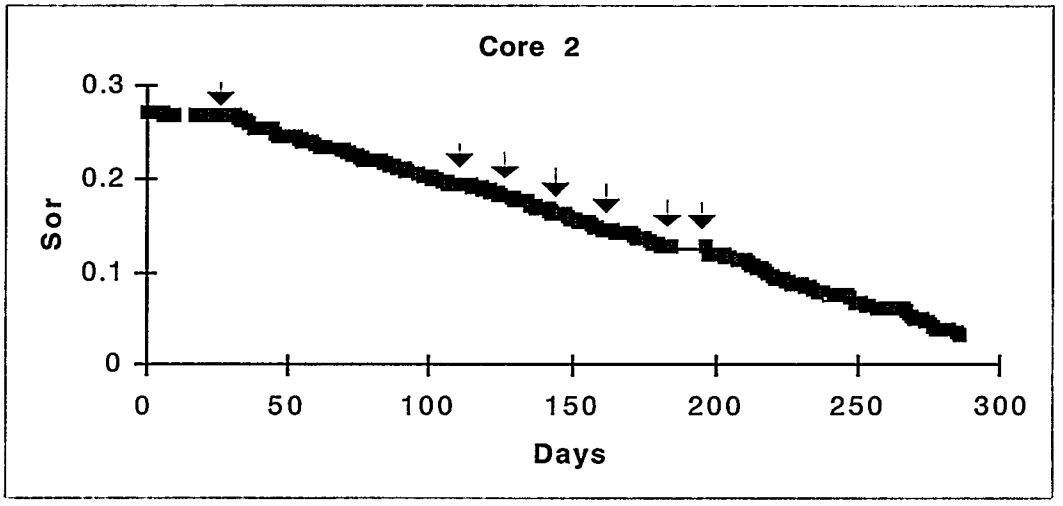

Figure 5 Oil Saturation As a Function of Time. Results for Experiment No 2. Arrows Indicate: (1) Inoculation of Core with Bacteria. (2) Core Flooded 8 Hours/D. (3) Core Flooded 4 Hours/D. (4) Core Flooded 2 Hours/D. (5) Core Flooded 1 Hour/D. (6) Flooding Stopped for 12 Days. (7) Flooding Resumed for 2 Hours/D.

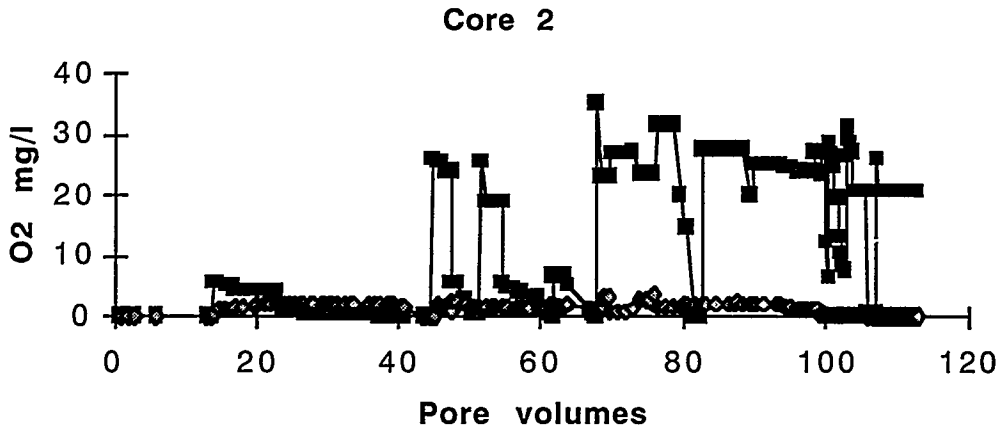

Figure 6 Oxygen Concentration in Medium into Core (■) and at Core Outlet (◊). Results from Experiment No. 2.

Improved Oil Recovery Using Bacteria Isolated from North Sea Petroleum Reservoirs

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Abstract

During secondary oil recovery, water is injected into the formations to sweep out the residual oil. The injected water, however, follows the path of least resistance through the high-permeability zones, leaving oil in the low-permeability zones. Selective plugging of these thief zones would divert the waterflood to the residual oil and thus increase the life of the well. Bacteria have been suggested as an alternative plugging agent to the current method of polymer injection. Starved bacteria can penetrate deeply into rock formations where they attach to the rock surfaces, and given the right nutrients can grow and produce *exo*-polymer, reducing the permeability of these zones. The application of microbial enhanced oil recovery has only been applied to shallow, cool, onshore fields to date. This study has focused on the ability of bacteria to enhance oil recovery offshore in the North Sea, where the environment can be considered extreme. A screen of produced water from oil reservoirs (and other extreme subterranean environments) was undertaken, and two bacteria were chosen for further work. These two isolates were able to grow and survive in the presence of saline formation waters at a range of temperatures above 50°C as facultative anaerobes. When a solution of isolates was passed through sandpacks and nutrients were added, significant reductions in permeabilities were achieved. This was confirmed in Clashach sandstone at 255 bar, when a reduction of 88% in permeability was obtained. Both isolates can survive nutrient starvation, which may improve penetration through the reservoir. Thus, the isolates show potential for field trials in the North Sea as plugging agents.

Introduction

Oil recovery is enhanced by the use of polymer-based methods to reduce permeability. An alternative method using bacteria, with particular relevance to the North Sea oil-bearing strata, is presented in this paper. The method is to inject specific starved bacterial cells into the oil-bearing strata via the injection wells. These are carried by the waterflood to the thief zones. The bacterial cells penetrate into the high-permeability thief zones and begin to block them, preventing water penetration. Next, appropriate nutrients are injected that cause the bacterial cells to grow and produce sticky exopolysaccharides. This substance is a natural product which assists growing bacteria to adhere to rock surfaces. The lysis products of older bacterial cells can also be trapped within the exopolysaccharide matrix. This process increases the effectiveness of the blocking and selectively reduces permeability.

Starved bacteria are prepared by depriving suitable bacterial cells of nutrient before injection into the well. Starved bacteria are also termed ultramicrobacteria.[1] In the context of enhanced oil recovery, starvation reduces the size of the bacterial cells. They also produce less exopolysaccharide material, which facilitates their penetration through the rock pores and into the thief zones. Starved bacteria, which are subsequently given nutrients, will form a deeper zone of reduced permeability within the rock strata than will unstarved bacteria.[2,3,4,5]

This approach is particularly appropriate for the North Sea fields. To date, microbial recovery techniques have been focused on shallow, low producing wells that provide a congenial environment for bacterial growth.[6–10] The North Sea environment is more hostile in terms of pressures, salinity, temperatures, and the absence of oxygen. Bacteria used to plug thief zones there will not only have to be effective in these hostile conditions but must also tolerate the contrasting conditions associated with transport on the surface and transfer to the rock strata. Recent work in our laboratory has identified bacteria that are able to survive in a wide range of such extreme environments.

Our work has been in support of the SIFT (Selectively Induced Flow Technique) Project. This project was to study bacteria that were suitable for selective permeability reduction techniques to enhance oil recovery in the North Sea. The three phases of the project were: 1) screening oil reservoir samples to identify suitable bacteria, 2) monitoring the responses of the bacteria within a simulation of the reservoir, and 3) field trials. This paper reports on the project's first two phases, which have now been successfully concluded.

Materials and Methods

Culture Selection

Suitable cultures for use in SIFT were identified. Sixteen samples were collected from eight different oil fields in the North Sea. These were enriched for bacteria for further work by growing in a range of media supporting anaerobic bacteria up to 75°C. They were also tested for the ability to grow in saline conditions at temperatures above 50°C. Two cultures, denoted EX299 and EX302, were selected for further study.

EX299 was obtained from the produced water of an offshore facility. It grows anaerobically between 50°C and 75°C and optimally at 70°C. EX302 grows aerobically or anaerobically between 25°C and 55°C and optimally at 50°C.

Nutrient Selection

Using the two selected cultures, growth was monitored in the presence of various media. The objective was to identify that recipe which would encourage the plentiful production of exopolysaccharide with a minimal cost implication. Two nutrient packages (medium E^{11} and medium G) were used and modified in trials to identify the optimum growth-inducing recipe.

Starvation

It is important that any bacterium used in the SIFT process be able to survive starvation and grow within the rock pores. Both EX299 and EX302 were starved of nutrients for extended periods. This was done by adding the cultures separately to artificial seawater and periodically removing samples to check whether they remained viable. Both cultures were starved in seawater at a range of temperatures and under aerobic and anaerobic conditions. The ability of the cultures to remain metabolically active was assessed by INT (p-iodonitrotetrazolium violet) and microscopy.

Penetration and Growth in Sandpacks

To model the effectiveness of the cultures to penetrate rock pores, the cultures were injected (using constant pressure of 5 psi) into one-meter sandpack columns containing Bunter sand within a high-temperature oven. First the cells were injected at their normal vegetative size, then the trial was repeated using starved cells. In a

third trial, starved cells were again injected but were followed by a nutrient injection to resuscitate the cells. The flow rate was measured during these injections to observe the permeability reduction. After each experiment, the columns were examined for bacterial penetration using scanning electron microscopy.

High Pressure Coreflooding of EX302

Culture EX302 had been isolated from a subterranean source and not a petroleum reservoir. An experiment was commenced to test the ability of the culture to survive high pressure and temperature conditions. This work was undertaken with the co-operation of AEA Winfrith, U.K.

The core used was made of Clashach sandstone and measured 30.3 cm by 3.8 cm. It was wrapped in PTFE tape to provide chemical inertness, followed by two layers of aluminum foil to provide a gas barrier. The core was then inserted into a rubber-sleeved Hassler Core Holder and an initial overburden pressure of 30 bar applied. This compressed the rubber sleeve onto the PTFE and foil to give a leak-free system.

The pore volume of the core was determined using the gas expansion technique to avoid using toxic chemicals within the test core. The core pressure was raised to 2 bar using nitrogen, followed by isolation from the rest of the system and de-pressurizing. The nitrogen released from the core was collected and measured. The effective pore volume was calculated, using Boyle's equation, to be 61.1 ml with a porosity of 17.7%.

The permeability of the core was determined by using seawater (brine). The brine did not contain any biocide and was filtered (0.45 μm) to remove bacteria. The brine was degassed with nitrogen prior to use. The core was initially filled with brine which flowed through the circuit at various flow rates ranging from 60 to 180 ml/hr and at a mean circuit pressure of 10 bar. This was continued until the gas content of the core had been reduced to 1 ml at atmospheric pressure. The brine was again flowed at a pressure of 10 bar at between 50 and 300 ml/hr, and the pressure drop across the core was noted for each flow rate. The pressure drop was used in Darcy's equation to obtain the absolute brine permeability. This was calculated to be 553 md at 10 bar. The core characteristics are listed in Table 1.

EX302 was prepared by growing overnight in 1-liter aliquots of anaerobic medium E at 50°C before centrifuging at 15,000 g for 10 minutes. The resulting culture pellets were resuspended in 1-liter aliquots of anaerobic artificial seawater in Duran bottles. The bottles were left at room temperature for 9 weeks to reduce the cell size. One week before coreflooding, samples were taken to confirm their viability.

The coreflooding system is as shown in the flow diagram (see Fig. 1). The core in its holder was immersed in silicone oil. The Jefri cylinder, or feed vessel, was used for the injection of the brine, the culture, and the nutrient into the core. This cylinder was driven hydrostatically by an Altex pump which was used to set the circuit pressure and was limited to 255.4 bar. When the brine and nutrient were injected, two 1-μm filters were inserted before the core to reduce microbial cross contamination.

The flow rate through the circuit was controlled by the Hydraton pump, which was set to extract fluid from the core at 2.6 ml/hr. The effluent from the core was collected in a PVT cell,which had a glass sight through which the collected volume could be measured using a vernier telescope. The PVT cell was subjected to an over pressure to prevent the sight from breaking. A 1-liter Jefri cylinder containing brine was placed between the PVT cell and the extraction pump to act as a buffer as the two pumps alternated. The pressure difference across the core was measured with the pressure transducers PT10 and PT11 and was recorded on a Magus 2,000 data logger. This also recorded the laboratory and oil bath temperatures.

To remove a sample from the rig, the PVT cell was isolated by closing valve V75. The pressure in the PVT cell was then reduced to atmospheric pressure to avoid aerosol formation during sample collection. Then, the sample vent was opened and the contents transferred to a sterile universal bottle. The PVT cell was then returned to the rig pressure before opening V75 and continuing the flood. The time taken to take a single sample was about one hour.

Prior to the experimental run, the risk of microbial contamination was reduced by filling the whole circuit with filtered brine and raising the temperature to 100°C for 4 hours. The feed vessel and line to valve V74 were sterilized with 2% Virkon for one hour, before rinsing three times with sterile distilled water.

The feed vessel was filled with the starved EX302 by pumping in with a Minipulse peristaltic pump (Gilson) to avoid aerosol formation. The feed line was reconnected to the circuit and the pressure slowly raised to 255 bar. Valves V33 and V34 were closed to ensure that the core was not bypassed, and the flow was started. 2.7 PV of EX302 were injected. At the end of culture injection, the feed line was disconnected and the contents of the feed vessel were pumped back into a Duran bottle. The vessel was again sterilized in Virkon before filling with anaerobic nutrient medium E. The line was reconnected, and 8.8 PV of nutrient were injected into the core. This was followed by 4.9 PV of brine, which was used to determine any change in permeability (K_{br}). Nutrient was again injected for another 5.0 PV, which was followed by another 2.2 PV of brine.

The total numbers of bacterial cells in the effluent before and during injection were monitored using epifluorescence microscopy and plotted against pore volumes passed. Viable counts were also performed on these samples and plotted against pore volumes.

A second flooding of brine through the core was used to determine any further permeability changes. The brine was flooded initially at 1 ml/hr before being raised to the experimental condition of 2.6 ml/hr after one pore volume.

At the end of an experimental run, the core were carefully removed and cut into four equal sections. Pieces were then taken from the start, first quarter, midpoint, third quarter, and end of the core. These were placed inside sterile universal bottles of known weight and reweighed to determine the weight of the core sample. The pieces were then placed in a sterile mortar with 10 ml of sterile 1/4 ringers solution and ground up using a sterile pestle. The resulting solution was diluted and spread onto nutrient agar plates for viable counts, and incubated at 30°C for two days. Viable counts were expressed as per gm of Clashach core. The total counts were also done on these samples using epiflourescent microscopy.

Pieces of the core were also taken and fixed in 3% glutaraldehyde cacodylate buffer. These were observed in a scanning electron microscope for the presence of bacteria and polymer.

Results and Discussion

Recipe and Culture Combinations

One nutrient package (medium E) supported good growth in EX302. Trials of modifications to this recipe showed that optimum exopolysaccharide production occurred when the yeast extract concentration and the sucrose concentration were respectively twice and half that used in the original recipe. However, this recipe did not support luxuriant growth of EX299.

Further work using another nutrient package (medium G) supported growth of both cultures. This suggests that an ideal SIFT culture is a mixture of cultures EX299 and EX302. This combination would be able to plug rock pores at the range of temperatures typically found below surface. They would be grown using a combination of nutrients as identified. However medium G would be optimum where temperatures in the thief zones range from 40°C to 70°C.

Starvation Responses

The starvation survival of both cultures was monitored using INT. Both cultures remained metabolically active in temperatures in the range 50°C–70°C under both aerobic and anaerobic conditions for over 28 days.

The ability of the cultures to revive following starvation in both aerobic and anaerobic conditions was also assessed by transferring to a suitable growth medium. Growth was assessed by an increase in culture turbidity (see Tables 2 and 3). Growth recommenced strongly after starvation, although EX302 was restricted to below 60°C.

The effect of starvation on the size of individual cells was measured by computer enhanced microscopy. The comparison of the sizes of growing cells and cells starved for 14 days showed that EX299 changed in size from 2.54 × 0.41 μm to 2.98 × 0.42 μm. EX302 changed from 3.31 × 0.27 μm to 2.78 × 0.43 μm.

Penetration of Sandpacks

The analysis showed that both cultures were able to penetrate along the length of the column. In further work, when nutrient package E was added to the starved EX302 cells within the column, confluent layers of exopolysaccharide were found over and between the sand particles. Similarly, when nutrient package G was added to starved EX299 cells within the column, large amounts of biomass were found throughout the column.

EX302 Pressure Injection

Pressure changes across the core during the initial injection of EX302 in the core were recorded (see Fig. 2). There was little change initially due to the circuit dead volume, but a pressure difference of 0.45 psi was achieved at 2.5 PV. Note the four sampling points where the pressure dropped to zero.

When the nutrient was initially injected, there was the normal lag phase associated with bacterial growth when there was little, if any, change in pressure across the core (see Fig. 3). After 4 PV, however, there was rapid growth of EX302 and a rapid increase in pressure differential. A maximum value of 20 psi was achieved.

The permeability of the core was assessed to determine if any change had occurred. This was performed by injecting brine as the reference (see Fig. 4). After an initial increase in pressure to 30 psi, the pressure differential declined to 0.3 psi. The

initial increase was probably due to the continued growth of EX302 in the nutrients still available. When nutrient was added for a second time, EX302 again showed a lag phase before the pressure differential across the core increased (see Fig. 5). The rate of increase was more rapid during this second nutrient feed because of the higher number of bacteria already present within the core. A total of 5 PV of nutrient were injected, with a maximum differential of 34 psi achieved.

The second brine flood showed that EX302 continued to grow while nutrient was still present, but the pressure differential declined again as it did during the first brine flood (see Fig. 6).

All stages of flooding are summarized in Figure 7, where it is apparent that each nutrient flood had a significant effect on the growth of EX302 in the core and on the pressure differential across the core. The flood conditions remained constant throughout the experiment, but oscillations in pressure differential did occur. This may be due to parts of the microbial biofilm being removed by shearing forces. These fragments moved further into the core before becoming attached again and growing further. The points where the pressure differential is at the lowest are the points when samples were taken from the rig for microbial analysis.

Analysis of the effluent from the rig showed large numbers of cells being swept out (see Fig. 8). It was interesting to note that the point where least total numbers were seen corresponds approximately with the start of the first brine flood, and the maximum value corresponded with the end of the second nutrient flood. The viable count dropped to below detectable levels after 7 PV. This may be due to cell death caused by rapid depressurization of the PVT cell as the sample times were accelerated to reduce rig downtime. Alternatively, the viable microbial population was firmly attached in the biofilm, and only dead cells were sheared off.

Figure 9 shows the distribution of the microbial population within the core at the end of the experiment. The total cell count showed that the microbes were evenly distributed throughout, whereas the viable cell count was marginally reduced towards the outlet. This was probably due to the bacteria at the front of the core utilizing most of the available nutrients at this low flow rate. It was apparent, however, that the core did contain large numbers of bacteria evenly distributed along its length, demonstrating permeability reduction throughout. These findings have been confirmed using electron microscopy. Figure 10 is from the core inlet face, where rod-shaped bacteria seem to have penetrated the pore spaces. This is again showed in the micrograph (see Fig. 11) taken 10 cm from the inlet. The magnification is lower, but penetration of the pore spaces can again be seen. Figure 12 is from a position 20 cm from the inlet. A thick layer of polymer can be seen in the top half of the micrograph, above the smooth surface of a freshly fractured sandstone area. These two distinct layers are divided by a thin line of bacteria two

or three cells thick, which produced the polymer. The outlet of the core is shown by Figure 13, where rod-shaped bacteria are visible on the surface of the polymer, which can be seen covering the sandstone. Typically, most of the bacteria would be hidden by the thick layer of polymer as depicted in the previous micrograph (see Fig. 12). These thick layers of polymer also provide evidence that growth had occurred in situ.

Conclusions

This paper reports on a research project for improving oil recovery using a micro-engineered approach. Two suitable candidates for the SIFT technique have been isolated and shown to have suitable starvation/revival/plugging characteristics. At the end of nutrient injection under test conditions, brine permeability had been reduced by 76% from an initial 559 md prior to EX302 injection, to 132 md after nutrient injection. A second injection of nutrient reduced this further to 69 md. This gave a total reduction of 88% overall. We suggest that these two bacteria are suitable candidates for flow diversion in the North Sea.

Acknowledgments

We gratefully acknowledge the financial support provided by the Oil and Gas Division of the Department of Trade and Industry for this project. We also acknowledge the help and assistance of the staff at AEA Winfrith with the core flood experiments and for most of the figures contained herein.

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Table 1 Characteristics of Clashach Core

Length	30.3 cm
Diameter	3.8 cm
Bulk volume	344.8 cm^3
Pore volume	61.1 ml
Effective porosity	17.7%
Absolute brine permeability	559 md

Table 2 Ability of EX302 to Resuscitate after Starvation at a Range of Temperatures

Temperature °C	Aerobic	Anaerobic
50	Yes	Yes
60	No	No
65	No	No
70	No	No

Table 3 Ability of EX299 to Resuscitate after Starvation at a Range of Temperatures

Temperature °C	Aerobic	Anaerobic
50	Yes	Yes
60	Yes	Yes
65	Yes	Yes
70	Yes	Yes

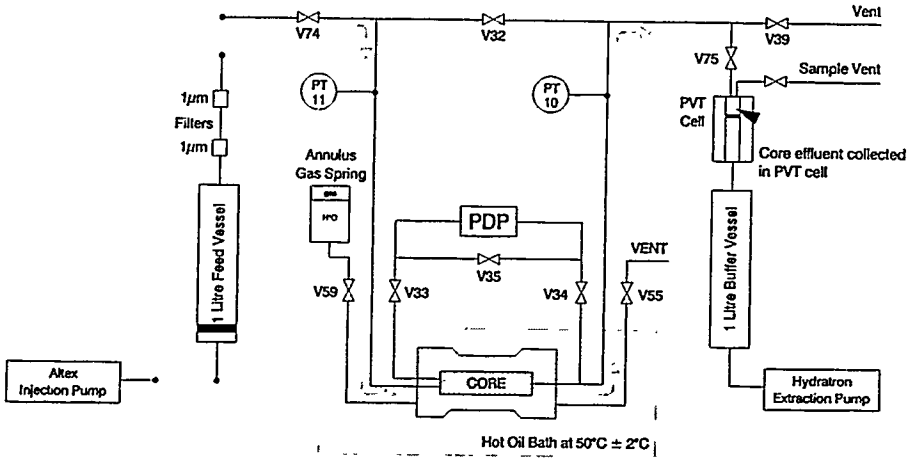

Figure 1 Flow Diagram of Test Rig

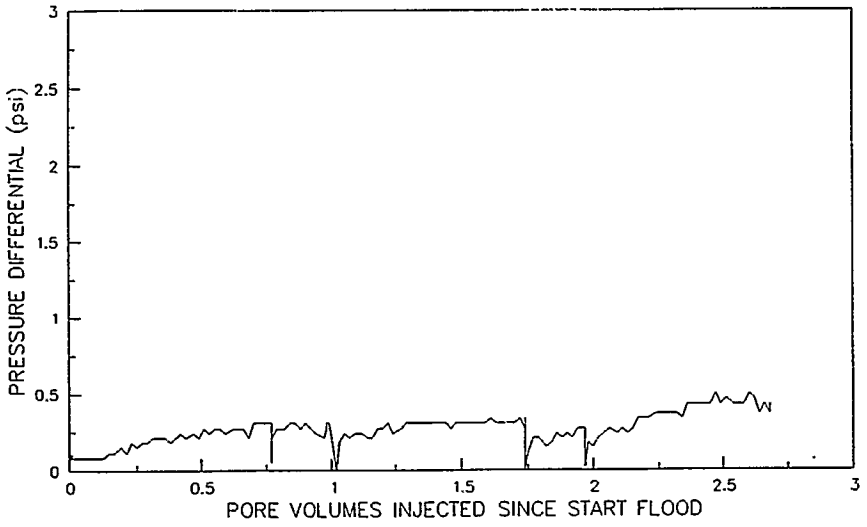

Figure 2 Pressure Differential across the Core during Initial Injection of EX302

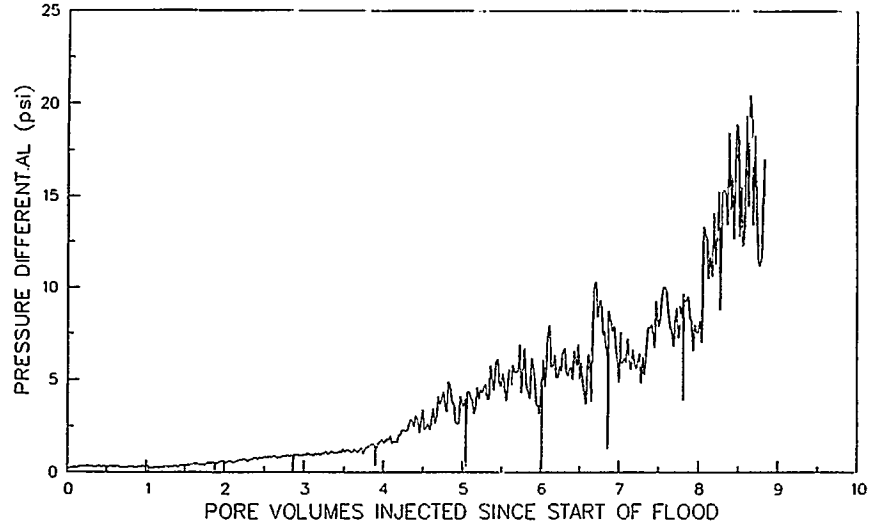

Figure 3 Pressure Differential across the Core during the Initial Nutrient Injection

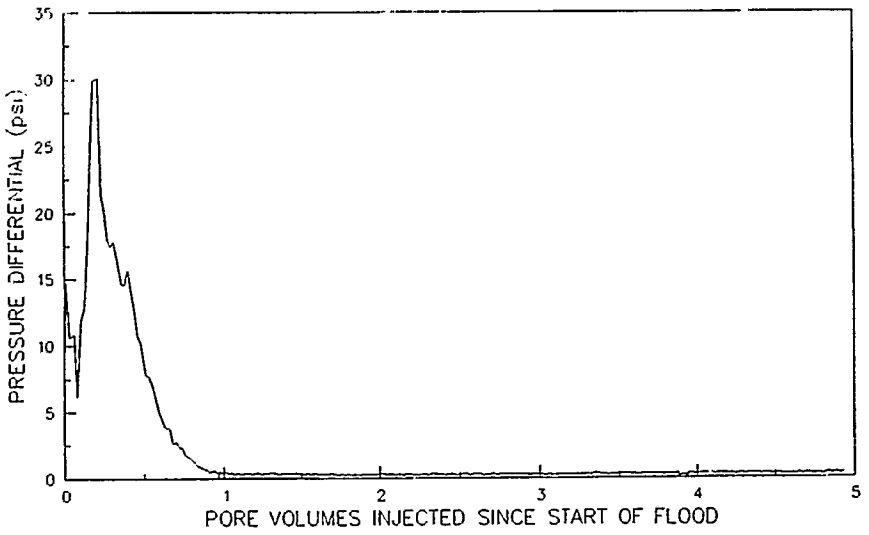

Figure 4 Pressure Differential across the Core during the Initial Brine Injection

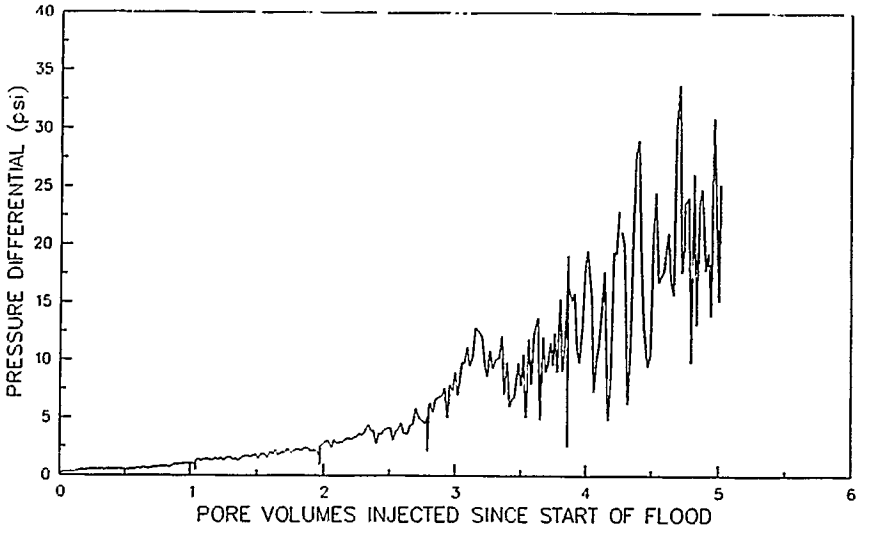

Figure 5 Pressure Differential across the Core during the Second Nutrient Injection

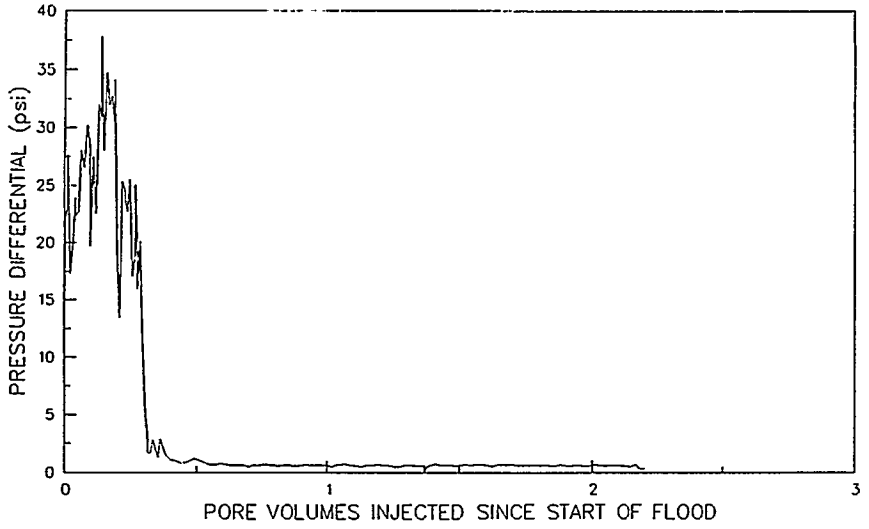

Figure 6 Pressure Differential across the Core during the Second Brine Injection

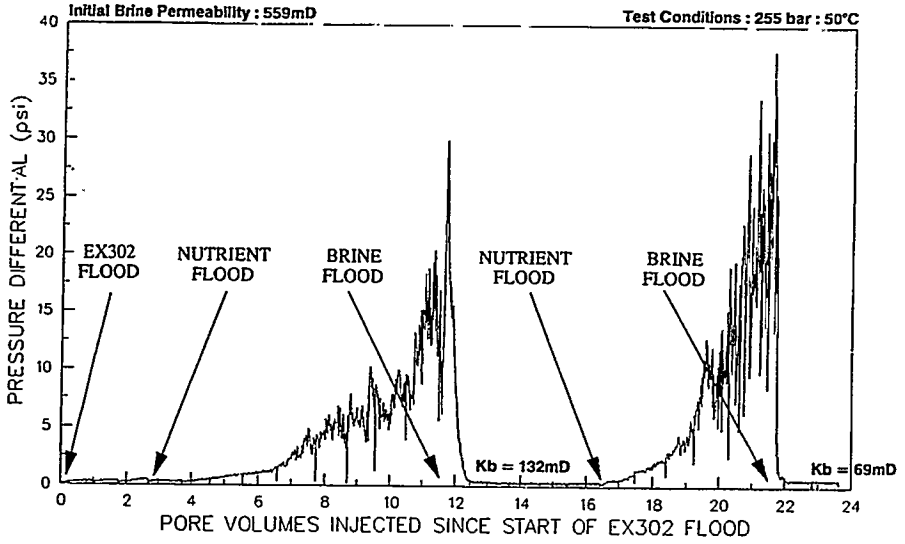

Figure 7 Summary of Pressure Changes across the Core during All of the Floods

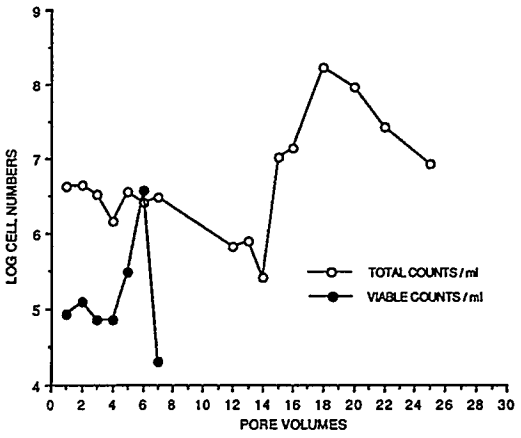

Figure 8 Total and Viable Cell Numbers per ml of Effluent vs. Pore Volumes

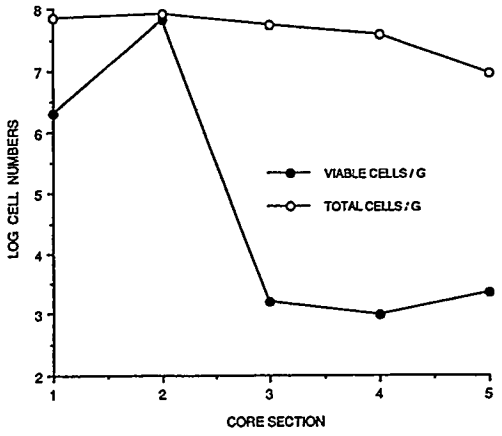

Figure 9 Total Cell Counts and Viable Cell Numbers per Gram of Sandstone along the Core. Position 1 Is the Inlet Face.

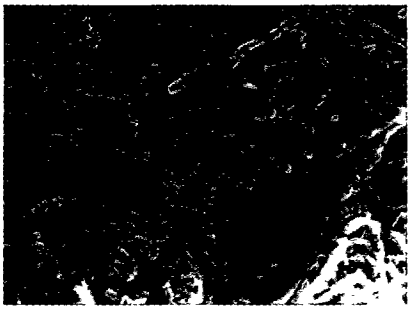

Figure 10 Electron Micrograph of the Core Inlet Face. Note the Rod-Shaped Bacteria Penetrating the Pore Spaces.

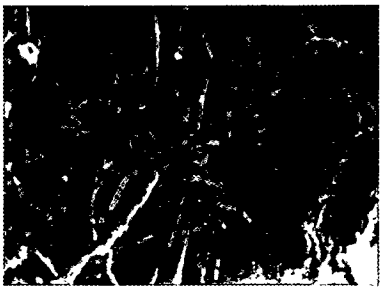

Figure 11 An Electron Micrograph of the Region 10 cm after the Inlet of the Core. Bacteria Can Be Seen Penetrating the Pore Spaces.

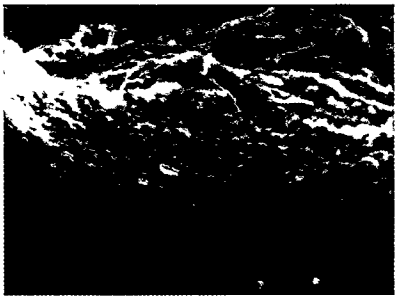

Figure 12 An Electron Micrograph of the Region 20 cm after the Start of the Core. Two Distinct Layers May Be Seen Divided by a Thin Layer of Bacteria. The Top Layer Is Polymer, the Lower Laery Sandstone.

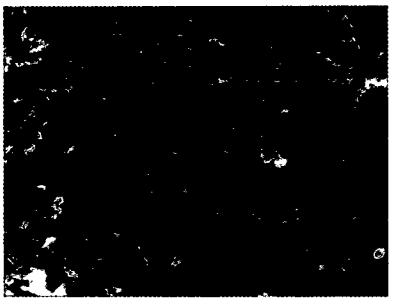

Figure 13 The End of the Core. Note That the Bacteria Have Traveled the Length of the Core As Shown by the Cells Visible on the Surface.

An Overview of Field-Specific Designs of Microbial EOR

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Abstract

The selection and design of an MEOR process for application in a specific field involves geological, reservoir, and biological characterization. Microbially mediated oil recovery mechanisms (biogenic gas, biopolymers, and biosurfactants) are defined by the types of microorganisms used. The engineering and biological character of a given reservoir must be understood to correctly select a microbial system to enhance oil recovery. This paper discusses the methods used to evaluate three fields with distinct characteristics and production problems for the applicability of MEOR technology. Reservoir characteristics and laboratory results indicated that MEOR would not be applicable in two of the three fields considered. The development of a microbial oil recovery process for the third field appeared promising. Development of a bacterial consortium capable of producing the desired metabolites was initiated, and field isolates were characterized.

Introduction

Microorganisms, most commonly bacteria, have been employed in the recovery of crude oil for decades.[1] Bacteria assist in oil recovery by the in-situ production of metabolites (i.e., byproducts as a result of growth) and biomass. Some bacterial products that may be useful in oil recovery include gases, surfactants, polymers, biomass, acids, solvents, and alcohols.[2,3]

Biogases, if produced in sufficient quantities, can reduce oil viscosity, displace immobile oil, and swell oil in place. Biosurfactants reduce interfacial tension; thus, improving pore–scale displacement efficiency. They can also alter wettability,[4] which may affect oil recovery.[5] Purified and concentrated biosurfactants have been reported to reduce interfacial tension between oil and water to 10^{-3} dyne/cm.[6]

Bacterial polymers and biomass are used to improve the sweep efficiency of waterfloods by plugging high-permeability strata or water-invaded zones. Recent field work has shown promise in the area of microbial plugging for improving sweep efficiency.[7,8]

Less is known about the effectiveness of acids, solvents, and alcohols produced by bacteria on oil recovery. Acids may improve permeability by altering the reservoir rock[9] and can also create CO_2 in situ by the dissolution of carbonate. Solvents may help remediate damaged wellbores resulting from paraffin deposition, dissolve crude oil, and act as cosurfactants. Alcohols may also assist oil recovery by acting as cosurfactants and solvents.

Every producing oil field possesses its own set of production problems. In order for microbial EOR to be successful, the treatment must be specifically designed to overcome problems associated with a given field. This paper idiscusses the methods used to evaluate three different fields for their suitability to microbial enhanced oil recovery.

Schuricht Field

Schuricht is a small single-well field in the Powder River Basin, Crook County, Wyoming. The well (21-24) was completed in 1983 in the Minnelusa "A" sand between 6,500 and 6,508 ft subsurface. Fluid expansion is the probable drive mechanism. The well is currently producing 100% oil at about 80 bbl/month and is near the economic limit. There is no associated gas or water production. Reservoir and crude oil characteristics are summarized in Table 1. Figure 1 shows the

production history of the well. There is no H_2S associated with Schuricht crude; however, there is 3.24 wt% elemental sulfur in the oil.

This field is similar to many Minnelusa fields in the Powder River Basin. If microbial EOR could be successfully demonstrated in this field, then the technology could hold promise for other fields in the area.[10] Moreover, this field has had no other EOR operations or off-pattern wells that could interfere with interpretation of results.

Demonstrating the effectiveness of a multiwell process in a single-well huff 'n' puff test is difficult. However, a technical success in this field would hold promise for economical successes in multiwell pattern floods elsewhere.

Determination of MEOR Method to Be Applied

A single-well pump-in/pump-out test of the microbial system is the only available option in the Schuricht field because there are no plans to drill a second well in this field in the foreseeable future. For this field, two options for microbial EOR were evaluated: in-situ biosurfactant production and in-situ gas generation. A simplified microbial soak process was evaluated using Buckley-Leverett fluid displacement principles to determine the results that could be expected under ideal circumstances. The Buckley-Leverett fluid displacement method uses relative permeability curves and viscosities of the flowing fluids as the basis of saturation front advancement.

Relative permeability data needed for the Buckley-Leverett simulations were not available for the Schuricht field, but initial and irreducible oil saturations were known. Relative permeability curves for the Schuricht field were taken from similar neighboring Minnelusa fields with similar end-point saturations.

Simplifying assumptions were made for both microbial options before running the simulations. The formation was assumed to be homogeneous and no bacterial plugging occurs. The bacterial injection slugs were dilute and the relative permeability curves for water were used during injection of the slug. No attempt was made to model bacterial transport, growth, or metabolite production. The following assumptions were made for the biosurfactant case: 1) Oil and water viscosity remain constant. 2) Relative permeability end points are altered after bacterial incubation corresponding to a 40% reduction in residual oil saturation resulting from biosurfactant production.

The following assumptions were made for the microbial gas production case: 1) No change in the relative permeability curve end points. 2) Enough CO_2 is produced to saturate all the contacted oil. Saturating the Schuricht crude oil with CO_2 would

swell the contacted oil and lower its viscosity from 15 cP to 6 cP.[11] These assumptions represent best-case scenarios and may not be representative estimates of actual field results.

Simulated incremental oil recovery for the microbial gas mechanism over the biosurfactant mechanism is shown in Figure 2. In this figure, normalized incremental oil recovery of the CO_2 process over the surfactant process is plotted against the normalized total fluid production. Normalized incremental oil production is calculated by the equation:

$$\text{Normalized incremental oil production} = \frac{\text{CO2 process} - \text{Surfactant process}}{\text{Contacted oil in place}} \quad (1)$$

Normalized total fluid production is calculated by the following equation:

$$\text{Normalized total fluid production} = \frac{\text{Total produced fluid volume}}{\text{Total injected fluid volume}} \quad (2)$$

Figure 2 is a good way of comparing one process to another. As can be seen, the option of saturating the contacted oil with CO_2 appears to recover more oil than generating a biosurfactant in situ for this particular single-well field. The reason for the poorer performance of the biosurfactant option is because the mobilized oil is in the high water saturation portion of the relative permeability curves. Oil mobility is low in this region and, consequently, the additional mobilized oil moves through the formation to the producing well very slowly. On the other hand, the microbially generated CO_2 lowers the viscosity of all the contacted oil, which changes its mobility or relative permeability allowing the oil to flow faster to the producing well. Swelling the oil by saturating it with carbon dioxide also aids in its recovery.

This simple analysis indicates that for the Schuricht field it would be advantageous to employ bacteria capable of producing enough CO_2 to saturate the contacted oil instead of bacteria that reduce interfacial tension between oil and water. Based upon this result, it was decided to find and evaluate bacteria that could produce a copious quantity of CO_2 or other similar gas to be applied in a possible microbial EOR demonstration in the Schuricht field.

Preparatory Microbiological Work for Field Test

A successful field application of microbial EOR is highly dependent on the selection of the bacterial strain or consortium to be used in the field. Enrichment procedures to isolate microorganisms indigenous to the Powder River Basin of Wyoming began by collecting and screening Schuricht field fluids and solids. Specific

characteristics sought for the enriched microbial cultures were: 1) growth at the reservoir temperature of 138°F (60°C), 2) growth with and without oxygen present (i.e., facultative anaerobe, microaerophilic, denitrifying), 3) salt tolerant to 3% KCl to match the reservoir rock and fluid compatibility, 4) compatible with Schuricht crude oil, and 5) production of large amounts of CO_2 under reservoir conditions.

Soil samples retrieved from the Powder River Basin were enriched for bacteria capable of gas production. A 1.0 g sample of oil-laden cow manure was added to liquid media [trypticase soy broth (TSB), potato dextrose yeast (PDY) medium (ATCC 337), and potato medium (ATCC 1126)][12] and incubated aerobically at 60°C. The medium was amended by adding 3% KCl and 1% $NaNO_3$. After 24 hr of growth, the organisms were subcultured to identical media and incubated anaerobically at 60°C. Growth (turbidity) and gas production (bulging septa) were observed within 24 hr. Potato medium continued to show a positive response for gas production and was used for further study. Organisms were streaked on agar plates, and colonies were picked and transferred to isolate the organisms. Four Schuricht enrichment cultures were obtained and identified as 1, 3a, 3b, and 4. All four isolates are similar; however, the number 1 isolate produced more gas and was singled out for further studies. This organism was renamed Powder River Basin #1 (PRB #1) and was stored in maintenance broth with 2% glycol for long-term storage.[13]

Medium Development. A potato base medium was used initially to study the bacteria because of the availability of a cheap carbon source (potato starch). The potato based growth medium was analyzed to determine bacterial utilization of the components. The carbon and energy sources provided in the potato medium were varied along with other constituents to determine the necessary components required for bacterial growth. Potato was added to provide minerals and a starch substrate (carbon source) for PRB #1; sodium nitrate served as the terminal electron acceptor of the electron transport chain, and yeast extract was added as a source of water-soluble vitamins, amino acids, peptides, and an additional source of carbon. Results indicated that PRB #1 requires yeast extract and sodium nitrate for growth and does not metabolize potato starch.

Because yeast extract and sodium nitrate are required nutrients for these organisms, a modified medium E (ATCC 1502 amended with nitrate and yeast extract) was used to cultivate the organisms. The modified medium provides mineral salts (molybdenum, iron, and copper) for enzymatic functions; phosphates for metabolic reactions; yeast extract for water-soluble vitamins, amino acids, peptides, and carbon; nitrate as a terminal electron acceptor; and fructose as the major source of carbon. The constituents of modified medium E per liter of water are as follows: 10 g fructose, 1 g $(NH_4)_2SO_4$, 0.51 g $MgSO_4$, 10.6 g K_2HPO_4, 5.3 g KH_2PO_4, 5 g yeast extract, 20 g $NaNO_3$, 30 g KCl, and 10 ml trace mineral salts.[14]

Characterization of PRB #1. PRB #1 is a thermophilic, gram positive, nonspore-forming rod. The organism does not decrease the surface tension of water and will not grow in a salt concentration above 10% KCl, but will grow and reproduce in 5% KCl. PRB #1 does not adhere to hydrocarbons and does not produce any organic acids or alcohols when grown on modified medium E. The organism will grow in a temperature range from 113°F to 162°F (45°C to 72°C). The organism does not produce ammonia, but does produce large amounts of carbon dioxide (CO_2) and nitrous oxide (N_2O) gas when cultivated on modified medium E. PRB #1 is a thermophilic bacterium capable of reducing nitrate to nitrous oxide under limited oxygen conditions.

Gas Production. Gas chromatography was used to analyze the culture headspace for the presence of CO_2 and N_2O in media containing fructose (sugar) and without fructose. Tests were performed at 138°F (60°C), and results indicate that PRB #1 produces more CO_2 than N_2O with fructose present in the growth medium. PRB #1 without fructose in the medium yields higher N_2O concentrations than CO_2. However, there was no significant difference in the total amount of gas produced with the two media. For example, in sealed serum vials, fructose media yielded 0.483 g/l of gas (CO_2 and N_2O combined) whereas, media without fructose yielded 0.556 g/l. Thus, while the overall amount of gas remains constant, the specific type of gas can be controlled by choosing the appropriate nutrient source.

Results of In-Situ Growth. A Berea sandstone core (1 in. diameter × 6 in. long) was used to determine if the organism could be transported with brine through sandstone rock, could grow within the rock matrix, and could produce the same quantity and mixture of gases inside a sandstone matrix as in a laboratory shake flask. The Berea sandstone core used in the experiment had a permeability of 650 md and a porosity of 23.5%.

Under a microscope, bacteria were clearly visible in the core effluent after injecting 1.5 PV of the bacterial suspension. Effluent from the core inoculated in fresh medium grew well, indicating cells were distributed throughout the core. A total of 2.0 PV of the PRB #1 cell suspension was injected into the sandstone core. A 60-ml syringe was attached to the outlet end of the core, the inlet end was capped, and the core and syringe were then incubated at 138°F (60°C).

The gas displaced into the syringe was qualitatively analyzed on a gas chromatograph to determine the type of gases produced. It was found to be predominately CO_2 with some N_2O present. This gas mixture is also the same mixture produced during incubation of PRB #1 cells in serum vials. The amount of gas produced from the core system was about 2.5 g/l or 5 times the amount produced from the sealed serum systems. However, calculations show that a yield

of 22 g/l is required to saturate the contacted oil for conditions at the Schuricht lease.

Permeability of Formation

At the time that the field was made available for a demonstration project, there was some uncertainty about the permeability of the reservoir. This characteristic is especially important when considering an application of in-situ MEOR. A formation permeability less than about 75 md is reportedly too tight to inject and deliver vegetative microorganisms into the reservoir.[15]

There were indications that the permeability of the field was 10 md or less, but the operators believed that this information was incorrect. A buildup test was run to confirm the reservoir permeability and other key properties such as skin and static reservoir pressure. Reservoir permeability calculated from a Horner analysis of the data was 2.2 md. This permeability value was substantially lower than the field owners and operators had estimated, but only slightly lower than the DST-calculated permeability of 10.2 md. A Fetkovitch decline curve analysis[16] indicated a permeability of 3.1 md. Other methods and analyses yielded permeabilities consistent with the pressure buildup calculated permeability.

Decision to Terminate the Schuricht Field Test

On the basis of the analyses indicating that the average permeability of the Schuricht reservoir was between 2.2 md and 10.2 md, which was much lower than thought by the operators, it was determined that the Schuricht field would be a poor candidate for a microbial EOR field demonstration. Such low permeability would not be expected to allow the successful injection and delivery of microorganisms into the formation.

Smackover Field

The operators of the Smackover field in southern Arkansas wanted to determine if MEOR could be successfully applied in this old, declining-production field. The average reservoir porosity is 36%, and the permeability in the clean, unconsolidated sand sections averages 5,000 md, but ranges as high as 12,000 md in the productive lenses. Connate water saturation averages 20%. The API gravity of the crude oil is 20°, and oil viscosity is presently 75 cP at the reservoir temperature of 110°F. The concentration of salt in the produced water ranges from 50,000 to 60,000 ppm TDS.

The discovery well for the field, the Murphy No. 1, blew out during drilling operations in 1922 and formed a crater 450 ft across and 50 ft deep. Blow-down of the gas cap and the absence of significant solution gas resulted in the almost complete dissipation of the reservoir energy in slightly more than five years. By 1930, vacuum installations were in operation, and the reservoir energy had been essentially exhausted. Production thereafter resulted from gravity drainage and bottom-water influx. Large volumes of sand were removed in the early life of the reservoir during the periods of high producing rate and created channels and voids in parts of the reservoir.

Of the approximately 500 million stock tank barrels (MMSTB) of oil originally in place (OOIP), about 35% has been recovered to date. Total reservoir produced water-cut was more than 80% as early as 1934 and has been controlled only through continuous remedial work and well abandonment. The reservoir is not well suited for secondary recovery because the vast majority of the field is not unitized, yet the volume of oil presently in place is greater than that found in many new fields, and every effort should be made to increase the ultimate recovery.

Microbial Oil Recovery Mechanism

During meetings with field operators, it was agreed to study the possibility of using indigenous bacteria to produce a biogas (CO_2) process to lower the in-situ crude oil viscosity as a mechanism of oil recovery. This process would be most effective in the areas of the reservoir with the greatest reservoir pressure because more CO_2 can be dissolved into the oil at higher pressures, lowering oil viscosity. It was also decided to consider the use of indigenous gas-producing bacteria, if possible, to produce the CO_2 because they would be best suited to reservoir conditions.

Microbial Isolation and Characterization

Bacteria were isolated from samples of produced brine collected from wellheads and from produced water tanks. Water samples were collected anaerobically in 1-liter glass bottles previously purged with nitrogen. In the laboratory, nutrients (phosphate, nitrate, and molasses) were added to the samples, and tests were conducted to enrich for gas-producing bacteria.

The nutrients were added to 100-ml serum bottles. The bottles were autoclaved and then placed in the anaerobic chamber for 2 to 3 days to allow for an exchange of anaerobic gases. A base of filter sterilized (0.2 μm) or nonsterilized Smackover brine was added to the bottles under anaerobic conditions. The serum bottles were then sealed and capped.

Data to monitor headspace pressure were collected using three different base brines: filtered (0.2 μm) reservoir brine, unfiltered reservoir brine, and a filtered synthetic brine. The synthetic brine is based on chemical analysis of field brine.

Results from these headspace pressure experiments are shown in Figure 3. The difference observed between the filtered and unfiltered brine samples may be caused by a secondary microbial population in the unfiltered brine that uses metabolites from the primary culture, utilization of insoluble nutrients that were filtered out of the filtered brine, or a combination of both. The data obtained from the synthetic brine indicate that it is suitable for experimentation if field brine is unavailable.

The field operator studied reservoir data and ran buildup tests to determine reservoir pressures at different locations in the field. Whereas the pressure did vary some throughout the reservoir, the highest pressure observed was 33 psia. From a published correlation of heavy crude oil and CO_2 mixtures,[11] it was found that saturating the oil with CO_2 would have a negligible effect on oil viscosity at reservoir pressure. For an immiscible CO_2 process to have a significant effect on oil viscosity, pressures in excess of 200 psia are required (see Fig. 4).

Except for gas production, bacteria enriched from the field brine were not studied extensively. However, the bacteria are known to produce n-propanol, ethanol, methanol, formate, acetate, and propionate. Their effect on oil recovery is not clearly understood. A sandpack made from produced sand collected at the Smackover field was used to determine the oil recovery potential of the enriched bacteria.

A sandpack filled with cleaned produced Smackover sand was brought to initial oil saturation conditions at reservoir temperature and then waterflooded with 4 PV of filtered (0.2 μm) field brine. It was then shut in for 2.5 weeks to simulate a bacterial incubation. This was followed by 2 PV of filtered brine and 4.3 PV of bacterial inoculum; then the sandpack was shut in to allow bacterial growth. The sandpack was incubated until the rise in pore pressure ceased (4 weeks). Pressure in the sandpack was maintained at 33 psia (reservoir pressure) by periodically bleeding the microbial gas into an attached syringe. Figure 5 illustrates the effect of bacterial growth and gas production on oil recovery.

Following the bacterial incubation period, both oil recovery and saturation followed the respective curves established during waterflood and bacterial injection. An increase in the slope of the oil recovery curve would be indicative of bacterial induced oil recovery. Sandpack recovery data after bacterial growth followed the same trend as the waterflood, which indicated no additional oil was recovered as a result of bacterial growth in the sandpack.

Smackover Work Stopped

The results of the sandpack experiment were disappointing, but expected. At such low reservoir pressure, saturating the oil with CO_2 is of little value. It was concluded jointly with the field operator that the Smackover field had little potential for successfully demonstrating microbial gas technology because of the low reservoir pressure and large gas cap.

Naval Petroleum Reserve 3

The Naval Petroleum Reserve 3 (NPR-3) is located 40 miles north of Casper, Wyoming, and produces from the Teapot Dome structure in the Powder River Basin. This structure contains nine producing zones ranging from 300 ft subsurface to 4820 ft subsurface. The Shannon sandstone is the shallowest and largest reservoir in the field with over 600 wells. Following discussions with NPR-3 engineers and studying the history of the field, it was decided that a small area in the Northern Second Wall Creek (NSWC) reservoir would be the best location for a MEOR field trial.

The NSWC reservoir is bounded to the northeast by the Salt Creek South unit and to the south by a sealing northeast-southwest trending strike-slip fault which separates it from the Southern Second Wall Creek reservoir. The eastern and western boundaries are the corresponding oil-water contacts.

Initial production was established from the NSWC reservoir on November 17, 1922. Development and production continued until December 31, 1927, when operations were discontinued by order of the United States District Court and the reserve was turned over to the Navy Department. Peak production of 2,542 barrels of oil per day (BOPD) occurred in 1923, and 2.338 MMSTB of the original 39.21 MMSTB oil in place had been produced from the NSWC reservoir before shut-in.

In response to concerns of lease drainage in the adjacent Salt Creek South unit, limited production began along the lease boundary. A waterflood was initiated in 1979; production peaked quickly at 1469 BOPD and began a rapid decline.

The Second Wall Creek reservoir at NPR-3 is naturally fractured, and the waterflood is performing poorly because much of the oil is bypassed by the fracture system. Natural gas huff 'n' puff has recently been proven successful at NPR-3 in the Shannon formation. Based upon that experience, it was decided to investigate the use of native bacteria that produce large quantities of gas in conjunction with other bacteria that produce biosurfactants. The combination of the two bacterial

products (gas and surfactant), along with any bacterial plugging of the fractures that may occur, appeared to have potential for recovering additional oil from NPR-3.

Microbial Enrichment

Produced water, injection water, produced oil, and wellhead soil samples were collected anaerobically from NPR-3. Soil samples, collected near wellheads, were transferred to Hungate tubes containing modified medium E (as mentioned above, with fructose as a carbon source) to enrich for native gas-producing organisms. A mixed consortia of microorganisms were enriched from the soil samples. The most promising bacterial culture was designated as NPR-3A. The culture is a facultative anaerobe and grows robustly within 48 hr at the reservoir temperature of 50°C (122°F).

Water samples were also collected at the wellhead and used to determine if sulfate-reducing bacteria (SRB) were present and to enrich for other gas-producing bacteria. SRB were found in two of the four wells sampled for water. Bacteria that did not produce gas were cultivated from the produced water, but no native gas-producing bacteria were grown from any of the produced water samples tested.

Microbial Characterization of NPR-3A

Microbial Physiology. The organism (NPR-3A) enriched from the NPR-3 site is a facultative anaerobe and reduces nitrate to nitrous oxide while producing carbon dioxide. Using a facultative anaerobic bacteria (capable of growth with and without oxygen present) for a field project is desirable because the reservoir matrix is anaerobic, while the surface facilities would be an aerobic environment. The organisms do not reduce surface tension, indicating that biosurfactants are not produced.

Gas Production. NPR-3A begins gas production sooner in sealed vials with oxygen present in the headspace than under completely anaerobic conditions (see Figs. 6 and 7). The total gas (combined CO_2 and N_2O) produced did not significantly vary with oxygen or without oxygen present in the headspace (0.444 g/l and 0.545 g/l respectively).

Surfactant Producing Organisms. The inclusion of a biosurfactant-producing bacteria, *Bacillus subtilis*,[17] in the proposed bacterial consortia for improving oil recovery in the Second Wall Creek reservoir of NPR-3 may be a more effective approach than applying gas-producers by themselves. *Bacillus subtilis* ATCC 21332 appears to grow well and produce surfactant at 50°C.

B. subtilis 21332 was grown at 50°C in medium E, trypticase soy broth, potato medium, and sugar beet medium to determine the most effective medium for surface tension reduction (biosurfactant production). Surface tensions were measured using the supernatants. The cell pellets were lyophilized to determine cell dry weights. *B. subtilis* generated similar amounts of biomass in each media type; however, the surface tensions were only decreased in potato medium, medium E with 1% yeast extract, and trypticase soy broth.

Coreflood. The average pressure of NSWC reservoir is 500 psia. A coreflood was run at reservoir temperature and pressure to determine if the bacteria can survive a sudden pressure increase, as would occur during injection operations. NPR-3A organisms were injected into a Berea sandstone core (700 md) to determine transport and growth efficacy of these bacteria at a reservoir temperature and pressure. Cells were injected into a brine-saturated core under anaerobic conditions at 500 psia and 50°C. Breakthrough of the cells occurred at 1.75 PV, indicating good microbial transport through the core. A gas sample was collected from the core after bacterial incubation and confirmed to be predominantly carbon dioxide and nitrous oxide, indicating that the bacteria grew well in the core at reservoir temperature and pressure.

Development of a Field Medium

A field medium was prepared using production water collected from the 45-AX-20 wellhead. Water analysis indicated that the produced water was deficient in phosphate, nitrate, and several minerals. Modifications to the reservoir water were necessary before a microbial population could be established. The water was amended with the following nutrients per liter: 20 g KNO_3, 5 g yeast extract, 1 g $(NH_4)_2SO_4$, 10 g fructose, 10 ml mineral salts (0.01 g $Na_2MoO_4 \cdot 2H_2O$, 0.1 g $CoCl_2 \cdot 6H_2O$, 0.01 g $CuSO_4 \cdot 5H_2O$, 0.1 g $ZnSO_4 \cdot 7H_2O$, and 1.0 g Na_2EDTA), 100 ml phosphates (53.3 g KH_2PO_4 and 106.0 g K_2HPO_4). The amended water would be used as the injection medium for the active culture during a field trial. Initial tests indicate positive growth and gas production from the NPR-3 consortia in the field medium.

Project Status

The microbial process for improving oil recovery at NPR-3 looks promising. However, funding for the project ended before the process design was completed for field testing. Additional work needed to complete the process design includes determining (1) the ability of bacteria to plug natural fractures, (2) the compatibility of the two microbial systems (NPR-3A and *B. subtilis*), and (3) the synergistic

effect on oil recovery of combining the two microbial recovery processes (biogas and biosurfactants).

Discussion

The application of microbial EOR to an oil field involves input from engineers, microbiologists, field personnel, and chemists. Engineers can help determine which microbial process will be of most value to the reservoir and the quantities of bioproducts required. Microbiologists are needed to select the application organism and to fine-tune growth conditions to achieve the quantities of bioproducts required. Field personnel and engineers who know the problems associated with a particular producing field can help steer the microbial EOR project away from poor candidate sites and help select a viable microbial oil recovery mechanism. The importance of knowing the field fluids chemistry becomes obvious when considering the application of living organisms.

The gas-producing bacteria that we worked with produced CO_2 and N_2O. It is well established that microbial denitrification proceeds from nitrate to N_2 (and in some cases to N_2O) with the help of various enzymes. The respiratory denitrification pathway is expressed as follows:

$$NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

When a bacterium lacks nitrous oxide reductase, the enzyme required to convert N_2O to N_2, the final product in the denitrification pathway is N_2O. The molecular weight of nitrous oxide is the same as carbon dioxide; and N_2O is slightly more soluble in water than CO_2. Other physical properties of N_2O are similar to CO_2.[18] Nitrous oxide is not commonly used in the recovery of petroleum; however, it may be of some value to oil recovery based upon the comparison of its physical properties with carbon dioxide.

The Schuricht field initially appeared like an ideal location for a microbial field test —small field, pristine conditions, no other wells to interfere with interpretation of results. However, the permeability of the formation was too low to allow effective injection and placement of the bacteria into the reservoir. If the permeability had been acceptable, then there was still the issue of producing enough gas to effectively recover oil.

Calculations indicate that on the order of 20 g of CO_2 per liter of injected inoculum would be required to saturate the contacted oil in the Schuricht field. The theoretical maximum CO_2 production from 10 g of fructose is 14.7 g of CO_2 based on stoichiometric chemistry for anaerobic metabolism.[19] Shake flask experiments

(batch) obtained 0.5 g (CO_2 and N_2O combined) per liter of inoculum. An experiment in a core obtained 2.5 g/l with a single nutrient slug. This number could be improved by optimizing growth conditions, increasing fructose concentration, and improving metabolite production kinetics, but reaching the goal of 20 g/l from a single nutrient slug injection is still questionable.

The Smackover field also held high initial promise for microbial EOR. High reservoir permeability, low temperature, and moderate salinity are ideal for bacterial growth. However, the production problems associated with that field appear to be too great to be overcome with microbial EOR. A multiwell flood was not feasible because of the very low reservoir pressure and the large gas cap overlying the reservoir, and because the field is not unitized. This left the single-well treatment as the only currently viable option for the field. The current recovery mechanisms for this field are gravity drainage and water drive. Reducing the viscosity of the heavy oil may increase the efficiency of the natural water drive enough to recover additional oil. The reservoir pressure, however, is too low for CO_2-producing bacteria to be effective at reducing oil viscosity.

NPR-3 Second Wall Creek reservoir appears to have a need that microbial EOR might be able to fill. There is an active waterflood that has poor sweep efficiency because of the naturally fractured reservoir, and gas huff 'n' puff has been successful in other reservoirs in the area. The application of bacteria to at least partially plug the fractures and at the same time produce CO_2 and biosurfactants in situ appears to be a promising recovery mechanism for this field. More testing of the systems and their interactions needs to be done before field application.

Obviously, one MEOR process cannot be successfully applied to every oil reservoir. Each field must be evaluated individually before choosing a microbial treatment. And not all fields are well suited for microbial EOR techniques.

The bacteria to be applied in the field must be compatible with field properties. Two of the most important field properties are temperature and salinity. Bacteria have a limited growth temperature range, and their optimal growth temperature range is much smaller. At higher temperatures, their metabolism may shift to produce other unwanted metabolites or shut down altogether. Salt concentration in the reservoir brine is also an important element in the metabolism of bacteria. High salt concentration is a natural preservative (or inhibitor of bacterial growth).

Complete modeling of bacterial systems in a porous medium is a daunting task. Besides the issue of transport, there is the complication of cell division and product formation. Decoupling these factors can be helpful in designing a microbial EOR system. For example, before deciding on a bacterial system to apply in the Schuricht field, two mechanisms were modeled abiotically using Buckley-Leverett

methods. Bacterial transport and growth were neglected, and assumptions of product formation and efficacy were based on laboratory results and productivity. It was determined that in-situ biogas generation would have a more distinguishable effect on oil recovery than an in-situ biosurfactant soak (see Fig. 2).

Conclusions

1. A field design of MEOR should involve the expertise of engineers, microbiologists, field personnel, and chemists.

2. Simple models and correlations that consider only the abiotic portion of MEOR can lead to important knowledge regarding what will and will not work.

3. Field-specific laboratory data on microbial growth and metabolite production, including corefloods, are vital to the design of a microbial field project.

4. Field properties such as temperature, salinity, and permeability are significant factors in MEOR design.

Acknowledgments

We would like to express our gratitude to K S L Enterprises, TIORCO, Phillips Petroleum Co., and the staff at the Naval Petroleum Reserve 3 for their support and guidance with regard to the various projects discussed. Thanks also go to the U.S. Department of Energy for funding the work (Contract Number is DE-AC07-76ID01570) and to Leonard Keay (DOE-ID) and Rhonda Lindsey (DOE-BPO) for their project management. We are also grateful to Lockheed Martin Idaho Technologies for allowing the publication of these findings.

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Table 1 Reservoir and Fluid Property Data, Schuricht 21–24, Minnelusa "A" Sand

Depth, ft subsurface	6,500
Sand thickness, ft	8
Porosity, %	12
Connate water saturation, %	37
Reservoir temperature, °F	138
Crude oil gravity, °API	25.4
Crude viscosity at reservoir temperature, cP	15
Original oil in place, MSTB	115
Recoverable oil, MSTB	115
Areal extent, acres	103
Number of wells	1
Cumulative oil produced, STB	8,300

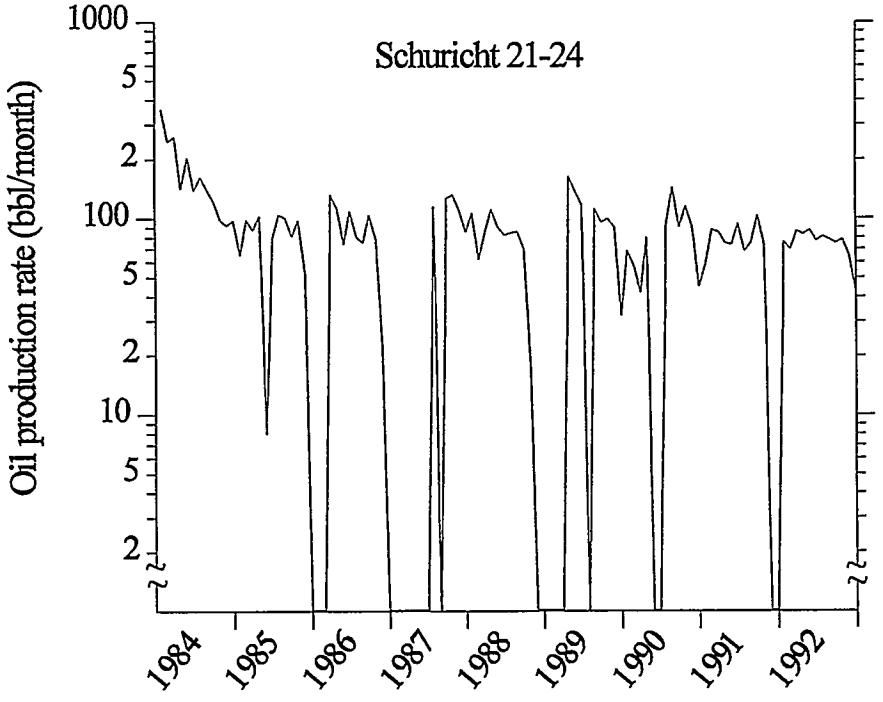

Figure 1 Production History of the Schuricht 21–24 Well

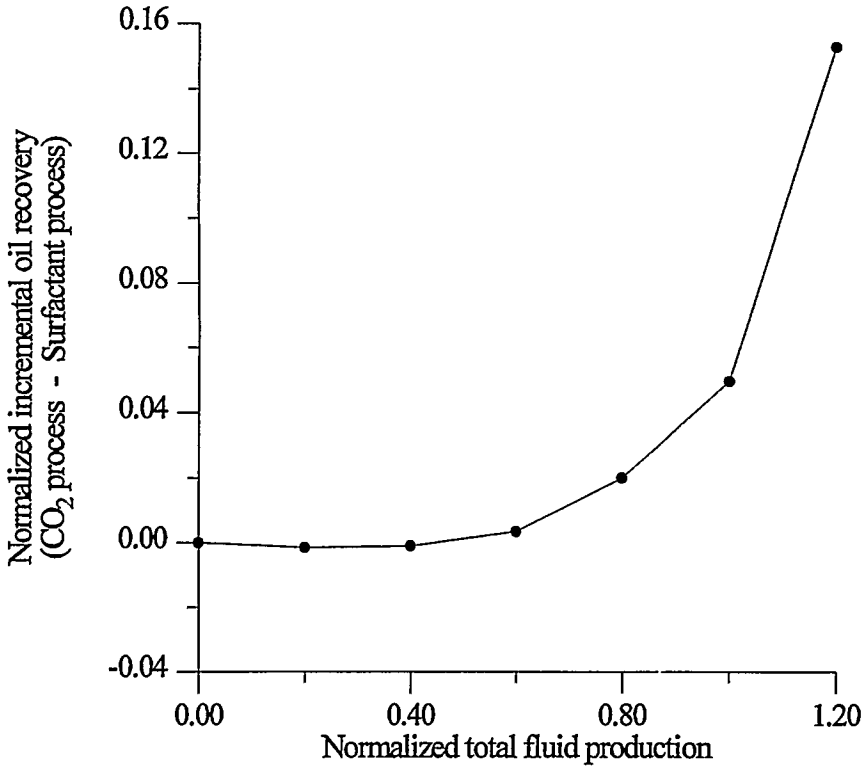

Figure 2 Comparison of Simulated Oil Recoveries from In-Situ-Produced CO_2 and Biosurfactant

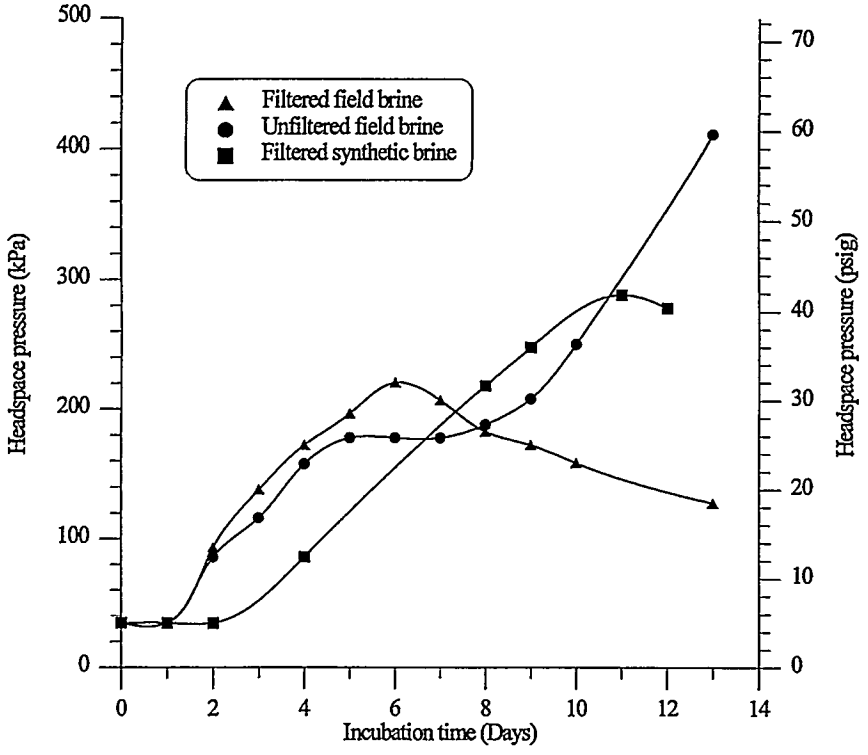

Figure 3 Headspace Pressure Data from Smackover Field Cultures

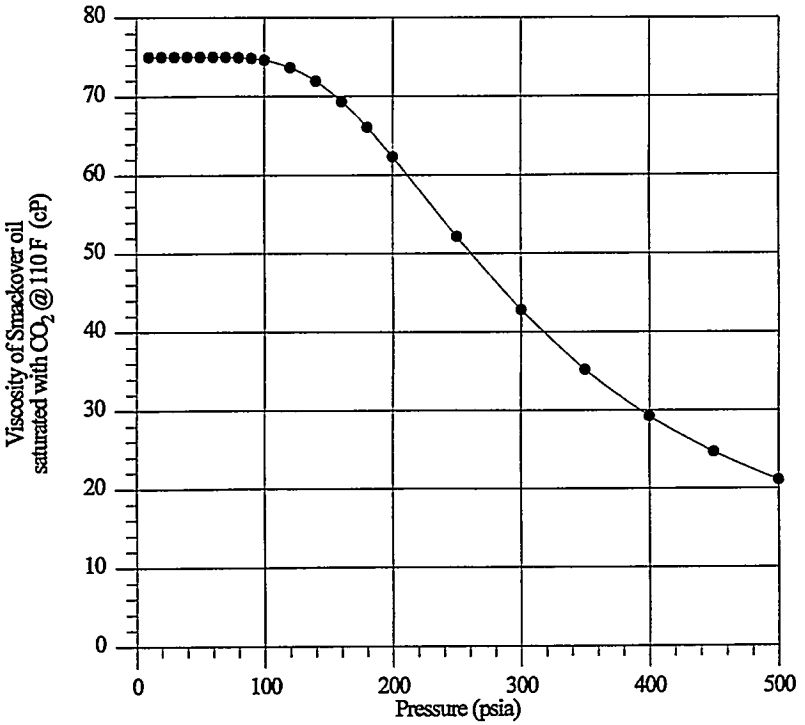

Figure 4 **CO_2-Saturated Smackover Crude Oil Viscosity Reduction with Increasing Pressure. Reservoir Temperature Is 110°F.**

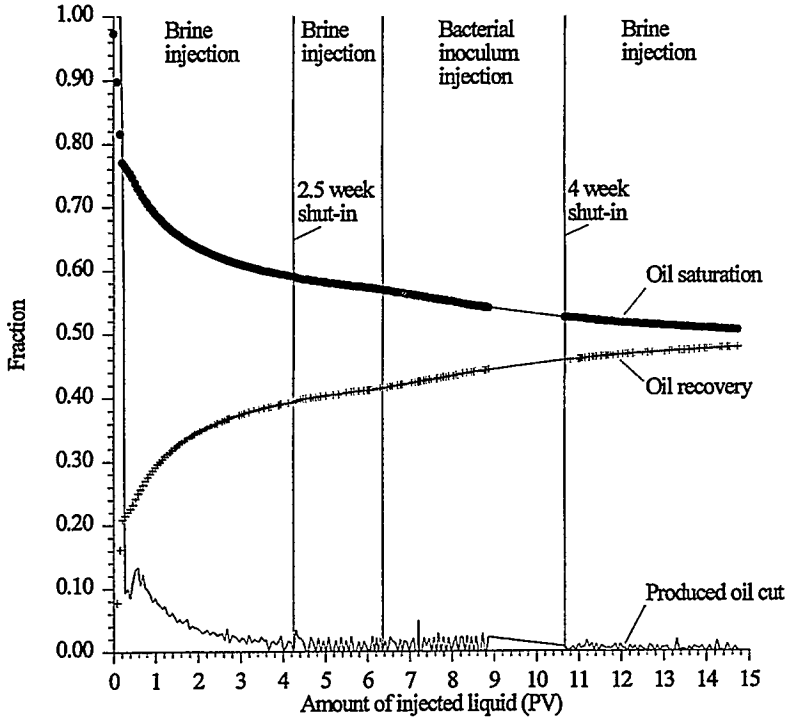

Figure 5 **Effect of Brine Injection, Bacterial Cell Injection, and Bacterial Growth on Oil Recovery and Saturation in an Oil-Saturated Sandpack**

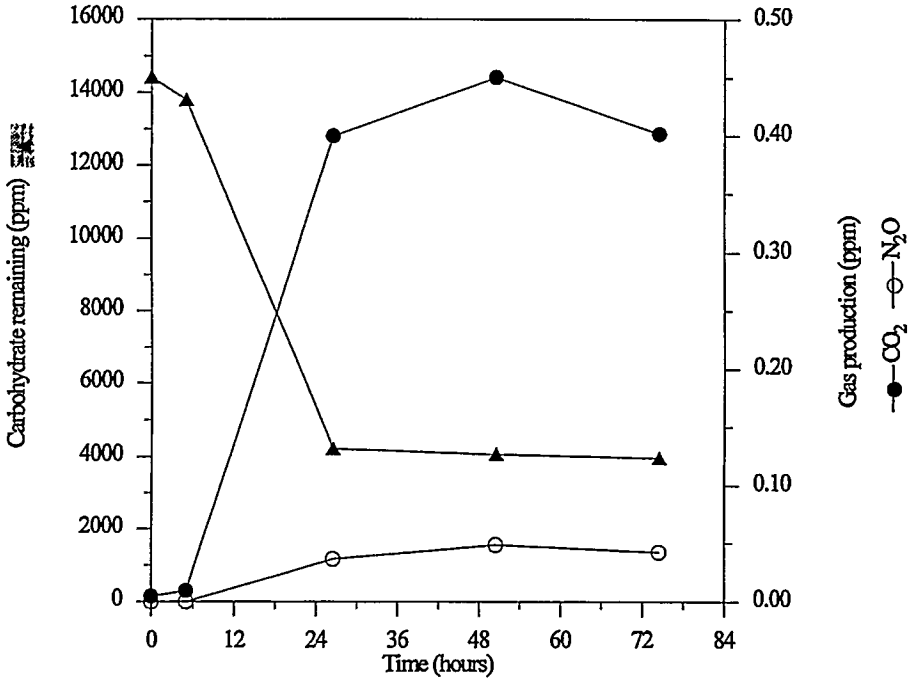

Figure 6 Growth of NPR-3A at 50°C in Capped Vial with Air Headspace

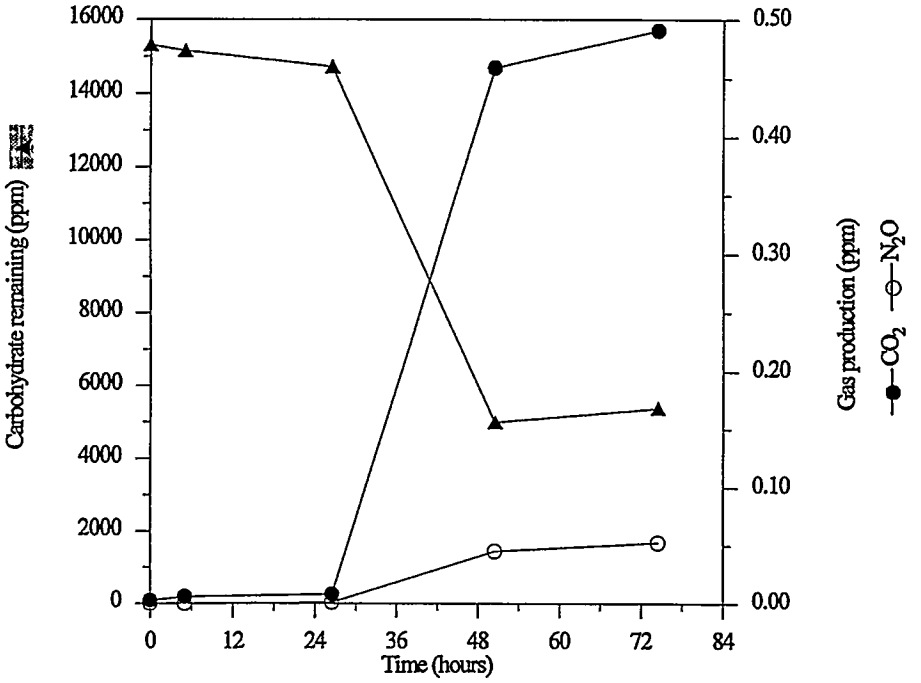

Figure 7 Growth of NPR-3A at 50°C in Capped Vial with Helium Headspace

BIOTECHNOLOGY TREATMENT OF FLUIDS AND SOILS

Bioremediation of Oil-Contaminated Soils: A Recipe for Success

Stuart A. Wittenbach
Kerr-McGee Corp.

Abstract

Bioremediation of land crude oil and lube oil spills is an effective and economical option. Other options include road spreading (where permitted), thermal desorption, and off-site disposal. The challenge for environmental and operations managers is to select the best approach for each remediation site.

Costs and liability for off-site disposal are ever increasing. Kerr-McGee's extensive field research in eastern and western Texas provides the data to support bioremediation as a legitimate and valid option. Both practical and economical, bioremediation also offers a lower risk of, for example, Superfund clean-up exposure than off-site disposal.

The goals of the research were to:

1. Verify that bioremediation is a satisfactory option for remediating crude-oil and lube-oil contaminated soils in E&P operations.

2. Determine the critical factors that control the bioremediation process.

3. Develop a bioremediation program recipe for EH&S and operations to meet a variety of field conditions.

All three goals were met. This paper includes both results and conclusions of the study.

Oil Spills

In the exploration and production of oil and gas, the accidental release of oil can occur at various stages. The industry installs safety and environmental controls, and develops operational procedures to prevent spills. However, despite a company's best efforts, spills can happen. Once a spill occurs, the company works diligently to clean the environment. The type of oil may be crude oil or refined product. In the past, the spilled product was "remediated" by one of the following methods: road spreading (where permitted), thermal desorption, or off-site disposal. The options of road spreading and off-site disposal created further potential liability and, therefore, did not always appeal. Thermal desorption was not always acceptable due to the lack of thermal desorption companies in the immediate area, the concern for air emissions, and the extremely high operational cost.

Bioremediation Study

In the last five to seven years, the successful use of bioremediation (aerobic or anaerobic) on various organic remediation projects led Kerr-McGee Corp. to investigate this option. Bioremediation, the degradation of organic substances by microorganisms, has been studied by scientists since the early 1950s (Riser-Roberts 1992).[1] However, the widespread use of bioremediation on crude-oil or lube-oil spills had not been proven in field situations. Therefore, Kerr-McGee's U.S. Onshore Region began a field test to determine the effectiveness of aerobic bioremediation for soils contaminated with crude oil and lube oil. Two active oil and gas sites in Texas were chosen for the study. The two sites had different environmental conditions (East Texas has a higher annual precipitation rate than West Texas). This allowed the study to compare the various environmental factors to the effectiveness of bioremediation. A random-block statistical design was used on both sites.

Study Results

The East Texas site involved a lube-oil-contaminated soil. The study began in the fall of 1990 and was completed in the fall of 1993. Two compressor sites were chosen for the lube oil study. The controllable factors were soil nutrients, organic material, oxygen, and oil and grease content. The following amendment rates were used: fertilizer rates (5 lb/100 ft^2 and 12 lb/100 ft^2) and organic rates (25 lb/100 ft^2 and 50 lb/100 ft^2). The treatment areas were tilled on two different schedules (two times per month or four times per month). This variation in tillage allowed Kerr-

McGee to determine tillage effect (the addition of oxygen to the soil/microbes) on the degradation rate.

Results of the three-year study (see Figs. 1 and 2) indicated that the optimum fertilizer rate and organic material rate for bioremediation of a lube oil soil was 12 lb/100 ft^2 and 25 lb/100 ft^2, respectively (at the 0.05 alpha level). It was also noted visually that the weekly tillage rate was more effective on the degradation rate. Additional tillage increased the oxygen content and produced additional microbes and/or provided a more acceptable environment.

The second study site was a former emergency blowdown pit located in West Texas. The pit was contaminated with crude oil and produced water. The study began in the winter of 1991 and was completed in the fall of 1993. The controllable factors at this site were: soil amendments, nutrients, water or moisture content of the soil, oxygen, and oil and grease content. Two fertilizer rates (4 lb/100 ft^2 and 10 lb/100 ft^2) and three organic material rates (25 lb/100 ft^2, 50 lb/100 ft^2, and 75 lb/100 ft^2) were used. Fresh water was available for irrigation, enabling the correlation of soil moisture with degradation rate.

Results (see Figs. 3 and 4) indicated that the optimum fertilizer rate and the optimum organic material rates for bioremediation of the crude oil soil were respectively, 14 lb/100 ft^2 and 75 lb/100 ft^2 (significant at the 0.05 alpha level). The irrigated plots exhibited a faster rate of degradation than the dry-land plots. The irrigated plots with a soil moisture content of 14% or greater provided the microorganisms with a more suitable environment.[2]

Conclusion

In conclusion, the study results showed bioremediation to be a good alternative for the remediation of petroleum-contaminated soils. From the study, generic amendment rate(s) were developed. The tillage rates should be no less than once per week to ensure an adequate amount of oxygen to the microorganisms. The soil moisture content should be monitored to ensure the water content is at least 14%. Further studies will be conducted in 1995–97 to determine the effectiveness of bioremediation in colder climates, such as in the Rocky Mountain states.

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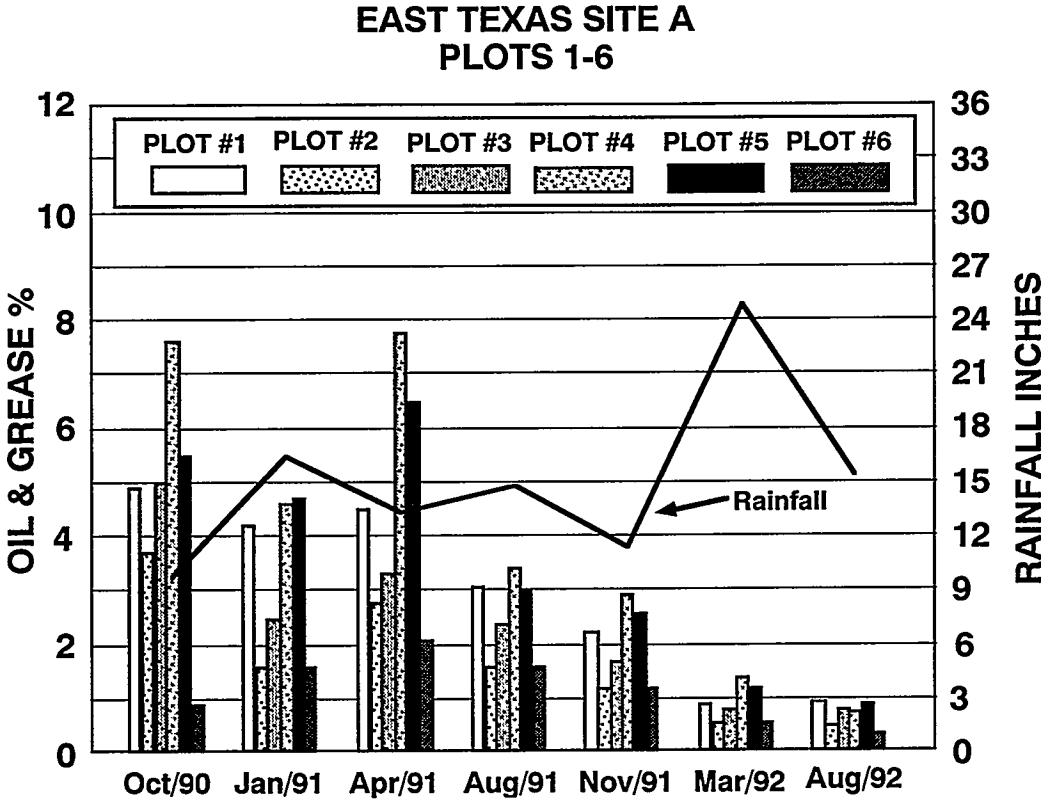

Figure 1 Results of Treatment of East Texas Site A Plots 1–6

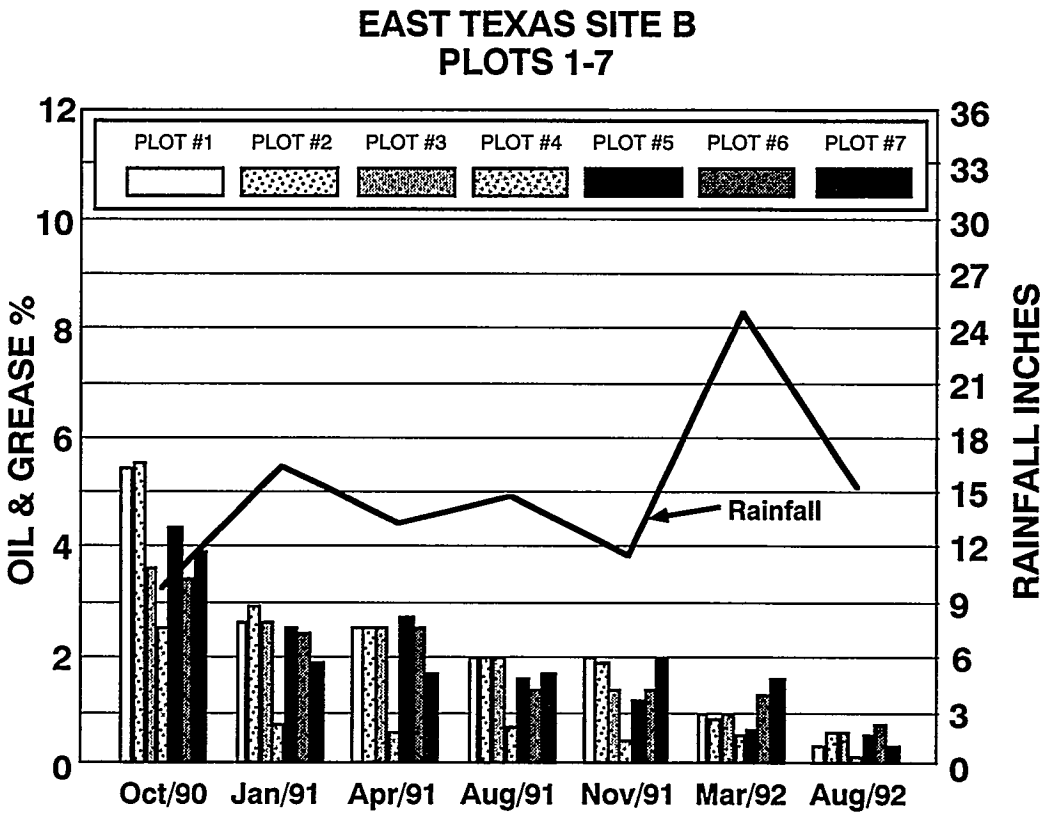

Figure 2 Results of Treatment of East Texas Site B Plots 1–7

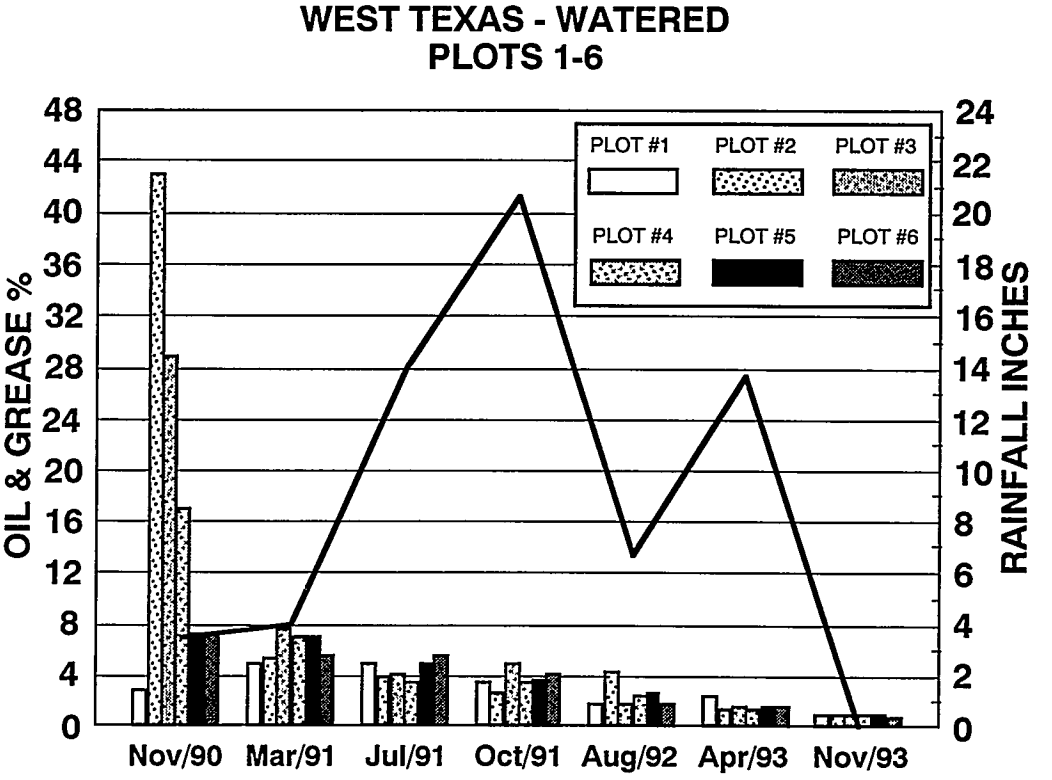

Figure 3 Results of Treatment of West Texas Watered Plots 1–6

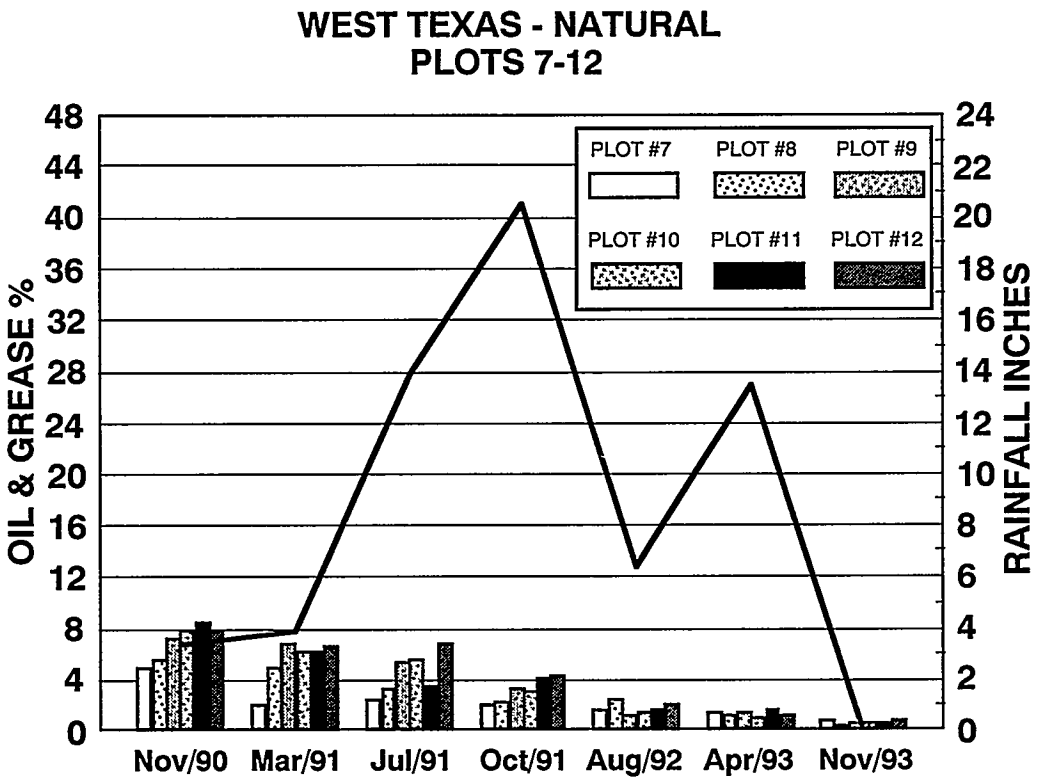

Figure 4 Results of Treatment of West Texas Natural Plots 7–12

Petroleum Storage Tank Cleaning Using Commercial Microbial Culture Products

Dennis Ray Schneider, Lee C. Entzeroth,
Andrew Timmis, Abby Whiteside,
and Brian C. Hoskins
Micro-Bac International, Inc.

Abstract

The removal of paraffinic bottom accumulations from refinery storage tanks represents an increasingly costly area of petroleum storage management. Microorganisms can be used to reduce paraffinic bottoms by increasing the solubility of bottom material and by increasing the wax-carrying capacity of carrier oil used in the cleaning process. The economic savings of such treatments are considerable. The process is also intrinsically safer than alternative methods, as it reduces and even eliminates the need for personnel to enter the tank during the cleaning process. Both laboratory and field sample analyses can be used to document changes in tank material during the treatment process. These changes include increases in volatile content and changes in wax distribution. Several case histories illustrating these physical and chemical changes are presented along with the economics of treatment.

Introduction

Oil storage tanks require periodic cleanings for several reasons including corrosion monitoring and inspection, waste disposal, and basic maintenance. Traditionally, cleaning has been accomplished through entering the tank and physically removing the "bottoms" or by heating and applying chemicals. Both processes raise safety questions. The first method requires personnel to spend long hours in the presence of toxic fumes in the tank. In the process of heating and applying chemicals in the second method, the contents of the tank are heated to temperatures that reach dangerous levels. Microbial treatments offer a safe and economical alternative way to clean petroleum storage tanks. The tank bottoms are made pumpable, and limited personnel entry or heating is required. As a result of treatment, the amounts of hazardous material for disposal are reduced, crude oil recovery is possible, and final cleaning is facilitated.

Tank "bottoms" are complex mixtures of a variety of substances which may include mineral scales, water, and other inorganics such as sands and clays, as well as hydrocarbons. Typically accumulating over multiple cycles of tank filling and emptying, the bottoms mirror the hydrocarbon types placed in the tank. Tank bottoms generally are not homogeneous, adding to the complexity of the problem. The hydrocarbons contained typically belong to types which more readily precipitate, aggregate, and form the organic portion of the bottom solid. These include the higher molecular weight alkanes and asphaltenes.

Bacteria have long been known to successfully control paraffin deposition in oil wells.[1] Early in the history of these treatments, it was observed that bottom accumulations in production tanks were reduced by continued microbial treatments. From this observation, the natural extension was made that accumulated bottoms such as those found in terminal or refinery storage tanks could be treated using microbial culture products.

These microbial culture products are composed of naturally occurring, nonpathogenic strains of bacteria. The bacteria and their metabolic byproducts act to solubilize paraffin, disperse paraffin, and prevent its deposition and formation. Among the byproducts generated are glycolipids, fatty acids, alcohols, and ketones. The biosurfactant glycolipids act to disperse paraffin and asphaltene aggregates and suspend them in solution. The fatty acids, alcohols, and ketones act as solvent molecules to help bring the paraffin and asphaltenes into solution.

Direct metabolic action on the paraffin components helps to reduce the amount of high molecular-weight alkanes. Through the synergistic action of these various effects, the bottom is loosened and brought into suspension and/or solution. A

typical treatment methodology might incorporate a carrier oil added in the tank to provide a fluid medium in which to solubilize and suspend the aggregate hydrocarbons.

Tank Bottom Testing

An important point of microbial tank bottom treatment is an adequate testing protocol. This is important for several reasons. Tank bottoms may exhibit toxicity to microorganisms due to introduction of biocides or other chemicals for corrosion control. Tank bottoms, being of unknown origin, may contain a significant fraction of materials (such as sands and clays) which are not susceptible to microbial treatment. A minimum preliminary characterization of the bottom would involve a treatability test, where a tank bottom sample is analyzed for its composition and is incubated with the microbial product to determine whether the sample is toxic to the microbial product. In this type of characterization, the hydrocarbons are profiled as to their carbon number and type by whole oil gas chromatography, and the hexane-insoluble solids portion is analyzed for its organic vs. inorganic contents. The latter analysis is particularly important, as some tank bottoms may have inorganic contents in excess of 50% by weight. The organic portion of these solids is typically high molecular-weight paraffins and asphaltenes.

The second stage of characterization is the determination of the type of microbial culture product to use in the treatment. The tank bottom is incubated with a variety of different products, as well as a control, and then the bottom is assayed for physical and chemical changes. Significant physical changes may include free oil breakout or the visible softening of the bottom, but the principal means of determining product dosage is gas chromatography of the treated and control tank bottoms. Two types of changes can be seen: reduction in the higher molecular-weight hydrocarbons with a corresponding shift to lower molecular weights and an increase in volatile content. Both or either changes can be seen with different bottom samples and different products. For example, in Table 1, a tank bottom analysis can be seen that shows a significant increase in volatile components with PB/S™ microbial culture product (48% from 34%) and with M-1000™ (47% from 34%) microbial culture product, but no significant change in the hydrocarbon composition.

A second tank bottom analysis shown in Table 2 indicates a significant trend of change in hydrocarbon composition with M-1000 microbial culture product supplemented with a specific nutrient formula (OSNF#1™). A consistent trend is seen in reduction in the C_{20}–C_{25} and C_{25}–C_{30} range with a corresponding increase in the C_{15}–C_{20} (soft wax) range. There was no change in volatile content.

Both of these tank bottoms would be considered treatable. Even though laboratory experiments cannot guarantee success, they can provide guidance in the successful selection of products for use and increase the likelihood of success. For example, a tank bottom analysis showing no change in either volatile or hydrocarbon content would likely be evaluated as not treatable.

The release of free oil from tank bottoms requires further discussion. While release may come from oil trapped in a solid bottom, it may also result from the breaking of emulsions present in the bottom. Microorganisms are well known as bridging agents between emulsified oil droplets to stimulate oil drop coalescence.[2] Also, the thinning effect produced by production of solvent-type molecules promotes coalescence by increasing the mobility of individual droplets.

Treatment Methodology

The application of the product is a straightforward process. Once the clean oil has been removed from the tank and the bottoms have been isolated, the treatment can begin. If the bottoms do not contain enough oil to carry the paraffins, asphaltenes, and other solids, a carrier oil is added. The microbial products can be applied to the tank and circulated with a pump through the suction and discharge valves. Fresh water may need to be added if the tank contents do not contain enough to accommodate the bacteria. Depending on the characteristics of the bottom, 1% water by volume is sufficient. The solution of bacterial product and bottom is circulated from three to six weeks until the bottom is fluid enough to be pumped out of the tank and treated through the operation's system.

Case Histories

Crude Oil Slop Tank in Louisiana

A crude oil slop tank in Louisiana contained 4,000 bbl of slop oil with an API gravity of 14°. The bottoms consisted of 78% basic sediments and water (BS&W) with 18% sediment. The tank was treated and circulated for eight days. The API gravity rose to 16.3°, the BS&W dropped to 12%, and the amount of sediment dropped to 4%. Roughly 3200 bbl of raw crude were reclaimed and sold.

Cleaning for Internal Inspection and Coating

A 10,000-bbl fixed roof storage tank with over 2 ft of bottoms (roughly 600 bbl) needed to be cleaned for the inspection and coating of the floor, walls, and roof. The sludge contained gas that made entering the tank hazardous because of the explosive atmosphere. The tank was treated with product and approximately 300 bbl of carrier hydrocarbon. This was allowed to sit overnight, and then the tank was circulated 5 hr/day for 18 days. The tank's sump recovered 200 bbl of water and 600 bbls of condensate. The remaining sludge was removed with a vacuum truck.

Brazilian Tank Sludge Treatment

A Brazilian crude oil tank was treated with product and circulated for 24 days. The treatment increased the volatiles and hydrocarbons, and reduced the solids. The bottom was pumpable after treatment because the volatile level increased, thus solubilzing the remaining paraffin. Hydrocarbon levels rose, indicating a shift in the n-alkane distribution towards shorter chains resulting in less paraffin to be solubilized. The solids that were removed or solubilized were the heavy, long-chain paraffins (C_{35+}) and some asphaltenes and resins. The hydrocarbon distribution shifted towards the lighter ends, and the paraffin content was almost half of what the original sample contained. The paraffin reduction was mostly in the C_{20}–C_{30} range (see Fig. 1).

Conclusion

Tank cleaning using microorganisms is a new application for microbial paraffin control technology. Laboratory testing can document the changes in volatility and hydrocarbon profile produced by the microbial products. This technology has been used on hundreds of oil storage tanks of various types and in various producing regions. It is an example of the maturity of the commercialization of microbial culture technology and represents a new tool in the petroleum industry's armamentarium for solving production problems.

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Table 1 Tank Bottom Characterization

Product*	PBS	M-1000	PBS+OSNF	Control
Bulk Composition				
Volatiles	48	47	36	34
Hydrocarbons	28	31	34	39
Solids	22	23	29	26
Hydrocarbon Composition				
C_{15}–C_{20}	40.4	32.1	29.1	39.9
C_{20}–C_{25}	28.9	34.9	36.2	29.1
C_{25}–C_{30}	19.0	22.0	25.0	19.7
C_{30+}	11.7	11.0	9.7	11.4
Paraffins	51.3	47.0	44.7	50.4

*PBS, M-1000, OSNF, and Ben-Bac are trademarks of Micro-Bac International, Inc.

Table 2 Tank Bottom Characterization

Product*	Ben-Bac	M-1000*	M-1000+OSNF	Control
Bulk Composition				
Volatiles	54	47	49	49
Hydrocarbons	13	15	17	16
Solids	34	39	35	36
Hydrocarbon Composition				
C_{15}–C_{20}	11.5	32.7	36.1	23.9
C_{20}–C_{25}	56.3	43.7	47.0	54.3
C_{25}–C_{30}	24.7	20.0	11.5	16.8
C_{30+}	6.5	3.5	5.3	5.0
Paraffins	32.1	26.2	33.9	34.4

*PBS, M-1000, OSNF, and Ben-Bac are trademarks of Micro-Bac International, Inc.

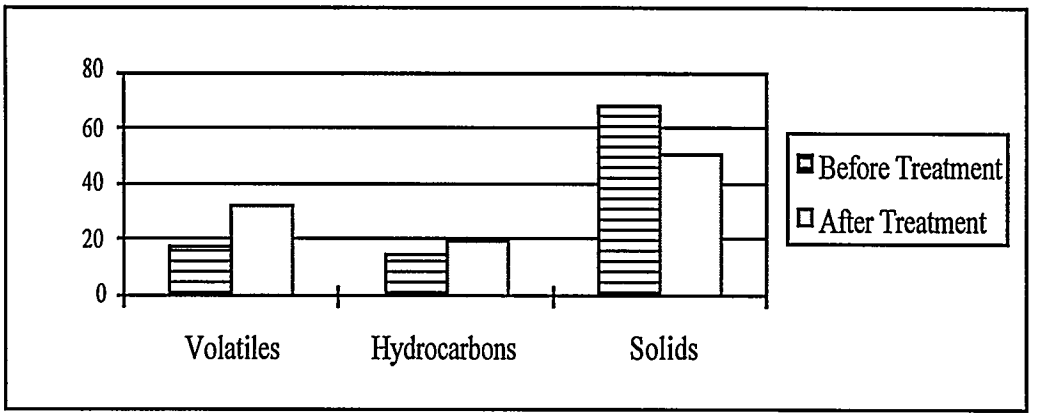

Figure 1 Bulk Composition of Brazilian Tank Sludge

Biological Treatment Process for Removing Petroleum Hydrocarbons from Oil Field Produced Waters

Gilbert Tellez
U.S. Environmental Protection Agency
Nirmala Khandan
New Mexico State University

Abstract

The feasibility of removing petroleum hydrocarbons from oil field produced waters using biological treatment was evaluated under laboratory and field conditions. Based on previous laboratory studies, a field-scale prototype system was designed and operated over a period of four months. Two different sources of produced waters were tested in this field study under various continuous flow rates ranging from 375 l/D to 1,800 l/D. One source of produced water was an open storage pit; the other, a closed storage tank. The TDS concentrations of these sources exceeded 50,000 mg/l; total n-alkanes exceeded 100 mg/l; total petroleum hydrocarbons exceeded 125 mg/l; and total BTEX exceeded 3 mg/l. Removals of total n-alkanes, total petroleum hydrocarbons, and BTEX remained consistently high over 99%. During these tests, the energy costs averaged $0.20/bbl at 12 bbl/D.

Introduction

Produced water is the single largest volume of waste generated by the oil and gas industry. These waters are generated during crude oil and natural gas production at both onshore and offshore operations. The amount of produced water generated depends upon the method of recovery and the nature of the formation. In some formations, large volumes of water are pumped to the surface with the oil and gas in the early stages of production. In others, water is not produced until the formation has been significantly depleted. In others yet, water is never generated.

During onshore recovery, the crude oil/gas/water mixture is processed by gravity separation at the well site, and the water extract is discharged into surface pits or aboveground storage facilities for evaporation or subsequent reinjection into the subsurface for permanent disposal or secondary recovery. In offshore operations, these waters are discharged directly into the sea. Since produced waters are laden with a range of petroleum hydrocarbons, salinity, etc., such treatment and disposal practices have resulted in contamination of surface, ground, and coastal waterways.

Problems can occur at final disposal in subsurface reinjection, earthen impoundments used for evaporation, and surface-stream or offshore discharges. For example, surface water discharge practices were determined to be detrimental to a Nueces County, Texas, creek during a continuous produced-water discharge (approximately 8,500 gal/D) from an adjacent oil field site, according to a 1981 study by the Texas Parks and Wildlife Department. During discharge, the creek maintained an oil sheen and was devoid of native vegetation and fish, which were abundant in the upstream stretches of the creek. Total petroleum hydrocarbon concentrations in excess of 9.0 mg/l were extracted from the downstream waters.

The effects of produced waters discharged from oil and gas operations on shallow water coastal wetlands have recently emerged to be of significant environmental concern.[1] Direct discharge is permitted for produced waters from offshore oil and gas production platforms or shore-side treatment facilities designated for territorial sea discharge. Many of these facilities have been unable to comply with the Best Practicable Technology (BPT) methods for petroleum hydrocarbon separation from produced waters.[2] If not removed, hydrocarbon concentrations in produced water are known to impact species diversity in benthic communities close to shallow water discharges.[3,4] Neff et al.[5] found elevated total petroleum Hydrocarbon (TPH) concentrations less than 300 m from a shallow water discharge, and less than 100 m from a deep water discharge in Louisiana coastal waters. Neff[4] concluded that benthic communities within 20 m of both discharges were influenced by sediment contamination, probably due to produced water discharge.

Produced waters contain both soluble and insoluble petroleum fractions of complex mixtures of organic compounds similar to those found in crude oils and natural gases. The individual constituents cover a broad range of boiling points, carbon numbers, chemical families, and structural isomers.[6] The hydrocarbon fractional groups commonly found in produced water include alkanes, alkenes, alkynes, aromatics, polynuclear aromatics, and complex hydrocarbon compounds containing oxygen, nitrogen, and sulfur.

The most commonly detected hydrocarbon groups are C_{10} to C_{30} straight-chain alkanes.[7] The n-alkanes which are present at the highest concentration are C_{14} through C_{18} alkanes, with concentrations gradually decreasing with increasing chain length to C_{34}.[8] Only 25% of the n-alkanes of produced water are of the higher molecular weight C_{21} through C_{34} n-alkanes. Produced waters from gas production operations generally contain higher levels of BTEX than waters from oil production.[9] Stephenson[10] obtained a mean benzene concentration range from 5.8 to 12.2 mg/l in gas operations and 1.3 to 8.7 mg/l in oil productions. Polynuclear aromatic hydrocarbons (PAHs) are present in crude oils and produced waters in moderate amounts. PAHs are considered to be hazardous compounds because of their carcinogenic characteristics in animal studies and potential health risks to man.

Literature on economic treatment and/or disposal of oil field produced water is very limited. The Gas Research Institute (GRI) of Chicago, Illinois, published two reports evaluating the relative cost of produced water treatment.[11,12] The range of cost covers nearly two orders of magnitude, from $0.03 to $3.00 per bbl. These extremes were derived for treatment applications using the least economical burden (surface discharge) preceded by the most basic treatment (aeration followed by sedimentation) to the most costly (evaporation). The cost range for deep well injection, including the cost for transporting the produced water to the disposal sites, is approximately $1.25 to $1.75 per barrel. Deep well injection costs may reflect regional differences as well as technical differences associated with converting producer wells vs. new well development. Cost for biological treatment followed by surface discharge, ranged from $0.03 to $0.25 per barrel. However, this technique is seldom used because of the insufficient technical and operational information available to the oil and gas industry.

Whereas the biodegradability of petroleum hydrocarbons has been well demonstrated, biological treatment of produced waters laden with such chemicals has not been attempted because of the very high TDS levels often found in produced waters. Our previous studies under laboratory conditions using produced water samples with TDS levels up to 100,000 mg/l demonstrated that biodegradation was possible after adequate acclimation.[13] In this study, those findings are extended in designing and evaluating the performance of a field-scale biological treatment system in removing petroleum hydrocarbons from two oil field

produced water sources. The performance of the proposed system is demonstrated in terms of removals of total petroleum hydrocarbon (TPH), total n-alkanes (TNA), and aromatic hydrocarbons (BTEX).

Materials and Methods

Produced Water

This field study was conducted on produced waters generated from oil and gas production wells located in the mineral-rich Permian Basin situated in portions of Lea, Eddy, Chaves, and Roosevelt Counties of New Mexico. In 1985, these four counties produced over 70.5 million bbl of crude oil and 462.3 bcf of natural gas from a total of 26,143 wells, while generating 4.4 bbl of produced water per barrel of oil.[13] Two distinct sources of produced waters were evaluated: in one case, the produced water was drawn from an open, aboveground storage tank; in the other, produced water was drawn from a closed storage tank. The TDS levels of both these sources were in excess of 50,000 mg/l, and their BTEX fractions were significantly different: 3 mg/l in the open tank vs. 8 mg/l in the closed tank due to volatilization to the atmosphere.

Biological Reactor Configuration

The reactor configuration consisted of a 4 × 10 × 4 ft 12-gauge steel tank with three compartments in series, followed by two 4 × 4 × 4 ft slow sand filters in parallel. The first compartment (945 liter; 250 gal) served as a completely mixed (no cell recycle) pre-aeration tank designed to increase the dissolved oxygen content of the produced water to an operating concentration of approximately 3 to 4 mg/l. This would allow for stimulation of any naturally existing aerobic microbial cultures. An air flow rate of 0.345 m^3/min (equivalent to 305 lbs/D of oxygen) was provided using a Fuji ring compressor to ensure complete mixing and adequate oxygen transfer.

The second compartment (945 liter; 250 gal), designated as the aeration tank, received the effluent from the pre-aeration tank. This tank was initially bioaugmented with a commercial culture (Petrobac-S®). Complete mixing of the cells was provided by delivering 0.431 m^3/min (15 cfm) of air (equivalent to 382 lb/D of oxygen) which maintained the bacterial cells in total suspension while providing an operating dissolved oxygen concentration in the range of 3 to 4 mg/l.

The effluent from the aeration tank then entered the third compartment (1,050 liter; 278 gal) which served as a clarifier. A biomass recycle system was installed between the clarifier and the aeration tank to maintain the desired MLSS (mixed liquor suspended solids) concentration in the aeration tank. The clarified effluent was discharged into one of the two parallel slow sand filters to remove any carryover of bacterial cells. The two filters were operated on an alternating basis, allowing for continuous flow of the waste stream during cleanup and backwashing of the filter media.

Naturally Occurring and Bioaugmented Cultures

Two types of microbial cultures were evaluated in this study. The pre-aeration tank was operated aerobically on a continuous-flow basis allowing for naturally occurring bacteria to grow. An initial bioaugmented culture product, Petrobac-S, was used in the aeration tank. This product is a hydrocarbon-utilizing consortium specially formulated for degrading crude or refined hydrocarbons in moderate saline environments under aerobic conditions.

As presented by previous research, acclimation of this commercial culture to the high TDS levels in the produced water is essential.[13] The acclimation procedure consisted of adding the commercial Petrobac-S seed to 945 liter of fresh water in the aeration tank. Acclimation was achieved by daily additions of 95 liter of produced water from the Hobbs, New Mexico, closed tank system. By discarding biomass at 95 liter per day, a 10-day solids detention time was maintained at an MLSS concentration of approximately 700 mg/l (dry cell weight). A cell yield of 0.38 mg MLSS/mg TNA was obtained during the acclimation period.

Total n-Alkanes (TNA) Analysis

Gas chromatography/mass spectrophotometry (GC/MS) techniques were used in the quantitation of the n-alkane and aromatic hydrocarbons. Daily GC/MS analysis were initiated by collecting 1 liter completely mixed produced water sample volumes across the treatment train. Samples were then vigorously agitated in a separatory funnel for two minutes with a 10:1 water to solvent ratio. Methylene chloride was used as the solvent. After allowing a 10-minute separation time between the water and solvent phases, the bottom organic solvent layer was then removed. The organic solvent extract was concentrated with dry nitrogen to bring the final volume to 10 ml. This small final extract volume eliminated the possibility of having too dilute or too small a hydrocarbon sample, which could produce false conclusions about the extent of biodegradation.

Upon completion of the extraction procedures, 2-μl of the extract was injected into the gas chromatograph/ion trap mass spectrometer (Saturn Varian Co.). The GC/MS was equipped with an RTx-5 30m × 0.32 mm diameter capillary column, 5% diphenyl, 95% dimethyl polysiloxane; carrier gas was helium at a flowrate of 1 ml/min and a split flow of 60 ml/min. GC/MS running conditions were 600 second filament/multiplier delay time; 35°C initial temperature with 5 min isothermal, 8°C/min ramp to a final temperature of 280°C with 15-min isothermal, for a total run time of 50.6 minutes per sample.

Positive identification of the n-alkanes ranging from decane to tricotane (unbranched carbons from C_{10} through C_{30} n-alkanes) was achieved, first by mass spectral identification and then by acquisition of standard n-alkanes and comparison with unknown retention times. Quantitation was performed by measuring peak areas of the individual compounds, and by correlating with a standard calibration curve. A correlation coefficient of 0.99 was used as an acceptance criterion. A quality control sample was run every 10 samples. The quality control was 10%; if the quality control sample did not pass, a new calibration curve was entered. TNA concentrations were determined by summation of all the n-alkane compounds present. TPH analysis, as described next, was performed on all field evaluations to ensure QA/QC for the GC/MS analysis methods and procedures.

Aromatic Hydrocarbons Analysis

Upon extraction of a 1–liter field produced water sample, as described in the above n-alkane section, aromatic GC/MS (Hewlett-Packard 5890 series II) analysis was via split injection with a 2-μl sample volume and a 30:1 split ratio. The injector was held at 280°C and the GC/MS interface at 260°C. The oven temperature was initially held at a temperature of 50°C for 1.5 min, then programmed to 290°C at a ramp rate of 7°C/min with a 15-minute hold at 290°C. The mass selective detector was operated in the selected ion mode at m/Z values of 57.1 for alkanes greater than butane, 78.0 for benzene, and 91.1 for toluene, ethylbenzene, and xylene. Aromatic hydrocarbons were quantified by running a series of 100 μg (600 μg total) BTEX concentration standards (mixture of benzene, ethylbenzene, toluene, and xylenes), from which a response curve was obtained. Aromatic hydrocarbon content of the produced water was then run against this BTEX standard to obtain appropriate concentrations.

Total Petroleum Hydrocarbons (TPH) Analysis

Environmental Protection Agency Method 418.1 (Spectrophotometric, Infrared) was used to determine total recoverable petroleum hydrocarbons. Unlike oil and grease determination, petroleum hydrocarbons is the measure of only the mineral oils, which include the aromatic hydrocarbons (i.e., benzene, toluene through naphthalene), n-alkanes, and all nonpolar hydrocarbons.

Procedures included collection of a 1–liter produced water sample followed by acidification to a pH less than 2. Acidified samples were then serially extracted with freon-113 in a separatory funnel. Polar compounds such as esters, alcohols, aldehydes, fatty acids, and sulfur-bound carbons were codissolved with the petroleum hydrocarbons in Freon-113, and were removed with a silica gel adsorbent. After the silica gel was added, an infrared spectrum of the extract (solvent plus solute) was recorded by placing it in a 100-mm path length cell and exposing it to the infrared beam scanning at the absorption band of 2,930 cm^{-1} (Perkin Elmer Model 1600). The spectrum for the solute alone was recorded and measured by a simultaneous subtraction of the solvent spectra. Produced water TPH quantitation was based upon comparisons to standard calibration curves which consisted of 15.0 ml n-hexadecane (n-alkane), 15.0 ml isooctane (branched alkane), and 10.0 ml chlorobenzene (aromatic).

Continuous-Flow Testing

Upon completion of the 10-day acclimation period as described above, the produced water from the closed tank system was brought online for continuous-flow treatment. Produced water flowrate was set at 1,890 liter/D (500 gal/D), to maintain an MLSS concentration of approximately 700 mg/l in the aeration tank. The pre-aeration and aeration tanks reached steady-state conditions in approximately five days. At this time, MLSS, TPH, n-alkane, and aromatic removal remained constant on a day-to-day basis. Each day, a sufficient amount of biomass was wasted to maintain fixed MLSS concentrations in the aeration tank. Growth rates in the pre-aeration tank were maintained by its hydraulic detention time.

The following operational and water quality parameters were measured daily for all treatment units from the pre-aeration tank to the sand filter: influent flowrate; influent petroleum hydrocarbons (TPH, n-alkanes, aromatics), effluent petroleum hydrocarbons (TPH, n-alkanes, aromatics), cell return line petroleum hydrocarbons (TPH, n-alkanes, aromatics), and MLSS.

The dissolved oxygen, pH, and temperature of the treatment units were also checked on a daily basis. After treating the closed tank system for 45 days, the treatment system was again brought to a steady-state initial MLSS concentration of 700 mg/l. At this time, the influent from the closed tank was terminated, and the open tank system was brought online at a flow rate of 1,890 l/D. Water quality sampling and analysis were again performed as described previously for an additional 45-day testing period.

Results

Typical dissolved oxygen (DO), total dissolved solids (TDS), total n-alkanes (TNA), total aromatics, and total petroleum hydrocarbons (TPH) profiles across the various units in the treatment system are presented in Table 1 for the two sources of produced waters tested. While the TDS levels remained the same throughout the system, the overall reductions in TNA, aromatics, and TPH exceeded 99%. The high TDS levels found at this site are common in the southeastern oil-producing regions of New Mexico.

The system was run at five different flow rates and biomass concentrations to evaluate removal efficiencies. Results of these five runs are summarized in Table 2. Again, the system achieved consistently high removals of TNA, BTEX, and TPH. These results were also used to determine the biokinetic parameters that describe the growth of the organisms in the pre-aeration and the aeration tanks. The biokinetic parameters determined from these runs using standard procedures are listed in Table 3. These data could be used for process modeling and analysis, reactor sizing, and scale-up purposes.

Conclusions

This field study demonstrates that petroleum hydrocarbons can be biologically removed from oil field produced waters even at TDS levels as high as 50,000 mg/l. Removals of total n-alkanes, BTEX, and total petroleum hydrocarbons exceeding 99% were achieved at various continuous flow rates up to 1,800 liter/D. Based on the performance of this system, it is estimated that the direct energy consumption is about 32 Kw-hr per day to treat 500 gal/D (12 bbl/D), translating to about $0.20/bbl to achieve 99% total petroleum hydrocarbon removal efficiency. This compares very favorably against other currently available treatment or disposal options for produced waters.

Acknowledgments

This work was supported, in part, by the DOE Waste-Management Education Research Consortium (WERC) of New Mexico.

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Table 1 Results of Water Quality Analysis across the Treatment Systems

		Produced Water Source	
		Open Tank	Closed Tank
Raw water characteristics			
Dissolved oxygen	[mg/l]	< 1.0	< 1.0
Total dissolved solids	[mg/l]	52,164	55,023
Total n-alkanes	[mg/l]	126	115
Total aromatics	[mg/l]	3.1	7.7
Total petroleum hydrocarbons	[mg/l]	135	126
Effluent from pre-aeration tank			
Dissolved oxygen	[mg/l]	3.1	2.6
Total dissolved solids	[mg/l]	54,256	57,329
Total n-alkanes	[mg/l]	51.1	56.5
Total aromatics	[mg/l]	ND	ND
Total petroleum hydrocarbons	[mg/l]	58.3	61.1
Effluent from aeration tank			
Dissolved oxygen	[mg/l]	3.9	3.8
Total dissolved solids	[mg/l]	54,229	57,128
Total n-alkanes	[mg/l]	0.04	0.16
Total aromatics	[mg/l]	ND	ND
Total petroleum hydrocarbons	[mg/l]	0.27	0.44
Effluent from settling tank			
Dissolved oxygen	[mg/l]	2.3	2.9
Total dissolved solids	[mg/l]	51,637	54,647
Total n-alkanes	[mg/l]	0.01	0.02
Total aromatics	[mg/l]	ND	ND
Total petroleum hydrocarbons	[mg/l]	ND	ND
Effluent from sand filters			
Dissolved oxygen	(mg/l)	2.7	2.8
Total dissolved solids	(mg/l)	51,234	54,196
Total n-alkanes	(mg/l)	0.01	0.03
Total aromatics	(mg/l)	ND	ND
Total petroleum hydrocarbons	[mg/l]	ND	ND

ND—not determined

Table 2. Performance of Treatment System under Various Operating Conditions

		Produced water source			
		Open Tank		Closed Tank	
		Pre-aeration tank	Aeration tank	Pre-aeration tank	Aeration tank
RUN 1					
Flow rate	[l/day]	378	378	378	378
MLSS	[mg/l]	61	737	56	737
TNA removal	[%]	92	99	89	99
BTEX removal	[%]	99	99	99	99
TPH removal	[%]	88	99	86	99
RUN 2					
Flow rate	[l/day]	756	756	756	756
MLSS	[mg/l]	52	651	46	632
TNA removal	[%]	72	99	69	99
BTEX removal	[%]	99	99	99	99
TPH removal	[%]	67	99	64	99
RUN 3					
Flow rate	[l/day]	1134	1134	1134	1134
MLSS	[mg/l]	48	550	37	560
TNA removal	[%]	60	99	54	99
BTEX removal	[%]	99	99	99	99
TPH removal	[%]	54	99	49	99
RUN 4					
Flow rate	[l/day]	1512	1512	1512	1512
MLSS	[mg/l]	31	483	23	449
TNA removal	[%]	45	99	35	99
BTEX removal	[%]	99	99	99	99
TPH removal	[%]	33	99	34	99
RUN 5					
Flow rate	[l/day]	1890	1890	1890	1890
MLSS	[mg/l]	22	401	9	408
TNA removal	[%]	28	99	12	99
BTEX removal	[%]	99	99	99	99
TPH removal	[%]	21	99	10	99

TNA- conc. of total n-alkanes; BTEX- conc. of benzene, toluene, ethylbenzene, and xylene; TPH- conc. of total petroleum hydrocarbons.

Table 3 Biokinetic Parameters from Field Studies

	Produced Water Source			
	Open Tank		Closed Tank	
	Pre-aeration Tank	Aeration Tank	Pre-aeration Tank	Aeration Tank
Yield, Y [mg/mg TNA]	0.59	0.62	0.64	0.47
Decay coeff., kd [1/day]	0.02	0.01	0.01	0.01
Half velocity const, Ks [mg/l]	47	2	73	4
Max. velocity, μmax [1/day]	1.88	0.27	1.98	0.21

Biodegradation of Oil Refinery Wastes under OPA and CERCLA

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Abstract

Land treatment of oil refinery wastes has been used as a disposal method for decades. More recently, numerous laboratory studies have been performed attempting to quantify degradation rates of more toxic polycyclic aromatic hydrocarbon compounds (PAHs). This paper discusses the results of the full-scale aerobic biodegradation operations using land treatment at the Macmillan Ring-Free Oil refining facility. The tiered feasibility approach of evaluating biodegradation as a treatment method to achieve site-specific cleanup criteria, including pilot biodegradation operations, is discussed in an earlier paper.[1] Analytical results of biodegradation indicate that degradation rates observed in the laboratory can be met and exceeded under field conditions and that site-specific cleanup criteria can be attained within a proposed project time. Also presented are degradation rates and half-lives for PAHs for which cleanup criteria have been established. PAH degradation rates and half-life values are determined and compared with the laboratory degradation rates and half-life values which used similar oil refinery wastes by other investigators (API 1987).[2]

Introduction

The 100-acre Macmillan Ring-Free Oil Company site is located in rural Norphlet Township, Union County, Arkansas. It is bordered by a residential subdivision and the Norphlet Public School on the west, Hayes Creek on the north and east, and Massey Creek and lowlands associated with the creek on the south. Crude oil processing at the site began in 1929. Unlined surface impoundments used to store crude oil wastes periodically overflowed and contaminated the two adjacent creeks. The Region 6 U.S. Environmental Protection Agency—Emergency Response Branch (EPA-ERB) performed site investigations under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) which indicated that the majority of the contamination was contained within 10 lagoons. Waste material generated by water treatment was a Resource Conservation and Recovery Act (RCRA) listed waste K048—Dissolved Air Flotation (DAF) consisting of volatile and semivolatile organic compounds. The DAF generated during waste water treatment process was discharged into four of 10 on-site lagoons. Applying the mixture rule, the DAF has contaminated sediments in these four lagoons. The remaining six on-site lagoon sediments were contaminated with non-DAF crude oil wastes. Volume calculations indicated that approximately 32,000 yd^3 of lagoon sediments had been contaminated with DAF and approximately 13,000 yd^3 of lagoon sediments had been contaminated by non-DAF oil refinery waste. The primary contaminants of concern are PAHs.

Since both hazardous and nonhazardous wastes were present on-site, an innovative mixed funding approach was implemented to remediate the site. The six lagoon sediments contaminated with non-DAF oil refinery waste were classified as nonhazardous oil refinery wastes, and Oil Pollution Act (OPA) funding was obtained through the U.S. Coast Guard. The OPA funding was utilized to construct a two-acre Land Treatment Unit (LTU) to biodegrade the oil refinery wastes which threatened nearby waterbodies. The LTU was designed to maximize waste degradation and immobilization and to control surface water runoff. CERCLA funding was obtained for the construction of another two-acre LTU for the biodegradation of DAF contaminated sediments. Both land treatment units were designated as Corrective Action Management Units (CAMUs) for the treatment of wastes to Site Specific Cleanup Criteria (SSCC). As of May 1995, full-scale biodegradation operations have treated 9,000 yd^3 of OPA wastes and 3,000 yd^3 of CERCLA waste.

Full-scale aerobic biodegradation LTU construction began in the summer of 1994 and was completed that fall. Components of the operation include an LTU, a bacteria-culturing bioreactor, and an irrigation/bacterial application system using water from the former fire-water pond (see Fig. 1).

An existing waste water treatment system based on dissolved air flotation is currently being utilized for leachate treatment. The bioreactor is used to culture indigenous and commercially available freeze-dried bacteria. The irrigation system is used to apply the cultured bacteria strains to supplement the LTU sediment bacteria and augment the biological activities. An initial batch of approximately 3,400 yd^3 of OPA-regulated lagoon sediments and 2,900 yd^3 of CERCLA-regulated contaminated sediments was placed into each LTU for biodegradation. A comprehensive ambient air monitoring program was implemented prior to and concurrently with lagoon excavation and biodegradation operations to monitor worker exposure and to document migration of airborne contaminants. Due to the large size of the LTUs, and the documented study of volatilization during treatment in the laboratory (API 1987),[2] no attempt was made to measure the long-term degree of volatilization of contaminants from the LTUs.

Biodegradation Operations

Biological Monitoring Program

An on-site laboratory was established to perform the first four tests listed in Table 1, including soil moisture content, particle size analysis, soil nutrient analyses (including nitrate, nitrite, ammonia nitrogen, phosphorus, potassium, and pH), and microbiological analyses including total heterotrophic bacteria plate counts.

Microbiological and nutrient testing are conducted to assist in maintenance of the LTU operational parameters (see Table 2). The goal of the monitoring is to ascertain that environmental factors and concentrations of nutrients are optimized to expedite degradation of contaminants.

Nutrient content relates to the nutrients available for use by the microorganism. Since steady release of nitrates benefits microorganisms, frequent nitrogen supplement are applied. Application rates are determined through on-site biweekly testing. Enumeration of both the heterotrophic and the hydrocarbon utilizing bacterial population is performed to verify that the population is sufficient for degradation and to correlate population densities with contaminant degradation results. Bacterial strains are identified to ensure the species present are consistent with those identified in treatability testing as known hydrocarbon degraders. Table 2 lists the ideal operational parameters of LTU soil.

Soil Sampling and Analysis Program

There are two principal objectives for the soil sampling and analysis of the soil being treated in the LTUs: monitoring the biological parameters to optimize biological degradation of the targeted compounds; and monitoring the chemical degradation of these compounds to ascertain the attainment of SSCC. A 25-ft sampling grid is superimposed over both LTUs to facilitate statistical significance and representativeness, and to increase sampling location reproducibility (U.S. EPA OSWER Directive 9360.4-10).[7] In addition to the initial microbial and nutrient characterization, three soil samples were collected from each LTU at randomly selected grid nodes to establish a baseline contaminant concentration. Soil sampling is typically conducted biweekly to monitor the LTU operational parameters and degradation of contaminants of concern. To verify the attainment of SSCC, nine confirmation samples are collected at the completion of each treatment period at a proportion of one composite or grab sample per 300 yd^3 of soil. The SSCC target PAHs and their cleanup criteria are benzo(a)pyrene 12 mg/kg; chrysene 9 mg/kg; fluorene no criteria established; naphthalene 42 mg/kg; phenanthrene 34 mg/kg and pyrene 36 mg/kg. The samples were analyzed for the compounds for which cleanup criteria had been established.

Results

Environmental variables which were beyond the scope of engineering controls (i.e., soil temperature) are listed as they were recorded. All other partially controllable variables are listed in Table 3 as generally observed and/or amended in the field. Approximately one pound of prilled ammonium nitrate was applied per yd^3 of the LTU soils every two to three weeks (as nutrient testing indicated and moisture content allowed).

Table 3 lists the observed and/or amended parameters for the LTUs.

Indigenous soil bacteria (including *Pseudomonas stutzeri*) were cultured on-site in a 1,500 gal bioreactor. The bacteria strains were applied to the OPA soils using a wheel-line irrigation system as illustrated in Figure 1. In addition, freeze-dried bacteria were cultured for their ability to degrade the more recalcitrant petroleum hydrocarbon constituents. No bacterial applications were made to the CERCLA LTU since the bioreactor which supplies that LTU was not installed at that time. Tilling of the contaminated soil generally was performed every other day until initial homogenization of the LTU soils and reduction of soil aggregate size had been accomplished. After initial homogenization, the tilling was performed on a weekly basis for oxygenation of the soils and distribution of nutrient and bacterial

amendments. This operation was very important to achieve the project goals for aeration, which introduced oxygen used as a terminal electron acceptor during contaminant degradation. Oxygen depreciation is a common biologically limiting factor. Since lagoon sediments were saturated immediately after excavation, it became impractical to till and oxygenate OPA LTU sediments for the first few days of LTU loading. However, CERCLA LTU sediments were within desired moisture range and were tillable after 24 hr of loading. In addition, soil type (per visual soil classification) varied from very fine sandy silt to silty clay in the OPA LTU to fine sandy silt in the CERCLA LTU. The soil type exhibited an important role in draining the LTU soils and achieving the suitable LTU operation moisture content in a short period of time. The ratio of air and water in the soil strongly influences microbial activities.

Monitoring of the first full-scale batch of soil treated in the OPA LTU indicated that the bacterial population fluctuated dramatically as environmental and biological factors changed. Upon excavation and loading of the contaminated soil into the LTU, there was an increase in bacterial activity (10^6 to 10^7) due to aeration and nutrient application. High moisture content and low nutrient concentrations explained the resultant decrease in bacteria to pretreatment levels during the first week. Subsequent nutrient application and tilling for aeration increased bacterial population growth seven orders of magnitude (10^7 to 10^{14}). This increased bacterial presence dropped over a period of four weeks due to wet weather conditions and the inability to aerate the soils.

Monitoring of contaminant degradation indicated that the most significant degradation occurred within the first four weeks of treatment (see Fig. 2). Analytical results show reductions in volatile compounds ranging from 94% (ethylbenzene) to 98% (benzene) during a treatment period of 100 days. Semivolatile compound concentration reduction ranged from 85% (chrysene) to 99% (naphthalene). Total target PAHs decreased from 95 mg/kg to 0.93 mg/kg (97%) during the 100-day treatment period. Figure 2, which shows the degradation of the six target PAHs, graphs the concentration target PAHs vs. time (Arrhenius Plot). Ambient air sampling data generated during full-scale biodegradation activities indicated minimal (< 3 ppm) volatile contaminant emission during the loading and homogenization operations of the lagoon soil. Real-time air monitoring data using an Organic Vapor Meter (OVM-580S) indicated that volatile contaminant concentrations of concern were below Occupational Safety and Health Administration (OSHA) requirements and National Ambient Air Quality Standards (NAAQS). Once again, long-term air monitoring was not conducted due to the enormous size of the LTUs.

Individual semivolatile compound concentrations in the CERCLA LTU were reduced in a range from 62% to 92% over a period of 71 days. Total target PAHs

decreased 70.6 mg/kg to 7.50 mg/kg (89%). Values of PAH less than detection concentrations (as reported by the laboratory) were assumed to be equal to the detection limit. Figure 3 illustrates the degradation of the six target PAHs in the CERCLA LTU vs. time. Unlike the OPA LTU, the degradation rate of the CERCLA LTU remained almost constant throughout the treatment period.

Degradation rate constants and half-lives have been calculated for total target PAHs, as well as the individual PAHs pyrene and fluorene using linear regression analysis on data gathered from four (initial, two interim, and final) and three (initial, interim, and final) sampling events (OPA and CERCLA respectively) (see Figs. 4–9). Half-lives were calculated using the first-order degradation rate constant. Table 4 presents the first order degradation rate constants and the corresponding half-life values for OPA and CERCLA loadings. These values are compared to treatability studies conducted on similar waste and found to be consistent with the literature with the exception of pyrene (API 1987).[2]

Although the biological and physical treatment of wastes in the two LTUs differed significantly, the degradation rates and half-lives were relatively similar. The average half-life of the six target PAH compounds treated in the OPA LTU was 22 days. The average half-life of the six target PAH compounds treated in the CERCLA LTU was 21 days. These observations correlate well with half-lives estimated for Total Carcinogenic PAHs (TCPAHs) on ex-situ creosote contaminated soil being treated on another Superfund site in Libby, Montana.[5]

The half-life for pyrene (28 days) varies only 13% from the estimated half-life reported by Coover and Sims, 1987 (32 days)[6] at temperatures which were very similar (30°C) and Loehr and Sims[2] in 1987 using Nunn Clay Loam at an oil and grease (O&G) loading concentration of 2%. The degradation rate for pyrene treated in the OPA LTU (0.024/D) is almost exactly the same value as reported by Loehr and Sims[2] in 1987 for Nunn Sandy Loam with an O&G loading concentration of 2%. Fluorene half-lives (17 and 19 days) are on the same order of magnitude as those found by Loehr and Sims[1] in 1987 using Kidman Sandy Loam at 2% O&G loading and Nunn Clay Loam at 4% O&G loading.

Discussion

Treatability testing indicated that using biodegradation to reduce contaminant concentrations of Macmillan Ring-Free Oil Company wastes is feasible and met EPA's criteria for inclusion in the potential remedies for the site. During the pilot biodegradation project, significant reductions of target compounds were achieved and SSCC were attained under field conditions. Full-scale biodegradation operations have been standardized and SSCC were attained during the first

treatment batches. Half-lives calculated using first-order degradation rate kinetics are consistent with other values reported at the laboratory and similar full-scale levels of PAH treatment.[2,5] Soil type did not have significant effect on PAH degradation. Toi date, full-scale operations have treated 12,000 yd^3 of oil-refinery-waste-contaminated soil, which is more than one-third of the site wastes. The Macmillan project is contributing to the U.S. EPA call for full-scale biodegradation data for inclusion in several EPA treatment results databases (EPA 1994).[4]

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Notice

The information presented in this paper has been funded by the U.S. Environmental Protection Agency under the Technical Assistance Team Contract No. 68-WO-0037. It has been subjected to peer and administrative reviews by the Region 6 EPA Emergency Response Branch and has been approved for publication. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use.

Table 1 Summary of Biodegradation Test Methods

Parameter	Test Method
Moisture content	Soil moisture meter
Particle-Size Analysis of Soils	ASTM: D 422–63 Standard Test Method for Particle-Size Analysis of Soils
Nitrate nitrogen, nitrite nitrogen, ammonia nitrogen, phosphorus, potassium, pH	Combination Soil Test Kit
Heterotrophic bacterial population, colony forming units (CFUs)	Standard Methods for the Examination of Water and Waste water. APHA:AWWA:WPCF Method 907
Hydrocarbon-utilizing bacterial population (HCCFUs)	Bushnell & Haas media plus hydrocarbon substrate
Bacterial identification (typing)	Bacterial Identification System
Volatile aromatic compounds (BTEX)	EPA SW-846 Method 8020 Gas Chromatography/PID detection
Base neutral/acid extractable compounds (BNAs)	EPA SW-846 Method 8270 Gas Chromatography/Mass Spectroscopy

Table 2 Land Treatment Unit Ideal Parameters

Moisture Content of LTU Soil (% of Field Capacity)	pH of LTU Soil	Soil Temp. (°F)	Organic Carbon:Nitogen:Phosphorous (C:N:P)
70–80	6–8	70–90	100–120:10:1

Table 3 Land Treatment Unit Operating Parameters

Land Treatment Unit	Treatment Period (1994–95)	Soil Temp. (°F)	Nitrate Nitrogen (ppm)	Bacteria Applied (gallons)	LTU Tilling (hr)
OPA	August–January	30–90	50–150	15,900	166
CERCLA	November–January	30–60	50–150	none	65

Table 4 Biodegradation Kinetics

Contaminant	Initial Concentration (mg/kg)	Degradation Rate (1/D)	Percent Reduction (%)	Half-Life (days)
OPA				
TPAHs	93.0	0.032	97	22
Pyrene	14.0	0.024	94	28
Fluorene	15.7	0.041	99	17
CERCLA				
TPAHs	70.6	0.032	89	21
Pyrene	16.0	0.037	92	19
Fluorene	16.0	0.037	92	19

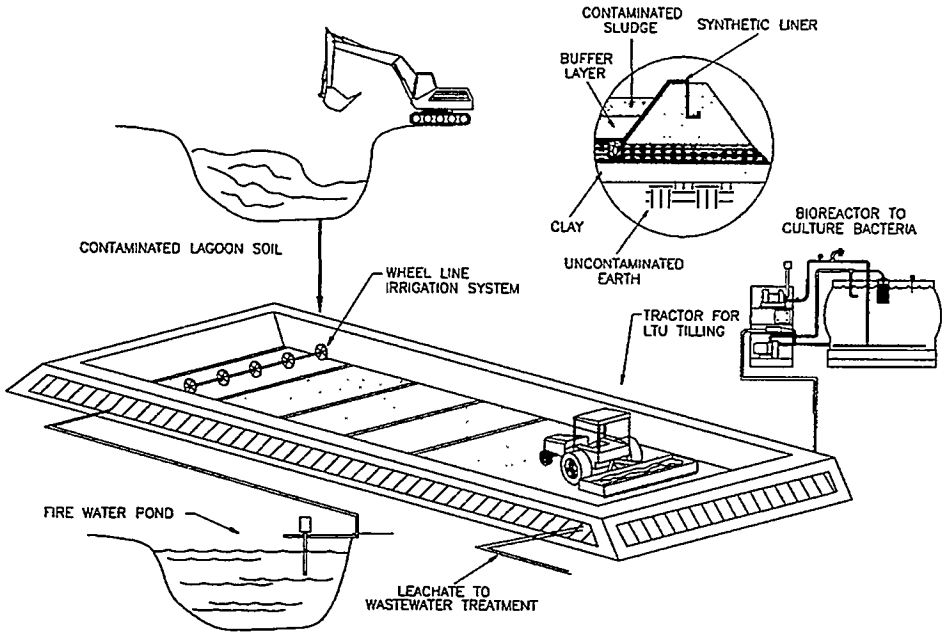

Figure 1 Biodegradation Operations Schematic

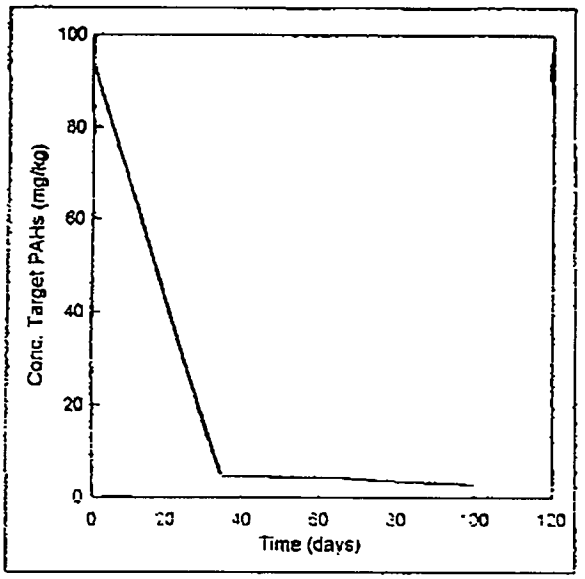

Figure 2 Degradation of Target PAHs in OPA LTU (Arrhenius Plot)

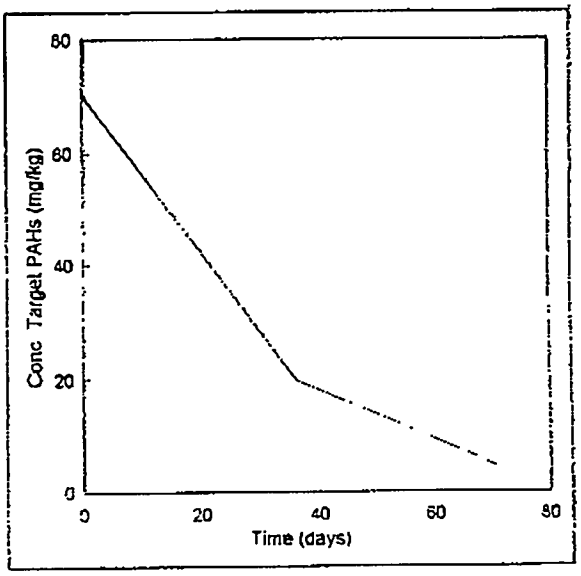

Figure 3 Degradation of Target PAHs in CERCLA LTU (Arrhenius Plot)

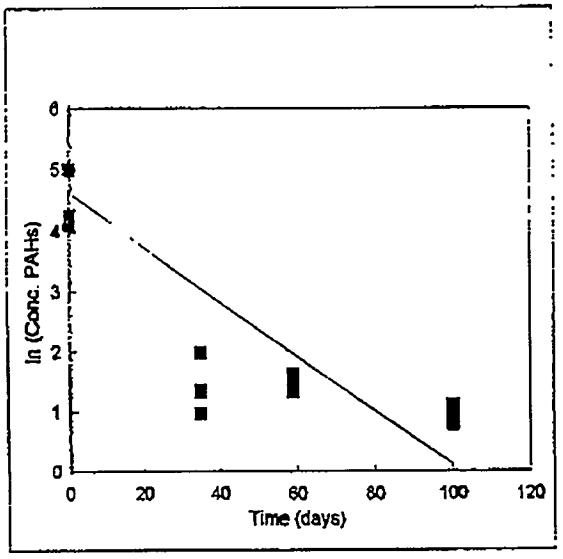

Figure 4 Degradation of Target PAHs in OPA LTU (Linear Regression Plot)

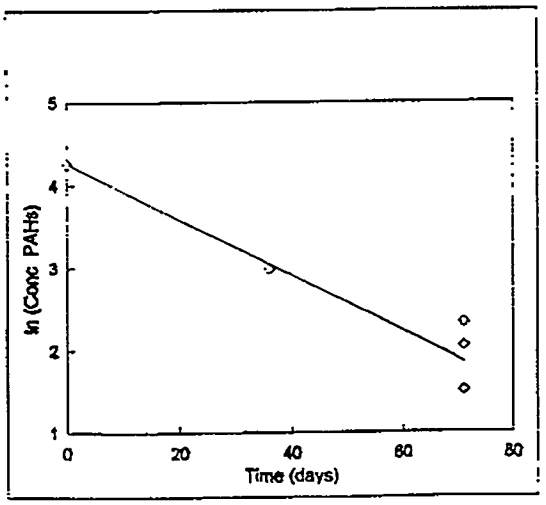

Figure 5 Degradation of Target PAHs in CERCLA LTU (Linear Regression Plot)

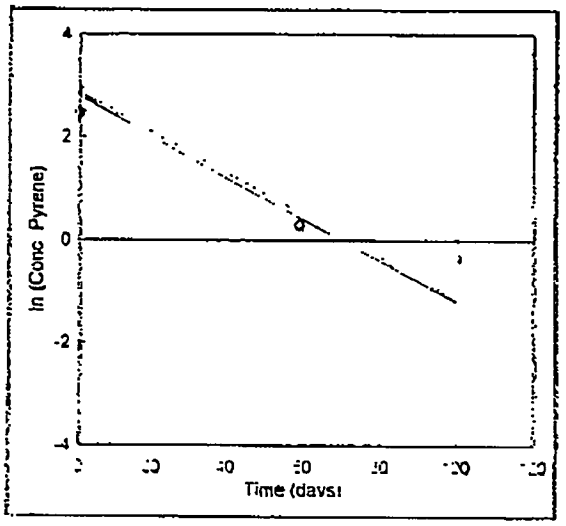

Figure 6 Degradation of Pyrene in OPA LTU (Linear Regression Plot)

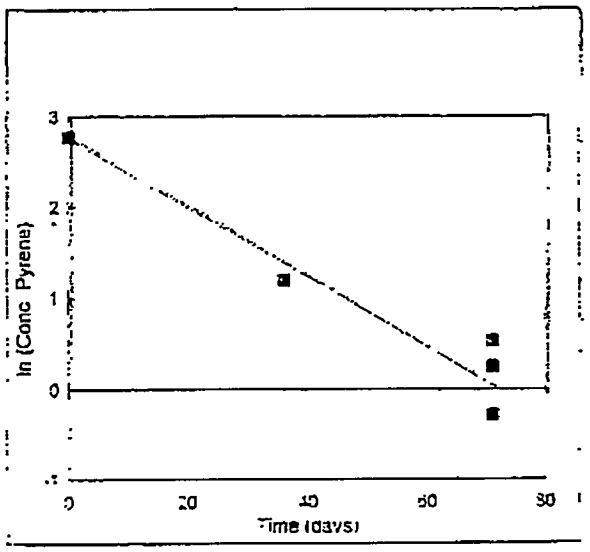

Figure 7 Degradation of Pyrene in CERCLA LTU (Linear Regression Plot)

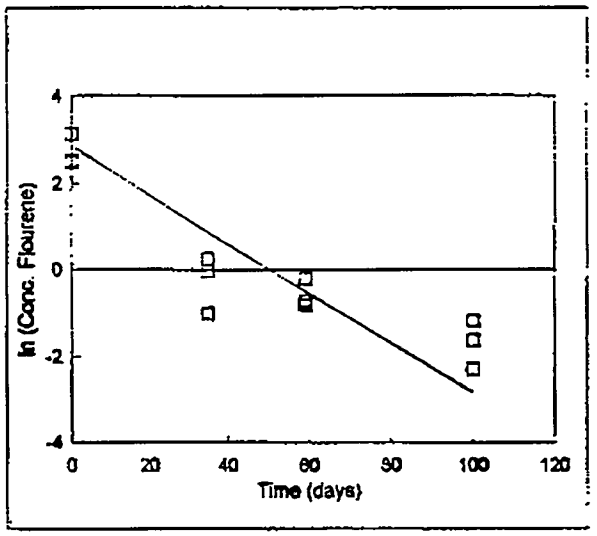

Figure 8 Degradation of Fluorene in OPA LTU (Linear Regression Plot)

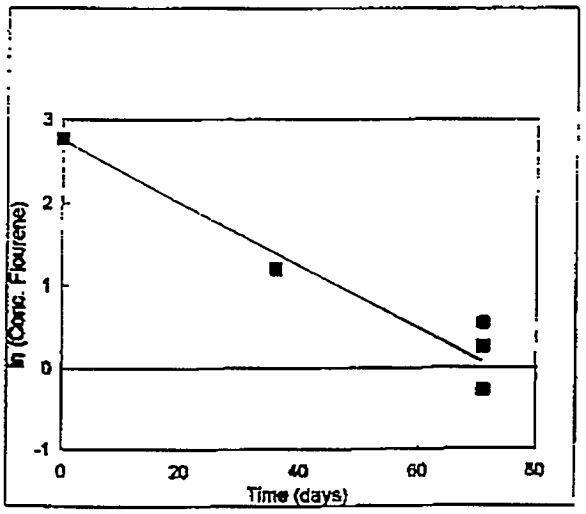

Figure 9 Degradation of Fluorene in CERCLA LTU (Linear Regression Plot)

Investigations on Potential Bacteria for the Bioremediation Treatment of Environments Contaminated with Hydrocarbons

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Abstract

In Romania after more than 135 years of oil production and processing, some severe environmental pollution problems have accumulated. In this context a joint research group from Institute of Biology Bucharest and S.C. Petrostar S.A. Ploiesti became involved in a research project on bioremediation of an environment contaminated with hydrocarbon waste.

In the first stage of this project, investigations on microbial communities occurring in environments contaminated with oil were carried out.

In the second stage, the hundreds of bacterial strains and populations isolated from soils, slops, and water sites contaminated with waste oil and water waste oil mix were submitted to a screening program, to select a naturally occurring mixed culture with a high ability to degrade hydrocarbons.

This paper presents and discusses the results of these investigations and explores the possibilities of some bioremediation applications. The natural bacteria consortia selected proved to have a high capacity for hydrocarbon degradation. Namely, the hydrocarbon waste from soils, slops, and water/waste oil mix have been degraded up to 72.75% by bacterial consortia isolated from soil and slop samples collected from several sites in oil fields and up to 82.94% in the case of bacterial consortia isolated from water/waste oil mix samples, also collected from oil field sites. Paraffin deposits have been degraded by such bacterial consortia up to 36.63%.

Introduction

Biological elimination of environmental pollutants is one of the major interdisciplinary goals of research. Over the last decades, significant quantities of hydrocarbon waste, as well as all kinds of industrial chemicals, have been released into the environment. Many of the wide range of organic waste compounds are rapidly degraded by naturally occurring microorganisms, compared to compounds with novel chemical structures or substituents (xenobiotics), which are degraded slowly and tend to accumulate.[1] In countries with a long history in oil production and processing, such as Romania, the environment has been contaminated over many years with sizable quantities of hydrocarbon waste, mainly generated by oil spills. Now biotechnology is generally accepted as one of several competing technologies for the maintenance of environmental quality.

The biotechnological methods have several advantages compared to physico-chemical ones: cost-effectiveness, reduced risks, and minimal environmental impact since the microorganisms degrade polluting substances to nontoxic end-products, such as carbon dioxide and microbial biomass. Bioremediation can be called a "green solution."[2] In the most cases, bioremediation relies upon naturally occurring microorganisms that are usually indigenous to the contaminated sites. But often the degradation activities of indigenous microorganisms are limited by factors such as the availability of molecular oxygen and some mineral salts which can be used to support microbial growth. By improving aeration and adding fertilizers (nitrogen and phosphate sources), the activities of indigenous microorganisms can be stimulated, and the rates of pollutant degradation accelerated. In the cases of inadequate genetic diversity within the microbial community, "seed cultures" can be added. Seed cultures may be an undefined mixture of microorganisms, such as those that occur at polluted sites, or may be specific microbial cultures with defined metabolic capacities including genetically modified microorganisms.[2,3,4]

The development of effective biotechnological processes for biodegradation of hydrocarbons as environmental pollutants has been in many instances based on microorganisms now present at contaminated sites.[14,15,16] In such cases efforts are directed towards identification and optimization of the parameters, including in-situ treatment processes. As reported by Harvey,[4] microbial technologies for remediation are often sought because they are less expensive, the hydrocarbon pollutants serves as a growth substrate for the growth of microorganisms, and their selection will then naturally enhance both the degradation rate and dispersal. Thus, strategies to detect and recover the microorganisms with the desired features from nature at the polluted sites were developed. When the classical enrichment technique is not satisfactory to recover all of the microorganisms of interest, new strategies such as using multidimensional gradients, the organism positioning mechanisms,

nucleic-acid-based tracking techniques, and new methods of physical separation should be explored.[5]

This paper presents the performance in hydrocarbon waste degradation of naturally occurring bacterial mixed cultures isolated and selected from hydrocarbon pollutant sites located in several Romanian oil fields.

Material and Methods

Characteristics of Samples Collected from Sites Contaminated with Hydrocarbons. Samples of environmental sites contaminated with hydrocarbons were represented by: soil, sludge, and water/waste oil mix collected from representative hydrocarbon polluted sites in several oil fields. Paraffin deposits were collected from the wells, pipelines, or tanks after cleanup operations, also from several oil fields. The content of total hydrocarbon waste contained in the samples studied has ranged between 11.0% and 98.46% for solid samples and between 105 and 150 mg/l for liquid samples (water/waste oil mix). Details on the hydrocarbon content in samples are presented in Table 1.

Microbiological Analyses of Samples. All the samples mentioned above were submitted for microbiological analyses looking for the occurrence of several physiological groups of bacteria as: aerobic, facultatively anaerobic, and anaerobic heterotrophic bacteria, hydrocarbon-oxidizing, sulfate-reducing, sulfoxidizing, and iron-oxidizing bacteria, as well as microscopic fungi (see Table 2). During this analysis, routine culture media were used.

The Methodology for Isolation and Selection of Natural Bacterial Consortia. For the isolation and selection of natural bacterial consortia from hydrocarbon-contaminated sites, the enrichment culture method has been used. Selection of such consortia has been a result of a screening program. The selected bacterial consortia should have a high capacity to degrade hydrocarbon waste, as well as be good producers of biosurfactants and solvents on samples which have hydrocarbon waste as the sole carbon source. The production of biosurfactants and solvents was proved using the kerosene test for biosurfactants[6] and the nigrosin test for solvents.[7]

The initial content in total waste hydrocarbon of each sample studied, as well as the percentage of microbial degradation of waste oil, has been determined by extraction with benzene or petroleum ether using separation funnels[8] in the case of liquid samples or Soxhlet extraction equipment[9] in the case of solid samples.

Next, the performances of natural bacterial consortia in waste hydrocarbon degradation were established both in aerobic and facultative anaerobic conditions. Several experimental procedures were used for this purpose. Details about these procedures, nutrients, aeration, and bacterial inoculum are presented in Tables 3, 4, 5, and 6.

Results and Discussions

Bacterial Occurrence in Hydrocarbon Polluted Sites and Isolation of Natural Bacterial Consortia (NBC). Table 2 presents data on the occurrence of bacterial communities in hydrocarbon polluted sites (soils, slops, water/waste oil mix, and paraffin deposits). It was found that a rich microflora occurs in waste-oil-contaminated sites. This proved that naturally occurring mixed cultures of interest for the bioremediation processes could be isolated and selected from such sites. This gave us the confidence that from such oil-polluted sites, bacterial consortia could be obtained, and they may be necessary to carry out different steps in the pathway of biodegradation, or they may be important because of the different phenotypic features maintained in the mixture, such as a range of kinetic properties effective at different substrate concentrations or tolerance to different conditions. Such natural mixtures or consortia are likely to yield more robust processes because of the diversity that exist in such bacterial communities. For this reason, in this study we have been interested in considering not single microorganisms for degradation of hydrocarbon pollutants, but a consortium. This is consistent with the results or opinion of other authors.[2,3,10,11,12,13]

Figure 1 lists the main steps followed to isolate and select naturally occurring mixed cultures having the ability to degrade hydrocarbon pollutants from the bacterial communities already existing. We called such mixed cultures obtained natural bacterial consortia.

Following the steps mentioned in Figure 1, which are based on classical enrichment techniques, a number of natural bacterial consortia with evident abilities in waste hydrocarbon degradation (see Table 1) were recovered from hydrocarbon-polluted site samples.

The Performances of NBC in Waste Hydrocarbon Degradation. Table 3 presents the performances, in degrading hydrocarbon waste contained in aqueous solutions, by several NBC isolated from water/waste oil mix samples. It was found that three of the five NBC tested have a high rate of hydrocarbon degradation, namely between 64.4% and 82.9%.

Table 4 supplies the degradation percentages of hydrocarbon waste contained in soil samples using five NBC isolated from soil on oil-polluted sites. Three treatment procedures were used. No increase in hydrocarbon degradation rates was noticed when supplementary inoculum or fertilizers were added during the incubation period. The efficiency in waste hydrocarbon degradation reached up to 72.2%.

The results in Table 5 concern the biodegradation of hydrocarbon waste contained in slops collected from oil storage tanks in several oil fields. The efficiency of hydrocarbon degradation is higher in procedure III with the addition of fertilizers, namely 66.5% to 72.8% compared to 54.5% to 53.8% (procedure I), or 53.7% to 59.3% (procedure II).

Table 6 presents the results of paraffin hydrocarbon degradation by several natural bacterial consortia isolated from paraffin deposit samples. The pretreatment of paraffin deposits with solvents should significantly increase the ratio of alkane biodegradation, but the percentage of paraffin degradation is not substantially higher compared to untreated samples. This could be explained by the high capacity of the natural bacterial consortia used to produce important quantities of biosurfactants and solvents, which facilitate a better access of bacteria to the paraffinic substrate.

Such bacterial consortia should be of interest for preventing the accumulation of paraffin deposits during the oil paraffin production, transport, and storage. A pilot experiment is in progress to investigate this possibility and to prevent the accumulation of paraffin deposits.

By using the appropriate strategies, it becomes easy to detect and recover the suitable bacterial consortia (those with the desired features for bioremediation of the oil polluted environments) from the bacterial diversity occurring at waste hydrocarbon polluted sites. Classical enrichment techniques have proven satisfactory for recovering the microorganisms of interest for bioremediation of an oil-polluted environment.

Conclusions

1. The microbiological analyses of samples collected from hydrocarbon polluted sites (soils, slops, water/waste oil mix, paraffin deposits) proved the existence of abundant microbial communities, including bacteria with high capacity for hydrocarbon degradation.

2. An important number of natural bacterial consortia of interest for bioremediation of oil-polluted sites have been isolated from the hydrocarbon-polluted site samples following several steps based on classical enrichment culture techniques.

3. For some of the natural bacterial consortia isolated, investigations have been done on their ability to degrade hydrocarbons contained in samples collected from oil polluted sites. The efficiency in waste hydrocarbon degradation was up to 82.9%, the rate being a function of the sample nature (soil, slop, water/waste oil mix, or paraffin deposits).

4. Pilot experiments using natural bacterial consortia for bioremediation of some environments contaminated with waste oil are under progress.

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Table 1 Environments Polluted with Hydrocarbon Waste from Which Natural Bacterial Consortia Were Selected

Environments Contaminated with Hydrocarbon Waste Submitted to Microbiological Analyses	Content in Hydrocarbon Waste	Number of Bacterial Consortia Isolated
Soils contaminated with waste oil	11.0–30.0%	82
Sludge from oil fields	29.8–52.1%	81
Waste waters from oil fields	105.0–150.0 mg/l	79
Paraffin deposits from oil fields	77.84–98.46%	66

Table 2 Bacterial Contamination of Waste Hydrocarbon Samples Collected from Polluted Sites

Hydrocarbon Polluted Sites	Occurrence of:* HOB	AHB	AnHB	SRB	SOB	IOB	FM
Soils	1.5×10^2 5.5×10^5	4.5×10^4 6.3×10^8	5.2×10^4 6.8×10^8	+++	+	+++	+++
Slops	2.5×10^3 6.5×10^5	9.5×10^4 7.5×10^6	1.4×10^4 2.5×10^5	++	-	++	++
Water/ waste mix oil	1.2×10^3 5.4×10^4	1.2×10^2 6.2×10^7	1.2×10^5 2.4×10^7	+++	+	+++	++
Paraffin deposits	3.0×10^2 7.2×10^5	2.5×10 7.2×10^6	1.7×10 1.4×10^6	++	+	++	+++

*HOB: Hydrocarbon-oxidizing bacteria
AHB: Aerobic, facultativly anaerobic, heterotrophic bacteria
AnHB: Anaerobic heterotrophic bacteria
SRB: Sulphate-reducing bacteria
SOB: Sulphur-oxidizing bacteria
IOB: Iron-oxidizing bacteria
FM: Microscopic fungi

Table 3 Performances of Several Natural Bacterial Consortia (NBC) in Bioremediation of Aqueous Solutions Contaminated with Crude Oil

Procedure of Microbial Treatment	Natural Bacterial Consortia Used As Inoculum (Collection Indicative)*	Bacterial Degradation of Waste Oil Contained in Aqueous Solutions (%)
Aqueous solution represented by mineral solution** with addition of 10% crude oil*** and 20% bacterial inoculum; incubation at 28°C for 7 days	WwNBC 1	49.5
	WwNBC 2	64.4
	WwNBC 3	55.5
	WwNBC 4	76.6
	WwNBC 5	82.9

* WwNBC1-5 = Waste water Natural Bacterial Consortia isolated from water and waste oil mix samples

** Koronelli mineral medium

*** Crude oil of nonparaffinic type

Table 4 Performances of Several Natural Bacterial Consortia (NBC) in Bioremediation of Soil Contaminated with Waste Oil

	Procedure of Microbial Treatment of Soil Sample*	Natural Bacterial Consortia Used As Inoculum (Collection Indicative)**	Bacterial Degrada-tion of Waste Oil Contained in Soil Sample (%)
I	Add 20% bacterial inoculum, followed by 7 days incubation at 28°C	SoNBC 1	65.1
		SoNBC 2	68.0
		SoNBC 3	67.6
		SoNBC 4	67.9
		SoNBC 5	72.2
II	Add 20% bacterial inoculum, followed by daily addition of 2% bacterial inoculum for 7 days	SoNBC 1	62.3
		SoNBC 2	54.2
		SoNBC 3	61.9
		SoNBC 4	52.9
		SoNBC 5	64.6
III	Add 20% bacterial inoculum, followed by daily addition of 2% mineral solution*** for 7 days	SoNBC 1	59.0
		SoNBC 2	59.8
		SoNBC 3	62.1
		SoNBC 4	63.4
		SoNBC 5	50.0

* Soil sample with a content in waste oil of 15.5%

** SoNBC1-5 = Soil NBC isolated from soil contaminated with waste oil

*** Koronelli mineral medium

Table 5 Performances of Several Natural Bacterial Consortia (NBC) in Bioremediation of Sludge Contaminated with Waste Oil

	Procedure of Microbial Treatment of Slop Sample*	NBC Used As Inoculum (Collection Indicative)**	Bacterial Degradation of Waste Oil Contained in Slop Sample (%)
I	Add 20% bacterial inoculum and 10% mineral solution,*** and incubate at 28°C for 30 days	SlNBC 2	45.9
		SlNBC 3	46.4
		SlNBC 4	48.3
		SlNBC 5	54.5
		SlNBC 6	53.8
II	Add 20% inoculum and 10% mineral solution. After 14 days, add 2% inoculum and incubate at 28°C for 30 days	SlNBC 2	45.8
		SlNBC 3	47.2
		SlNBC 4	51.5
		SlNBC 5	53.7
		SlNBC 6	59.3
III	Add 20% inoculum and 10% mineral solution. After 14 days, add 2% mineral solution and incubate at 28°C for 30 days	SlNBC 2	46.3
		SlNBC 3	57.8
		SlNBC 4	60.2
		SlNBC 5	66.5
		SlNBC 6	72.8

* Slop sample with a content of 36% waste oil and a sand addition of 50%

** SlNBC2-6 = Slop NBC isolated from sludge samples

*** Koronelli mineral medium

Table 6 The Performances of Several Natural Bacterial Consortia (NBC) in Degradation of Paraffin Deposits

	Procedure of Microbial Treatment of Paraffin Deposit Sample*	Paraffin Deposit: Inoculum: Mineral Solution** Ratio	NBC Used As Inoculum (Collection Indicative)* **	Bacterial Degradation of Hydrocarbons from Paraffin Deposits (%)
1	Treatment of paraffin deposit by addition of mineral solution and bacterial inoculum; incubation at 30°C for 14 days in aerobic conditions	1 : 5 : 15	PfNBC 4	38.7
			PfNBC 5	38.9
			PfNBC 6	51.7
			PfNBC 7	26.1
			PfNBC 8	39.8
2	Same as 1, but paraffin deposit has been treated with solvent (1: 2)	1 : 5 : 15	PfNBC 4	45.9
			PfNBC 5	46.7
			PfNBC 6	62.8
			PfNBC 7	42.1
			PfNBC 8	49.6
3	A thin layer of paraffin deposit in a Petri dish covered with a layer of mineral solution and inoculum; incubation at 37°C for 21 days in facultative anaerobic conditions	1 : 2 : 1	PfNBC 4	27.4
			PfNBC 5	20.9
			PfNBC 6	27.8
			PfNBC 7	30.3
			PfNBC 8	25.9
4	Same as 3, but paraffin deposit has been treated with solvent (1 : 2)	1 : 2 : 1	PfNBC 4	36.2
			PfNBC 5	22.7
			PfNBC 6	30.0
			PfNBC 7	36.7
			PfNBC 8	34.5

* Samples of paraffin deposits were collected from several oil fields (wells, pipe lines, tanks, etc.)

** Koronelli mineral medium

*** PfNBC4-8 = Paraffin NBC isolated from paraffin deposits collected from several oil fields

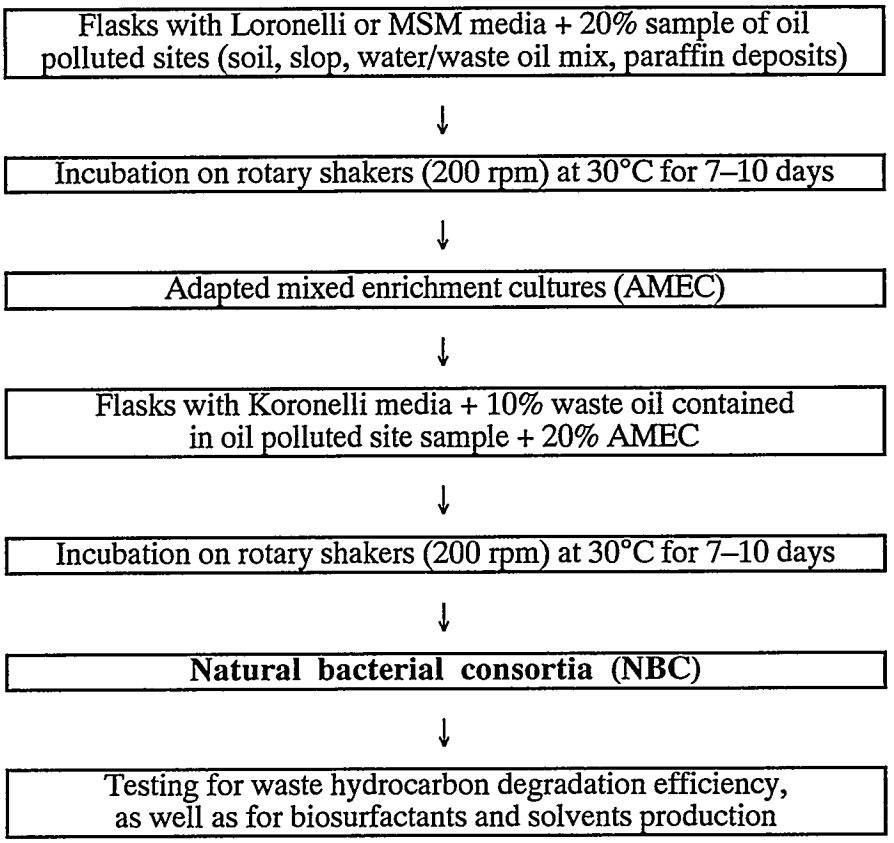

Figure 1 Main Steps of Isolation and Selection of Natural Bacterial Consortia from Hydrocarbon Polluted Sites

Characterization and Behavior of Microbial Systems

Microbial Interactions in Crude Oils: Possible Impact on Biochemical Versatility on the Choice of Microbial Candidates

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Abstract

Experimental data gathered over the past several years show that the interactions of microorganisms with crude oils are variable and depend on the microbial species and the chemical composition of crude oils. The variations can be observed in terms of the extent of emulsification, changes in the hydrocarbon composition of crude oils, and duration of biotreatment. All of these factors indicate that the interaction of microbes with crude oils involves multiple chemical reactions resulting from the biochemical interactions between microbes and oils. Different interactions may influence the efficiency of processes in which single or mixed microbial species are used for the oil treatment and may also suggest possible combinations of biological and chemical technologies. Some of these concepts will be discussed in this paper.

Introduction

Extensive studies dealing with the biochemical interactions of certain microorganisms (thermophilic and thermoadapted) on selected types of crude oils at elevated temperatures and pressures have shown that, as a consequence of microbial action, significant chemical changes occur in the composition of crude oils.[1-6]

Analysis of data generated from the studies of biochemical action of several species of microorganisms on crude oils from California, Alabama, Arkansas, Wyoming, Alaska, and Venezuela show that biotreatment causes (1) emulsification, (2) acidification, (3) qualitative and quantitative changes in the light and heavy fractions of crudes, (4) decrease in organic sulfur content and chemical changes in oil fractions containing sulfur compounds, (5) decrease in organic nitrogen content with concurrent biochemical conversion of polar nitrogen containing compounds, and (6) decrease in the concentration of trace metals. The qualitative and quantitative changes in the hydrocarbon composition depend on the microbial species and the chemistry of the crudes. There is also a distinction between "biodegraded" and "bioconverted" oils. The former term is more suitable for changes which occur under natural conditions over geological periods of time, and the latter is more applicable to changes brought about by deliberately introduced microorganisms acting over short periods of time. Further, preliminary results indicate that the introduced microorganisms may become the dominant species in the bioconversion of oils.[3] These studies have also generated information which supports the view that the biochemical interactions between crude oils and microorganisms follow distinct trends characterized by a group of chemical markers. Such markers are useful in the prediction of bioprocessing efficiency prior to and during the biochemical treatment of crudes regardless of upstream or downstream applications. Further, the microbial species considered in these studies use predominantly indigenous matter as their energy source, which implies that simultaneous intra- and inter-molecular chemical interactions must be involved, resulting in multiple chemical effects. In such complex chemical reaction mixtures, the use of chemical (biochemical) markers has obvious advantages. Systematic monitoring, analysis, and characterizations of chemical markers allow us to follow process variables such as kinetics, yields, economics, and others essential to the development and application of the bioprocesses in either oil recovery or oil upgrading technology. Several biochemical markers of diagnostic significance have been identified. These include total distribution of hydrocarbons, organic sulfur and nitrogen compounds, and organometallic compounds, as well as total sulfur, nitrogen, and trace metal contents. Furthermore, corroborative evidence of chemical changes can also be obtained from the distribution of asphaltenes, saturates, aromatics, and resin contents of crudes. The use of these markers in the choice of microbial species and

oils will be discussed. In a companion paper,[7] the application of chemical markers in the economic analysis of biochemical upgrading of crude oils is also discussed.

Materials and Experimental

Growth of microorganisms, experimental conditions, protocols, spectroscopies, and chemical analyses have been reported in detail elsewhere[1,3,5] (and references therein) and will not be discussed here. Any variation and modification of the referenced procedures will be noted in the bulk of the text.

Methods, Results, and Discussion

The use of "molecular fossils" (i.e., organic natural products originally derived from living organisms, whose molecular structures have persisted over geological time) in the exploration and correlation of petroleum, petroleum source rocks, sediments, and sedimentary organic matter has been successful over the past several years.[9] An extensive database is now available for comparative studies. The experience gained also stresses the importance of using several types of markers for correctional purposes. Analogous to the use of molecular fossil markers, the current information regarding the use of chemical markers as correctional and diagnostic tools in the study of biochemical processing of oils by different microbial species also points to the usefulness of the multiple chemical markers analysis. The applications of these will be discussed in the next several paragraphs. In Tables 1–3, sulfur, nitrogen, nickel, and vanadium concentrations in four oils serve as chemical markers indicative of the biochemical conversion of these oils by several microorganisms (biocatalysts).

The overall effect on the chemical markers due to the bioconversion of oils is a decrease in the concentrations of sulfur, nitrogen, vanadium, and nickel. However, this effect is not uniform and varies with the oil and the biocatalyst used, as is further emphasized in the example reported in Table 4 in which Cerro Negro, a heavy crude, was treated with several other biocatalysts.

Results shown in Tables 1–4, indicate that in terms of chemical markers and the experimental conditions used, some microorganisms are more or less efficient in their action on different oils. How do these oils compare and/or differ? For the purposes of this discussion, the four oils described above include two Venezuelan and two Californian oils. Both sets are compared with another set of Monterey, California, oils, A836, A837, and A851.[3] All of these oils are heavy with API gravities ranging from 12 to 19. The hydrocarbon distribution for all of the oils to be considered in this discussion is given in Table 5. It is to be emphasized that the

analytical methods used (i.e., chromarod and solvent/column chromatography) generates results which can only be compared qualitatively because of the use of different solvents and fractionation techniques. These techniques are sensitive to the effects of solvents on the relative concentration of saturates, aromatics, resins, and asphaltenes. However, regardless of the solubility bias, the relative distribution of major fractions reflects the history of the oil. Low (<20) API gravity oils are low in gasoline and high in residuum, which means that as the oils become heavier, the H/C ratios decrease as the oils change from light to dark heavy oils, while the NSO/C ratios increase and the oils become richer in asphaltenes.[8] Thus, the heavier fractions of oils are richer in resins and asphaltenes, as well as polar compounds, containing heteroatoms (O,N,S) and metals. Chemically, this also means changes in the concentration and distribution of paraffinic, naphthenic, and aromatic compounds with a progressive increase in the concentration of polyaromatic and heterocyclic compounds, with the highest concentration of the heterocyclics present in resins and asphaltenes. Further, these condensed polyaromatic chemical structures contain free radical sites with highly reactive unpaired electrons. These sites are involved in complexion of metals as well as inter- and intra-molecular reactions and molecular rearrangements. The polarity of asphaltic structures plays also an important role in hydrogen bonding and charge transfer complexes.

The combined effects of the mentioned functionalities (i.e., those due to reactions such as complexing at O,N,S sites and those due to unpaired election rearrangements, such as redox reactions) affect also viscosity and micellar structures. These chemical properties play decisive roles in the behavior of crude oils in reservoirs as well as their behavior in upstream and downstream processing. It is these properties that also influence the biochemistry associated with the interactions of microorganisms and crude oils. Mechanistically, microorganisms can then be considered as catalysts entering colloidal, micellar and molecular solutions which react with active sites,[1] initiating a dispersion of micellar organization which leads to a "deploymentation" of the heavy crude polymer as shown in Figure 1, where the dark dots represent heteroatoms and other reactive sites. Such mechanisms are consistent with the well studied mechanisms of microbial interactions with particulate matter, mineral surfaces, membranes, and other chemical surfaces involving active sites.[13–15]

Thus, the chemical markers discussed in this paper represent both the extent of the heaviness of the crude and the degree of biochemical conversion of the crude. For example, the oils listed in Table 5, in addition to being heavy, have other common characteristics. Monterey A851 (a California crude) and Cerro Negro (a Venezuelan crude) are heavy because they are "biodegraded," meaning, biodegraded over geological periods of time under reservoir conditions. Venezuelan Boscan crude and the California crudes, Monterey A836, A837, and OSC, are heavy, because they are immature. The sample of Midway Sunset Crude (also a California heavy

crude) was subject to secondary steam recovery which contributed to chemical alteration.[11]

The oils under discussion are complex mixtures representing different types of oils; however, all falling into definable categories. Chemically and biochemically caused changes in these categories involve multiple reactions within a complex mixture which follow distinct trends that can be followed by chemical markers. Thus, gas chromatographic analyses (see Fig. 2) of two biodegraded crude oils from different geographic localities (California Monterey 851 crude and Cerro Negro crude from Venezuela), when treated with the same biocatalyst, BNL-NZ-3, show a similar response, however differing in detail. Bioconversion of both leads to an enrichment in lighter hydrocarbons (shorter retention time). However, the relative distribution of hydrocarbons differs. Likewise, as shown in Figure 3, treatment of M851 with three different biocatalysts show a similar enrichment in lighter hydrocarbons as well as a similar hydrocarbon distribution. Since the data have been generated under identical experimental conditions using different microorganisms, the bioconversion of the biodegraded oils by BNL-NZ-3 may be more efficient. The corresponding gas chromatography and mass spectrometry analyses of organic sulfur compounds have been reported elsewhere.[3–5]

Table 6 shows the efficiency of organic sulfur removal by several different microorganisms on the same Monterey 851 biodegraded oil. It is to be noted that the changes in the sulfur concentration are due to the introduced microorganisms and not due to indigenous microorganisms responsible for the original formation of the heavy biodegraded oil.

The effect of induced bioconversion of this oil on four major fractions is shown in Table 7. While compared to the control (i.e., M851 untreated) the relative concentrations of saturates, aromatics, resins, and asphaltenes vary, the overall trend is an increase in saturates, a decrease in aromatics, and an increase in resins. Comparable results for OSC and MWS using two of the seven biocatalysts are shown in Table 8. It is to be noted that in most cases the concentration of asphaltenes decreases except in the BNL-NZ-3/M851, BNL-TH-31/M851, and BNL-4-22/OSC cases. Since this increase is very small relative to changes in other components, this may be within the experimental error or due to the initial extent of activation in the bioreactor or due to some chemistry peculiar to the particular biosystem used. Further studies will clarify these possibilities. Corresponding comparative analyses of the immature Venezuelan Boscan oil with the California immature OSC and steam-treated MWS crudes are given in Figures 4, 5, and 6. Consistent with previous results, bioconversion of these oils leads to an enrichment in lighter hydrocarbons and an overall redistribution of these hydrocarbons, as shown by the peak clusters as retention times of 20–25 minutes and 35–40 minutes. Corresponding chromatographic analyses of organic sulfur compounds using a

sulfur specific detector shown in Figures 7 and 8 show a decrease in the total signal, consistent with the analyses given in Table 1. OSC is a high sulfur content oil (4.4%), whereas MWS is much lower (1.1%) in sulfur. The overall lowering in concentration of organosulfur compounds and a concurrent redistribution of residual organic sulfur compounds is significant. These changes have been accomplished in a single pass per batch process. An important consideration in the application of the biochemical upgrading of heavy crudes is discussed elsewhere.[7]

Extension of the chemical marker analyses to major fractions of crude oils emphasized further the importance and the utility of the markers as diagnostic signals in process evaluation and the understanding of underlying mechanisms. Thus, pentane precipitation of asphaltenes showed that the heavy, nonvolatile asphaltenes residuum of OSC (see Fig. 9) and MWS (see Fig. 10) differ and both require pyrolysis gas chromatography to analyze their hydrocarbons. Relative increase in the more volatile components of the biochemical treatment with BNL-4-22 and BNL-4-23 is evident for both residues as shown in Figures 9a, 9b, 10a, and 10b. Corresponding sulfur-specific analyses, shown in Figures 11 and 12, indicate major changes in organosulfur composition. These analytical results also add further proof that the biochemical attack on crude oils does occur in the asphaltene fractions.

Nitrogen-specific analyses of the OSC and MWS crude are also consistent with data given in Table 2 and indicate a decrease in the content of organic nitrogen compounds as shown in Figures 13 and 14. Results of preliminary analyses of the pentane extracts of OSC and MWS for sulfur and nitrogen shown in Figures 15 and 16 further support the mechanism(s) which indicate that in the bioconversion of heavy crude oils, biochemical reactions occur in the heavy ends of crudes resulting in conversion, solubilization, concentration, and distribution of crude oil fractions containing organic sulfur and nitrogen compounds.

Conclusions

The use of chemical markers in the monitoring of the interactions between different microorganisms and various crude oils allows us to determine the efficiency of the biochemical conversion of the crudes. Concurrently, a database is generated which indicates that the biochemical mechanisms by which microorganisms interact with crude oils involve reactions at heteroatoms (i.e., N, S, O) and organometallic compounds and other active sites leading to:

1. Reduction in sulfur concentration (20%–45%)

2. Reduction in nitrogen concentration (15%–45%)

3. Reduction in trace metals concentration (16% –99%)

4. Conversion of heavy fraction of crudes into lighter fractions

5. The optimum reaction condition depends on both the microbial species used and the chemical composition of the oil.

Acknowledgments

This work is supported by the U.S. Department of Energy, Division of Fossil Fuels, under Contract No. AS-219-ECD, and Contract No. DE-AC02-76CH00016 with the U.S. Department of Energy. We wish to express our gratitude to Ernie Zuech of the U.S. DOE Bartlesville office for the supply of OSC oils, and to the Synfuels Research Corp. and the Santa Fe Energy Resources, Inc. of Houston for the supply of MWS oil. We also wish to acknowledge Mark McCaffrey of Chevron for the samples of M851, 836, and 837 oils, Yao Lin of Brookhaven National Laboratory for the graphic materials, and T. F. Yen and his group at the University of Southern California for the chromarod analyses and valuable discussions.

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Table 1 Variations in the Concentrations of Sulfur As a Function of Biocatalyst Treatment of Four Crudes: Boscan (BOS), Cerro Negro (CN), Midway Sunset Oil (MWS), and Offshore California (OSC)

Oil	Initial Concentration of S %	Treatment Biocatalyst	Reduction in Concentration of S %
BOS	5.49	BNL-4-22	25
CN	4.37	BNL-4-23	25
CN	4.37	BNL-4-24	29
MWS	1.1	BNL-4-23	50
OSC	4.4	BNL-4-23	45

Table 2 Variations in the Concentration of Nitrogen As a Function of Biocatalyst Treatment

Oil	Initial Concentration of N %	Treatment Biocatalyst	Reduction in Concentration of N %
MWS	0.79	BNL-4-22	25
MWS	0.79	BNL-4-23	15
OSC	0.66	BNL-4-22	20
OSC	0.66	BNL-4-23	45

Table 3 Variation in the Concentration of Nickel and Vanadium As a Function of Biocatalyst Treatment

Oil	Metal	Initial Concentration ppm	Treatment Biocatalyst	Reduction in Concentration of Metal %
CN	Ni	247	BNL-4-22	95
	V	494	BNL-4-22	99
CN	Ni	247	BNL-4-23	35
	V	494	BNL-4-23	58
MWS	Ni	63	BNL-4-22	19
	V	24	BNL-4-22	20
MWS	Ni	63	BNL-4-23	25
	V	24	BNL-4-23	36
OSC	Ni	80	BNL-4-22	28
	V	202	BNL-4-22	33
OSC	Ni	80	BNL-4-23	20
	V	202	BNL-4-23	16

Table 4 Variation of Nickel and Vanadium Contents in Cerro Negro Crude As a Function of Several Different Biocatalysts

Biocatalyst	Metal	Initial Concentration	Reduction in Concentration of Metal %
BNL-4-24	Ni	247	25
BNL-4-24	V	494	38
BNL-TH-29+	Ni	247	32
BNL-TH-31	V	494	57
BNL-2-45+	Ni	247	51
BNL-3-26	V	494	68

Table 5 Comparison of the Heavy Ends of Crudes

Oil	OSC*	MWS*	A836**	A837**	A851*	CN**	BOS**
Saturate %	17.3	19.2	10.3	12.8	19.2	11.7	10.7
Aromatic %	39.1	44.9	8.7	5.8	45.2	18.3	14.4
Resin %	37.4	35.3	25.0	30.4	38.9	45.0	34.8
Asphaltene %	6.20	2.6	56.0	51.0	4.4	25	40

* Analyzed by the chromarod method (10)
**Analyzed by solvent and column chromatography method (3, 9)

Table 6 Initial Total Organic Sulfur Contents and % Sulfur Removed after Biotreatment

	UTM 851	BNL-NZ-3	BNL-TH-31	BNL-TH-29	BNL-4-21	BNL-4-22	BNL-4-23	BNL-4-24
Total %	1.84	1.29	1.56	1.54	1.63	1.47	1.29	1.30
% S Removed	-	30	15	15	16	20	30	30

UTM 851 = Monterey 851 untreated control

Table 7 Determination of Four Major Fractions by TLC-FID Using Chromarods

	UTM 851	BNL-NZ-3	BNL-TH-31	BNL-TH-29	BNL-4-21	BNL-4-22	BNL-4-23	BNL-4-24
Saturate %	19.19	23.62	22.01	24.72	32.29	28.72	34.42	29.21
Aromatic %	45.15	31.64	35.27	28.62	32.04	33.87	29.72	19.59
Resin %	31.23	38.94	37.88	43.44	32.00	33.31	32.71	38.20
Asphaltene %	4.44	5.79	4.84	3.41	3.67	4.09	3.57	2.99

UTM 851 = Monterey 851 untreated control

Table 8 Determination of Four Major Fractions by TLC-FID Using Chromarods

	Control untreated OSC	BNL 4-22 treated OSC	BNL 4-23 treated OSC	Control untreated MWS	BNL 4-22 treated MWS	BNL 4-23 treated MWS
Saturate %	17.3	45.5	51.6	19.2	33.7	66.3
Aromatic %	39.1	18.0	20.5	44.9	29.1	11.2
Resin %	37.4	30.1	22.3	35.3	34.2	19.3
Asphaltene %	6.20	6.43	5.65	2.60	2.97	3.18

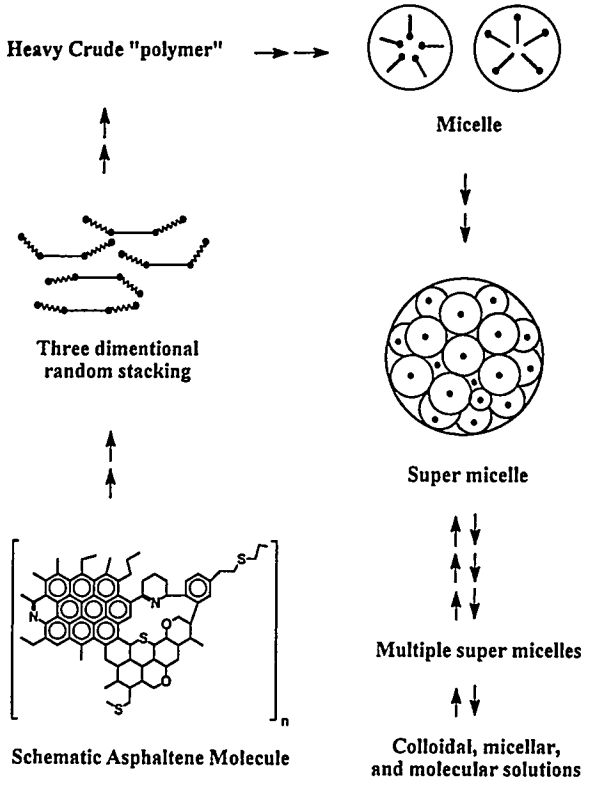

Figure 1 Formation of Heavy Oil Fractions from a Schematic Asphaltene Molecule (After 8 and 12). Dark Circles Represent Heteroatoms and Active Sites.

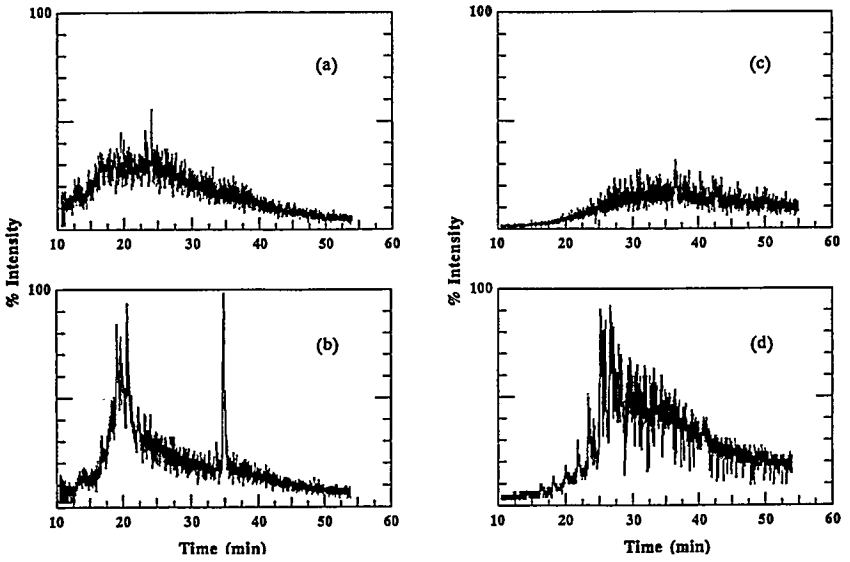

Figure 2 Gas Chromatogram, M/e 57 Trace, of (a) Control M851 Crude, (b) M851 Treated with BNL-NZ-3, (c) Control Cerro Negro Crude Oil, (d) Cerro Negro Crude Treated with BNL-NZ-3

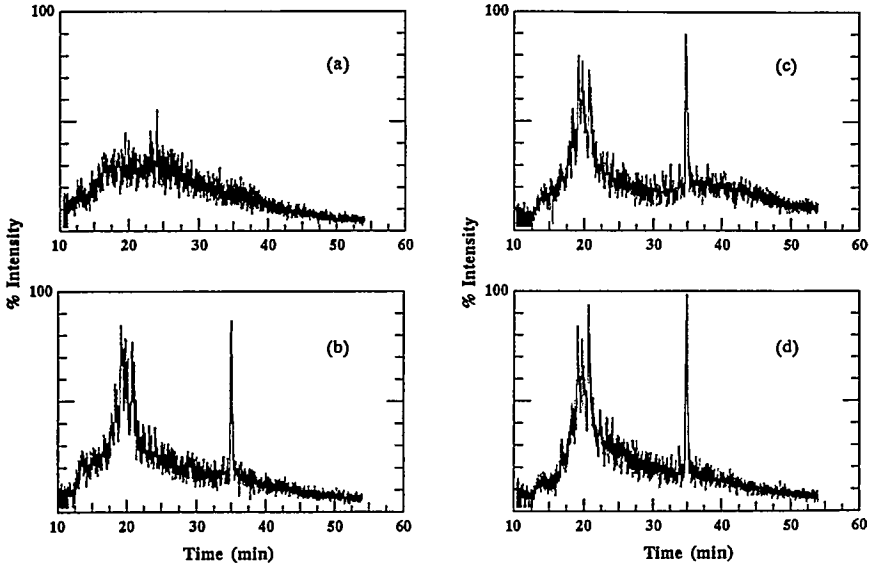

Figure 3 M851 M/e 57 Gas Chromatogram Trace of (a) Control, (b) Treated with BNL-4-22, (c) Treated with BNL-4-23, (d) Treated with BNL-NZ-3

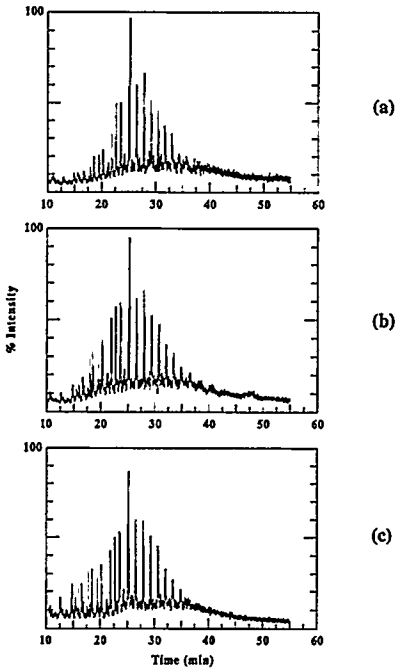

Figure 4 Boscan M/e 57 Pyrolysis Gas Chromatogram Trace of (a) Treated with BNL-NZ-3, (b) Treated with BNL-4-22, (c) Control

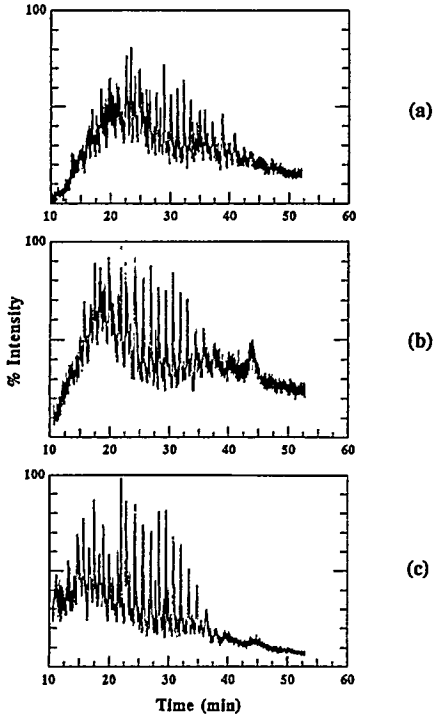

Figure 5 OSC M/e 57 Gas Chromatogram Trace of (a) Treated with BNL-4-22, (b) Treated with BNL-4-23, (c) OSC Control

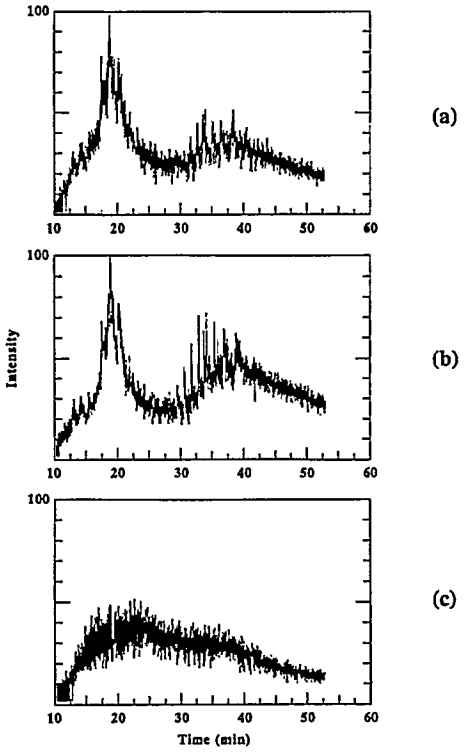

Figure 6 MWS M/e 57 Gas Chromatogram Trace of (a) Treated with BNL-4-22, (b) Treated with BNL-4-23, (c) MWS Control

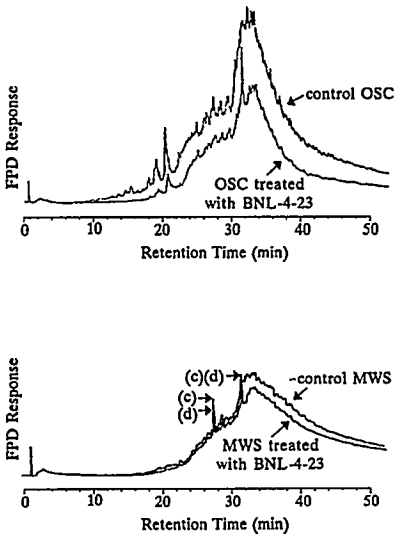

Figure 7 Sulfur Specific Trace (FPD) Chromatogram of (a) Control OSC, (b) OSC Treated with BNL-4-23, (c) Control MWS, (d) MWS Treated with BNL-4-23

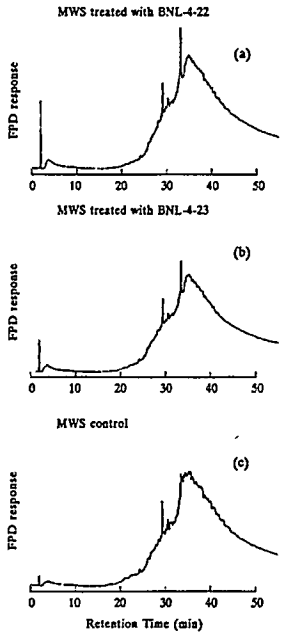

Figure 8 Sulfur Specific Trace (FPD) Chromatogram: (a) MWS Treated with BNL-4-22, (b) MWS Treated with BNL-4-23, (c) MWS Control

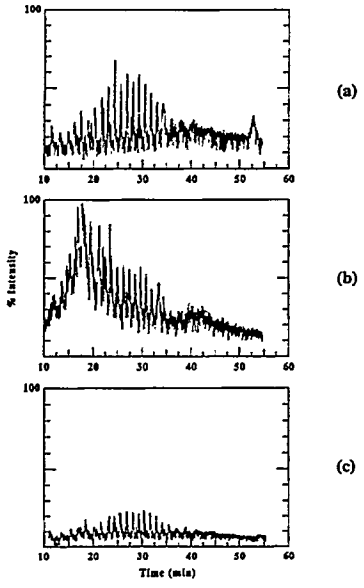

Figure 9 OSC M/e 57 Pyrolysis Gas Chromatogram of Asphaltenes: (a) Treated with BNL-4-23, (b) Treated with BNL-4-22, (c) Control

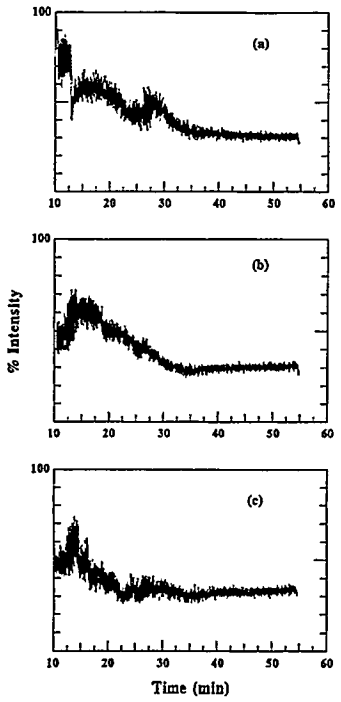

Figure 10 MWS M/e 57 Pyrolysis Gas Chromatogram of Asphaltene: (a) Treated with BNL-4-23, (b) Treated with BNL-4-22, (c) Control

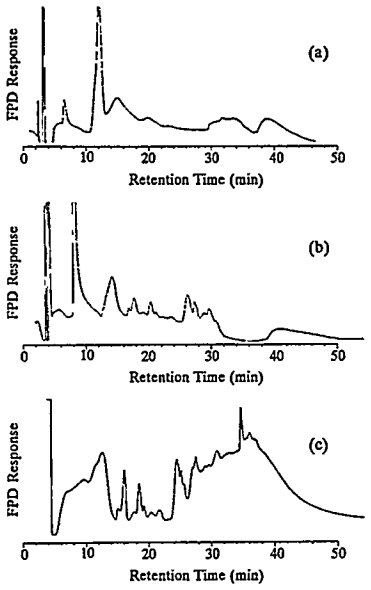

Figure 11 Pyrolysis Gas Chromatogram of Asphaltene of OSC Crude FPD Sulfur Specific Trace: (a) Treated with BNL-4-23, (b) Treated with BNL-4-22, (c) Control OSC

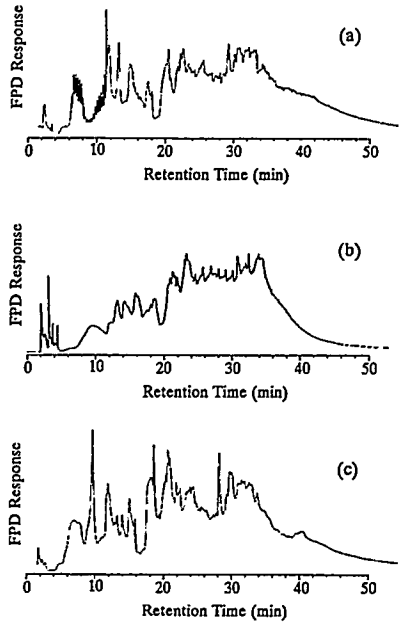

Figure 12 Pyrolysis Gas Chromatogram of Asphaltene of MWS Crude FPD Sulfur Specific Trace: (a) Treated with BNL-4-23, (b) Treated with BNL-4-22, (c) Control

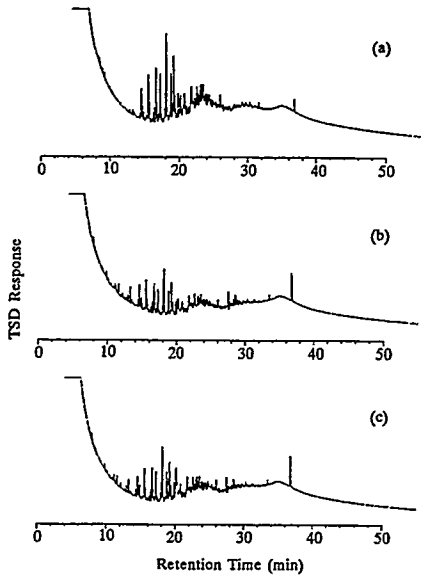

Figure 13 Nitrogen Specific Detector (Thermoionic Specific Detector, TSD) Chromatogram of (a) OSC Whole Oil Control, (b) OSC Treated with BNL-4-22 (c) OSC Treated with BNL-4-23

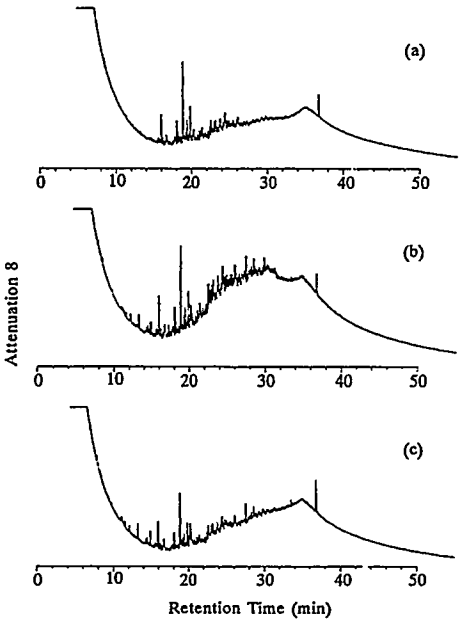

Figure 14 Nitrogen Specific Detector Chromatogram of (a) MWS Whole Oil Control, (b) MWS Treated with BNL-4-22, (c) MWS Treated with BNL-4-23

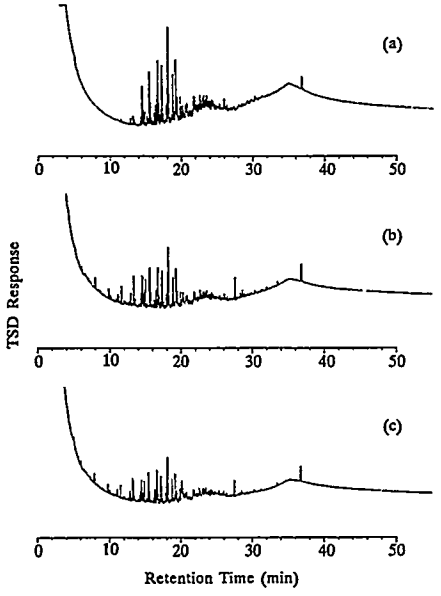

Figure 15 Nitrogen Specific Detector Chromatogram of Pentane Extract OSC, (a) Control, (b) Treated with BNL-4-22, (c) Treated with BNL-4-23

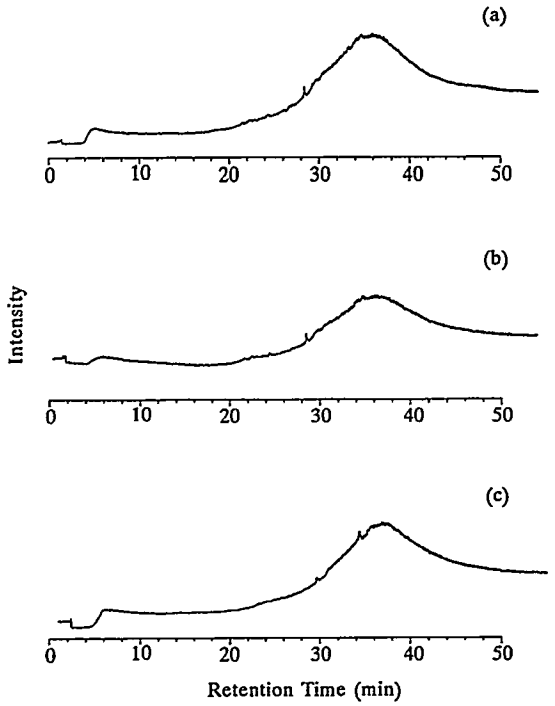

Figure 16 Sulfur Specific Detector Chromatogram of Pentane Extract MWS, (a) Control MWS, (b) BNL-4-22, (c) BNL-4-23 Treated MWS

Mineralization of a Malaysian Crude Oil by *Pseudomonas* sp. and *Achromobacter* sp. Isolated from Coastal Waters

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Abstract

Regarded as being a potentially effective tool to combat oil pollution, bioremediation involves mineralization, i.e., the conversion of complex hydrocarbons into harmless CO_2 and water by action of microorganisms. In practice, however, determination of the conversion by each of the oil degraders can be difficult in commercially available products as they consist of a mixture of microorganisms. Therefore, in achieving optimum effectiveness from the application of these products on crude oil in local environments, the capability of the bacteria to mineralize hydrocarbons was evaluated.

The microbial laboratory testing of mineralization on local oil degraders involved, first, isolation of bacteria found at a port located on the west coast of Peninsular Malaysia. Subsequently, these bacteria were identified by means of Biomereux's API 20E and 20 NE systems and later screened by their growth on a Malaysian crude oil. Selected strains of *Pseudomonas* sp. and *Achromobacter* sp. were then exposed individually to a similar crude oil in a mineralization unit and monitored for 16 days for release of CO_2. *Pseudomonas paucimobilis* was found to produce more CO_2 than *Achromobacter* sp. When tested under similar conditions, mixed populations of these two taxa produced more CO_2 than that produced by any individual strain. Effective bioremediation of local crude in Malaysian waters can therefore be achieved from biochemically developed *Pseudomonas* sp. strains.

Introduction

Biodegradation of crude oils by indigenous marine bacteria in Malaysian waters has not been comprehensively investigated. Apart from the work of Mahadi et al.[1] on the fate of crude oils in the marine ecosystem and the overall biodegradation in the west coast of East Malaysia, there was no work reported on mineralization of crude oil by marine bacteria in the coastal waters of West (Peninsular) Malaysia.

The use of commercially available products comprising mixed consortia of oil degrader strains has made the mitigation of oil spills in the ocean more convenient. However, not all applications have solved the problems, particularly when the strains were sourced from different part of the world and applied in different countries having different temperatures, nutrient requirements, etc. Consequently, this is becoming an important aspect of biodegradation research, especially when indigenous strains that degrade crude oils can be identified and be further developed.

This paper attempts to evaluate the relative mineralization capabilities (i.e., the complete conversion of oil into CO_2, water, and biomass) of indigenous marine bacteria isolated in Malaysian coastal water on a local crude oil. The investigation involved laboratory monitoring of CO_2 released under conditions similar to those found in the ocean.

Methods

Seawater at a jetty of a port located at the west coast of Peninsular Malaysia was sampled at two different depths (the surface and 1 m below the surface) by means of a T-joint sampler (Kahlsico J3 Bacteriological, USA). The port has had a history of pollution due to inevitable spillages occurring during routine operations throughout the years. It was assumed that based on previous investigations of Atlas and Bartha[3] and Zobell,[4] that the site was suitable for collecting hydrocarbon-utilizing microorganisms.

The characteristics of the seawater (temperature, pH, dissolved oxygen [DO], conductivity, oxidation-reduction potential, and salinity) at these two depths were measured in situ using Hydrolab equipment (Hydrolab Env. Data System, Sonde Unit, USA).

Two different media, unmodified and modified, were prepared using Difco Bacto Marine Agar 2216 for the purpose of isolation and enumeration. The unmodified marine agar, prepared by following the manufacturer's instructions, was used for

growth of heterotrophic marine bacteria (nondegraders); the modified media consisted of 0.5% crude oil and 0.003% phenol red[2] for determining growth and plate count of crude oil degraders. Inoculation of samples was performed through serial dilutions followed by incubation at 30°C for a minimum of 24 hrs.

The identification of marine bacteria strains was limited to gram negative rods, enteric, and nonenteric bacteria. Biomereux's API 20NE (nonenteric) was used for identification of 12 distinct colonies from both media, and this was followed with API 20E (enteric) for doubtful identification from the former. The species with the highest level of confidence during identification were chosen, individually or mixed, for the next step of mineralization.

The setup for the mineralization unit followed that of Atlas and Bartha,[3] comprising a rotating water bath (Grant SS40-D, Cambridge) that contained five 250 ml Erlenmeyer flasks, each connected with a tube filled with 50 ml of 0.1 M KOH for inlet flow of CO_2-free air, and with two similar tubes for outlet air flow as a result of mineralization. The flasks contained 100 ml of filtered (Millipore 0.22 μm filter membrane) seawater, 1 ml bacterial suspension, and 1 ml of crude oil. The water bath was set at 200 rpm with temperature similar of the seawater measured in situ (30°C). The CO_2-free air was introduced at 15 cc/ml into the flask.

The individual bacterial suspension was prepared by inoculating 1 loopful of *Achromobacter* sp. and *Pseudomonas* sp. colonies into 10 ml Marine broth 2216. After incubation at 35°C for approximately 48 hrs, the grown colonies were centrifuged (Hettich Rotanta/P, Germany) at 4,000 rpm for 15 minutes. The precipitated colonies were rinsed twice with Ringer's solution before being introduced into the flasks.

The released CO_2 from the flasks, arranged in duplicates, was absorbed in the tubes containing KOH solution. Periodically (24 hr intervals), the first tube was taken out for titration and replaced by the second. Consequently, a new tube containing 50 ml of 0.1 M KOH solution was attached in tandem of the second tube, and the determination of CO_2 was carried out for 15 days. On day 15, 1 ml 0.1 M sulfuric acid was added into each flask to decompose the inorganic carbonate and to release trapped CO_2.

The number of moles of CO_2 released, n, can be determined by Equation 1:

$$n = 2.5 \times 10^{-3} - 0.1V_h \quad (1)$$

where V_h = volume of H_2SO_4 required for titration (liter).

The detailed calculations arriving at Equation 1 are shown in the Appendix.

Results and Discussion

Distribution of Marine Bacteria

The distribution of heterotrophic marine bacteria (nondegraders) was relatively similar at both the surface layer and 1 m below the surface, whereby the population ranged from 1.5×10^7 to 2.0×10^7 cfu/ml.

The presence of oil degraders, however, was more dominant (1.7×10^{10} cfu/ml) and with concentration of 1,000 times greater than nondegraders at the surface. However, concentration of degraders at the surface layer was more than that in the deeper water column with a population of 1.0×10^6 cfu/ml.

Comparing the distribution of hydrocarbon degraders alone will indicate that degraders were present as much as 10,000 times more at the surface layer than at 1 m depth, which is more than the range (between 10 and 100 times more) reported by Crow et al.[5] This may reflect the distribution of hydrocarbon-utilizing bacteria in a tropical marine ecology of a site which has been burdened by organic contaminations over an extended period.

With the exception of pH and DO, the values for temperature, conductivity, and salinity measured by the Hydrolab were similar at the surface and 1 m depth. The temperature was 29.8°C, conductivity 47.5 mmhos/cm, and salinity 30.9 parts per thousand. The high population of degraders at the surface may be attributable to lower DO (4.49 mg/l) and higher pH (7.50) compared to the deeper location (4.70 mg/l and 7.43, respectively) as well as other factors not investigated in this study.

Identification of Degraders and Nondegraders

Twelve different colonies grown in modified and nonmodified Marine Agar 2216 were chosen based on morphology and color (white, yellow, and orange) for identification with API 20NE and API 20E.

Seven strains (i.e., 4 nondegraders and 3 degraders) were identified (with at least a good level of confidence) as *Achromobacter CDC gr. VD.*, *Pseudomonas paucimobilis*, *Pseudomonas mesophilica*, *Vibrio damsela*, and *Agrobacter radiobacter* (see Table 1). The remaining strains were identified by means of API 20E, but none were any identifiable strains.

API 20NE yielded similar results of *Achromobacter* sp. for white colonies from both nondegraders and degraders sampled at the surface and 1 m depth. Similarly,

the identification system identified *P. paucimobilis* for degraders isolated at both the surface and 1 m below.

P. paucimobilis, *P. mesophilica,* and *Achromobacter* sp. are known hydrocarbon degraders in the marine environment.[6,7,8,9] Since they were identified to present as both degraders and nondegraders in this study, they were chosen to be exposed to crude oil either individually or in a mixed ratio for mineralization.

Mineralization

Two stages of mineralization were each conducted for a duration of 16 days. The first stage comprised the runs of individual strains: *Achromobacter* sp. (degrader and nondegrader), *P. mesophilica* (degrader), and *P. paucimobilis* (nondegrader). Subsequent evaluation of mineralization comprised the mixtures of *Pseudomonas* spp. and *Achromobacter* spp. (i.e., degraders and nondegraders) by equal suspension volumes of two selected strains.

Individual Strains. Rates of CO_2 release by *Achromobacter* sp. (degrader) and *P. mesophilica* (degrader) were about equal until day 14, when a significant difference was observed. The CO_2 production was increasing in stages where the peak of gas release was higher for the degrader at 0.76 mM of CO_2 compared to the nondegrader at 0.72 mM (see Fig. 1).

In another individual run where *Achromobacter* sp. (nondegrader) and *P. paucimobilis* (nondegrader) were compared, the CO_2 production was generally observed at a decreasing rate until day 11, when a significant increase of gas releases was noted (see Fig. 2). In general, the degrader yielded a higher rate of CO_2 than the nondegrader throughout the mineralization period and achieved 0.84 mM CO_2 or 5% higher than *Achromobacter* sp.

For the duration of mineralization period, the total production of CO_2 was highest for *P. paucimobilis* (nondegrader) at 9.35 mM, followed by, in decreasing order, *Achromobacter* sp. (nondegrader) at 8.97 mM, *P. mesophilica* (degrader) at 8.93 mM, and *Achromobacter* sp. (degrader) at 8.72 mM.

Although the values for total release of gas for individual runs were quite close, they provide useful indicators for evaluating the relative performance of crude oil mineralization. Based on the above observations, nondegraders that were allowed to create their own artificial microbial ecology could degrade crude oil and were monitored to release more CO_2 than those achieved by the degraders isolated from modified marine agar. Similar observations were reported by Buckley et al.[10] for microorganisms isolated in an estuary.

Mixed Strains. The individual microbial ecology was diversified by the addition of another strain so that the resulting mineralization performance of mixed strains could be monitored. Dual strains comprising a degrader and a nondegrader were chosen with similar operational characteristics as mentioned previously. Equal bacterial suspensions of *P. paucimobilis* (nondegrader) and *Achromobacter* sp. (degrader) were introduced in one mineralization flask while another contained *P. mesophilica* (degrader) and *Achromobacter* sp. (nondegrader).

Both mixed strains yielded a steady increase of CO_2 production, but the combined strains of *P. mesophilica* and *Achromobacter* sp. produced a greater amount of CO_2 than the other combination after day 10. However, at the end of the mineralization run, both mixed strains were found to yield similar CO_2 at 0.72 mM (see Fig. 3).

The maximum daily CO_2 release for both combined strains was lower than their individual productions. For example, *P. paucimobilis* produced a maximum of 0.84 mM of CO_2, and *Achromobacter* sp. (degrader) produced 0.72 mM during the individual runs; however, a combination of equal volumes of these strains only yielded 0.72 mM. Commensalism between the two strains affected mineralization mechanisms and resulted in a low yield in gas release.

However, the highest achievable total CO_2 production for the 16-day mineralization from the combined strains was either similar or greater than those from their individual productions. The total CO_2 was 9.35 mM from the mixture of *P. paucimobilis* and *Achromobacter* sp. (degrader), whereas total CO_2 was 9.12 mM from *P. mesophilica* and *Achromobacter* (nondegrader).

Conclusions

Distribution of hydrocarbon degraders was 1,000 times greater at the ocean surface layer than nondegraders sampled at a site having a history of some pollution. Identification of bacterial strains by means of API 20NE and API 20E only yielded seven acceptable species from 12 isolated strains. The performance of individual strains can be ranked, in decreasing order, based on total production of CO_2: *P. paucimobilis* (nondegrader) > *Achromobacter* sp. (nondegrader) > *P. mesophilica* (degrader) > *Achromobacter* sp. (degrader). Combined mixtures of dual strains produced more CO_2 than that produced by their individual strains.

Recommendations

Effective bioremediation for the purpose of mitigating oil spill occurrences usually incorporates optimization of the performance of known hydrocarbon degrader strains by biochemical means. The isolated *P. paucimobilis* can be further developed biochemically by means of recombinant DNA technology and at the same time, known methods of addition of nitrogen and phosphorus should be attempted for both individual and mixed consortia of degrader strains.

Acknowledgment

The authors thank Suhaimi Omar, formerly at the Universiti Teknologi Malaysia, Kuala Lumpur, for his technical assistance in the study.

Appendix: Determination of CO_2 Released during Mineralization

CO_2, a byproduct of mineralization, is absorbed by the KOH solution contained in the tube connected to the mineralization flask through the reaction shown in Equation 2:

$$2KOH + CO_2 \rightarrow K_2CO_3 + H_2O \quad (2)$$

Upon titrating the solution by sulfuric acid, the products of titration are shown in Equation 3:

$$2KOH + H_2SO_4 \rightarrow K_2SO_4 + 2H_2O \quad (3)$$

From Equation 3, the number of moles of KOH that reacts with CO_2 is:

initial number of moles of KOH – moles of excess KOH

$$= [\,(50/100) \times 0.1\,] - [\,0.2 \times V_h\,]$$

$$= 5.0 \times 10^{-3} - 0.2\,V_h \quad (4)$$

where V_h = volume of H_2SO_4 required for titration (liter).

Since, from Equation 2, where 1 M of CO_2 requires 2 M of KOH, then from Equation 4:

The number of moles of CO_2 that is produced by degrader/ nondegrader

$$= [5.0 \times 10^{-3} - 0.2\ V_h] \times 0.5$$

$$= 2.5 \times 10^{-3} - 0.1 V_h$$

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Table 1 Identification of Marine Bacteria by Means of API 20NE

Depth	Degrader/ Nondegrader	Colony Color	API 20NE Identification Results		
			Species Identified	% Identification	Identification Profile
Surface	Nondegrader	White	*Achromobacter CDC. group VD.*	91.6	Good Identification
	Nondegrader	Yellow	*Pseudomonas paucimobilis*	99.7	Very Good Identification
	Degrader	White	*Achromobacter CDC group VD.*	96.9	Good Identification
	Degrader	White	*Pseudomonas mesophilica*	99.5	Very Good Identification
1m	Nondegrader	Orange	*Pseudomonas paucimobilis*	99.7	Very Good Identification
	Nondegrader	White	*Vibrio damsela*	99.5	Very Good Identification
	Nondegrader	Yellow	*Agrobacter Radiobacter*	99.4	Good Identification

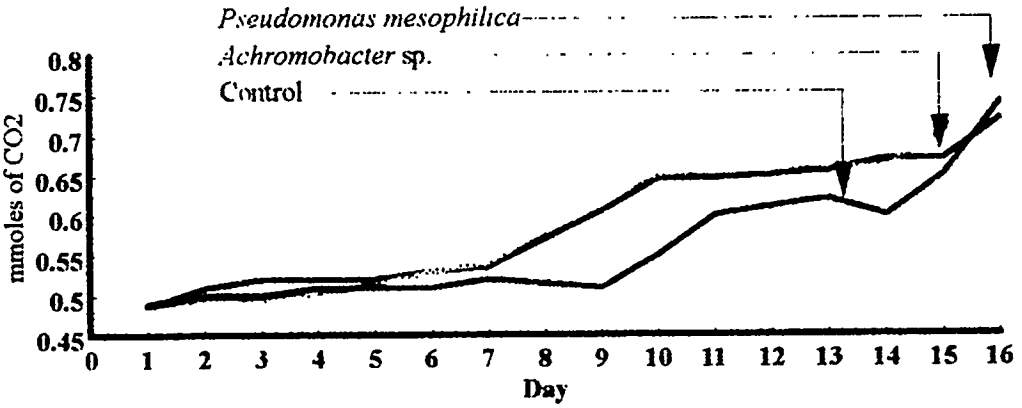

Figure 1 Mineralization Plot of *Archromobacter* sp. (Degrader) and *P. mesophilica* (Degrader)

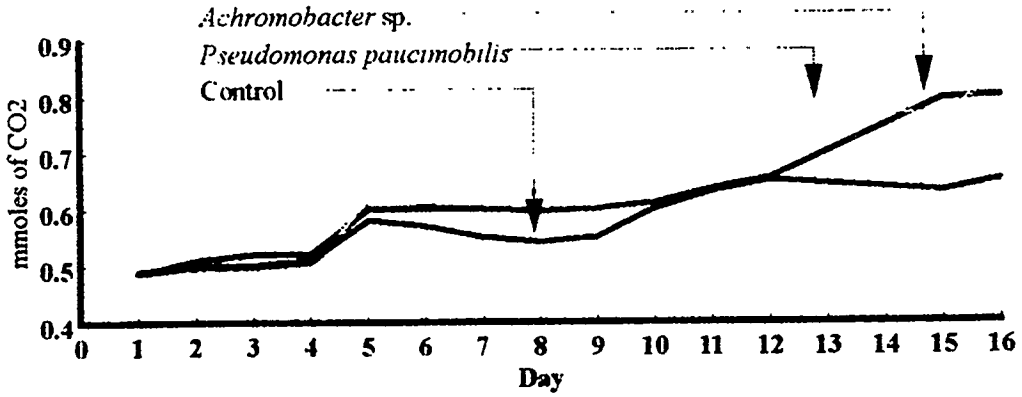

Figure 2 Mineralization Plot of *Archromobacter* sp. (Nondegrader) and *P. paucimobilis* (Nondegrader)

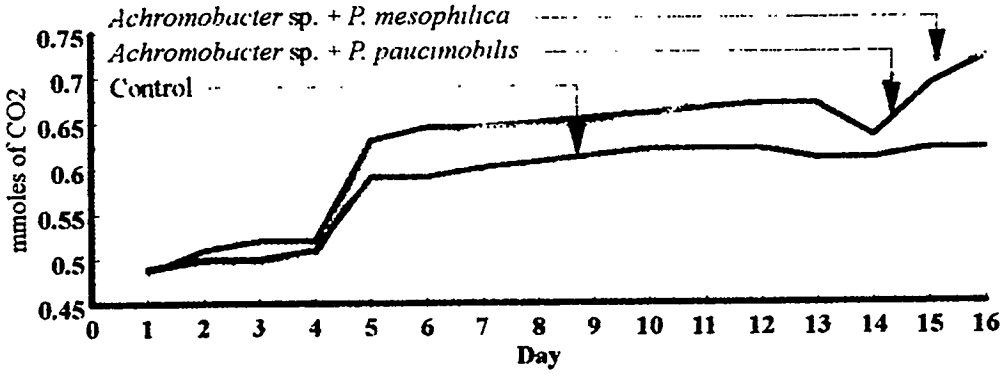

Figure 3 Mineralization Plot of *Archromobacter* sp. (Degrader) and *P. paucimobilis* (Nondegrader), and *Achromobacter* sp. (Nondegrader) and *P. mesophilica* (Degrader)

Assessment of Microorganisms from Indonesian Oil Fields

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Abstract

Petroleum resources have been the mainstay of the national development in Indonesia. However, resources are being depleted after over a century of exploitation, while the demand continues to grow with the rapid economic development of the country.

In facing the problem, EOR has been applied in Indonesia, such as the steamflooding project in Duri field, but a more energy efficient technology would be preferable. Therefore, MEOR has been recommended as a promising solution.

Our study, aimed at finding indigenous microorganisms which can be developed for application in MEOR, has isolated microbes from some oil fields of Indonesia. These microorganisms have been identified, their activities studied, and the effects of their metabolisms examined.

This paper describes the research carried out by LEMIGAS in this respect, giving details on the methods of sampling, incubation, identification, and activation of the microbes as well as tests on the effects of their metabolites, with particular attention to those with potential for application in MEOR.

Introduction

Crude oil is a strategic commodity which has important roles in the economics and national development of Indonesia, both as a source of energy for domestic consumption and as an export commodity or a source of foreign exchange.

In Indonesia, oil has been produced since 1885, when Telaga Tunggal well in North Sumatra started producing. Currently (1994), Indonesia is still producing oil at the rate of 1,534,000 bbl/D.

Indonesia has 60 sedimentary basins, located onshore and offshore of the 17,508 islands of the archipelago. Thirty-six basins have been explored; of which 14 produce oil and/or gas, 7 have proven to bear hydrocarbons, and 15 have had no discoveries. Twenty-four basins have yet to be explored.

The oil resource potential of Indonesia is currently estimated to be 48.40 billion bbl, of which 10.92 billion bbl is proven reserves, 32.52 billion bbl is reserves, and 4.96 billion bbl is potential reserves.

The average rate of recovery is rather low (35.4%), and this encourages the application of EOR techniques to improve recovery. One such technique, namely steamflooding, has been applied in Duri oil field in Central Sumatra.

Prior to the application of the technique, Duri oil field was producing at the rate of 60,000 bbl/D and a recovery of 8%. With the application of the technique, oil recovery has increased to 55% and oil produced to 300,000 bbl/D. However, 18% of the oil produced has to be consumed in situ to generate the needed steam.

With such high energy consumption, it is logical that we need better, more energy efficient, and more environmentally friendly techniques for enhancing oil recovery in the many other oil fields.

MEOR is one of the more potentially attractive techniques, particularly considering the large biodiversity in a tropical country such as Indonesia. We expect to find many varieties of species adaptable to an oil-bearing environment.

With the support of the PERTAMINA Research Grant Programme, LEMIGAS Research and Development Centre for Oil and Gas Technology has initiated a study on the collection, identification, and characterization of microbes found in the Indonesian oil environment for future development of their potential usefulness in the industry.

In this study we isolate, identify, determine, characterize, and make an inventory of "indigenous" Indonesian microorganisms. The specific objective of the present paper is to describe the methods of sampling, isolation, identification, and activation of the microbes, as well as tests on the effects of their metabolites, with particular attention to those with potential for application in MEOR.

Methodology

Samples of crude oils and formation waters were collected, as well as soil from 17 oil fields in five geographical areas (Cepu, Cirebon, Rantau, Prabumulih, and Jambi). These oil fields were selected to represent variations in geographical areas (Central Java, West Java, and Sumatra), reservoir rock types (carbonate and silicate), water formation salinity (low and high), and hydrocarbon types (paraffinic and asphaltic).

Liquid samples of oils and formation waters were drawn at the wellhead. They were first made to flow for a moment to clear the line. The samples were then collected in two 1-liter sterilized sample bottles filled to the top to reduce the effect of light and oxygen. They were transported in dark bags at room temperature.

Solid samples of soil were taken at several sampling points in the vicinity of the wellheads at shallow depths (1–2 m). Some 250–500 g of soil samples were taken from each sampling point and collected in plastic bags. Further handling and transportation were undertaken in a similar manner as the liquid samples.

Six wells were taken as the sampling points in Cepu, three in Cirebon, five in Rantau, six in Prabumulih, and eight in Jambi. Field data, including well depth, reservoir rock, temperature and pressure of the formation, oil type, water content of the reservoir fluid, salinity of formation water, and surface temperature were recorded.

Each sample was tested for physical properties and analyzed for its chemical characteristics and composition to provide an inventory of parameters related to the living environment of the microbes.

Microbiological analysis was effected by first doing the population count by the plate-count method at 30°C (in nutrient medium) and 55°C (in tryptone dextrose medium), followed by isolation, identification, and determination of selected colonies. Identification and determination were carried out on each isolate so that their genus and species could be recorded.

This was followed by activity tests at room temperature (30°C) and higher temperature (55°C) in nonhydrocarbon as well as in hydrocarbon media, which were conducted in a shaking incubator. The aim of this test was to know the isolates' capabilities in using the hydrocarbon as their carbon source. The active isolates were those that had a higher growth coefficient in hydrocarbon medium than in nonhydrocarbon. Nutrient medium and proteose peptone glucose ammonium salt (PPGAS) were used at 30°C, whereas tryptone dextrose and PPGAS were used at 55°C. For tests in the hydrocarbon environment, 1% crude oil was added to the media.

Specific tests on bioacid and biosurfactant producing capabilities were conducted in incubation media mixed with 50% crude oil. The effect on pH reduction and interfacial tension was measured. A processing tensiometer and a spinning drop tensiometer were used for interfacial tension measurement.

Results and Discussion

Identification and determination were conducted on isolates prepared from samples of formation waters, crude oils, and soils taken from oil fields in the Cepu, Cirebon, and Rantau areas. The isolates from Prabumulih and Jambi have not been identified and determined. Isolation of mesophilic microbes was made after incubation at 30°C and of thermophilic microbes at 55°C.

Identification and determination of isolates obtained at 30°C revealed the presence of microbes of the genus *Staphylococcus, Corynebacterium, Pseudomonas, Flavobacterium, Bacillus,* and *Enterobacter* in the samples from Cepu; *Corynebacterium, Pseudomonas, Flavobacterium, Bacillus, Neisseria, Staphylococcus, Alcaligenes,* and *Actinobacillus* from Cirebon; and *Corynebacterium, Flavobacterium, Bacillus, Enterobacter, Neisseria, Hafniae, Staphylococcus*, and *Chromobacterium* from the Rantau area (see Table 1).

Thermophilic microbes obtained at 55°C (see Table 2) are of *Bacillus* genus in Cepu, *Bacillus* and *Corynebacterium* in Cirebon, and *Bacillus* in Rantau. These were all obtained from formation water samples. It was also confirmed that more thermophilic microbes were obtained from oil fields in Cirebon, where the reservoir temperatures are quite high (up to 152°C) in comparison with the other oil fields.

Each species of the microbes was tested for its adaptability to hydrocarbon environment. Table 3 listed the microbes which showed higher growth rate in hydrocarbon medium at 30°C incubation: *S. epidermidis*, *Staphylococcus* sp., *C. pseudodiptheriticum, Corynebacterium* sp., *Pseudomonas* sp., *Flavobacterium* sp., *B. latherosporus, B. coagulans, Bacillus* sp., *Neisseria* sp., *Ch. lividum,* and *Al.*

faecalis. At 55°C, the microbes showing higher growth rates were *B. coagulans* and *B. licheniformis.*

The test result on the effect of the metabolism of the microbes on the pH of the media before and after a 24-hour incubation of the microbes at 30°C (20 species) and at 55°C (4 species) are presented in Table 4. One species of the isolates incubated at 30°C in the nutrient medium was found to have a slight reducing effect on the pH, but in the PPGAS medium there were five isolates that give a clear indication of pH reduction. All species that were tested at 55°C in the PPGAS medium had a reducing effect on the pH.

The test results on the effect of the metabolism of the microbes on the interfacial tension between the media and the oil are presented in Figures 1 to 4. Some of these results have been submitted for reporting in the 22nd Convention of the Indonesian Petroleum Association scheduled for October 1995. All species tested, except *A. faecalis,* had a reducing effect on the oil-medium interfacial tension, as observed after 5 days incubation at 30°C in the nutrient medium, whereas no exception was detected in the PPGAS medium. Incubation at 55°C in the PPGAS medium showed similar results, with *C. pseudodiphtheriticum* being the exception, whereas in the tryptone dextrose medium *B. coagulans* was the exception.

Reduction in interfacial tension indicated that the microbes biologically produced a surface active agent, and reduction of pH indicated that the microbes produced an acidic agent. But the results also show that the metabolites produced by the microbes are also dependent on the medium and environment of its growth.

Conclusion

We isolated and identified microbes of *Actinobacillus, Alcaligenes, Bacillus, Chromobacterium, Corynebacterium, Enterobacter, Flavobacterium, Hafnia, Neisseria, Pseudomonas,* and *Staphylococcus* genera present in liquid and solid samples of oil field environments in Indonesia.

Of these, *S. epidermidis, Staphylococcus* sp., *C. pseudodiphtheriticum, Corynebacterium* sp., *Pseudomonas* sp., *Flavobacterium* sp., *B. latherosporus, B. coagulans, Bacillus* sp., *Neisseria* sp., *Ch. lividum,* and *Al. faecalis* had higher growth rates in the hydrocarbon medium at room temperature. At the higher temperature (55°C), *B. coagulans* and *B. licheniformis* had higher growth rates.

Bacillus latherosporus reduced pH at 30°C in the nutrient medium. *Staphylococcus* sp., *C. pseudodiphtheriticum, Corynebacterium* sp., and *Flavobacterium* sp. reduced pH also at 30°C incubation but in the PPGAS medium. Similarly, *B.*

coagulans, B. alvei, and *B. licheniformis* reduced pH at 55°C incubation in the PPGAS medium.

S. epidermidis, P. aeruginosa, C. pseudodiphtheriticum, B. laterosporus, B. coagulans, and *Flavobacterium* sp. reduced oil/medium interfacial tension at 30°C incubation in both the nutrient and PPGAS media.

Similarly, *B. alvei* and *B. licheniformis* reduced oil/medium interfacial tension at 55°C incubation in the tryptone dextrose and PPGAS media. *A. faecalis* departed from the trends in the nutrient medium at 30°C; at 55°C, *B. coagulans* and *C. pseudodiphtheriticum* showed the same discrepancy in the tryptone dextrose and PPGAS media, respectively.

Those with promising MEOR effects will be improved and developed by application of appropriate biotechnology techniques to produce microbes with superior MEOR performance from indigenous Indonesian microorganisms. They will be thermophilic microbes which will be able to feed on in-situ hydrocarbons as their source of carbon and which will need only additional nitrogen as well as other elements from outside sources to increase their bioagent-production capacities.

Acknowledgments

The authors thank PERTAMINA management and staff for their support through its Research Grant Programme. Thanks are also due to the management and staff of LEMIGAS, particularly the members of LEMIGAS Biotechnology Group, Microbiology Laboratory, and Research Grant Team.

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Table 1 Results of Identification and Determination of Isolates Obtained at 30°C

Area	Code	Source	Field	Genus/Species
CEPU	GAN-1	Form. water	Nglobo	*Staphylococcus sp.*
	GAL-1	Form. water	Ledok	*Staphylococcus epidermidis*
	GAK-1	Form. water	Kawengan	*Corynebacterium sp.*
	GAK-2	Form. water	Kawengan	*Staphylococcus sp.*
	GAK-1	Crude Oil	Kawengan	*Psuedomonas aeruginosa*
	GMN-1	Crude Oil	Nglobo	*Flavobacterium sp.*
	GMN-2	Crude Oil	Nglobo	*Bacillus sp.*
	GMN-3	Crude Oil	Nglobo	*Enterobacter sp.*
CIREBON	GAJ-1	Form. water	Jatibarang	*Corynebacterium pseudodiphtheriticum*
	GAT-1	Form. water	Tugubarat	*Alcaligenes faecalis*
	GAC-1	Form. water	Cemara	*Staphylococcus sp.*
	GAC-2	Form. water	Cemara	*Bacillus sp.*
	GAC-3	Form. water	Cemara	*Flavobacterium sp.*
	GAC-1	Form. water	Cemara	*Flavobacterium sp.*
	GAC-2	Form. water	Cemara	*Bacillus sp.*
	GAC-3	Form. water	Cemara	*Flavobacterium sp.*
	GMC-1	Crude Oil	Cemara	*Neisseria sp.*
	GMT-1	Crude Oil	Tugubarat	*Neisseria sp.*

Table 1 Results of Identification and Determination of Isolates Obtained at 30°C (Continued)

Area	Code	Source	Field	Genus/Species
CIREBON	GMJ-1	Crude Oil	Jatibarang	*Actinobacillus sp.*
	GTJ-1	Soil	Jatibarang	*Pseudomonas sp.*
RANTAU	GAR-1	Form. water	Rantau	*Bacillus latherosporus*
	GAR-2	Form. water	Rantau	*Bacillus coagulans*
	GAR-3	Form. water	Rantau	*Flavobacterium sp.*
	GAR-4	Form. water	Rantau	*Pseudomonas aeruginosa*
	GAS-1	Form. water	Kualasimpang	*Neisseria sp.*
	GAS-2	Form. water	Kualasimpang	*Flavobacterium sp.*
	GAS-3	Form. water	Kualasimpang	*Bacillus sp.*
	GAS-4	Form. water	Kualasimpang	*Enterobacter sp.*
	GAP-1	Form. water	Pangkalansusu	*Flavobacterium sp.*
	GTP-1	Soil	Pangkalansusu	*Hafniae alvei*
	GMR-1	Crude Oil	Rantau	*Corynebacterium sp.*
	GMP-1	Crude Oil	Pangkalansusu	*Staphylococcus sp.*
	GMS-1	Crude Oil	Kualasimpang	*Chromobacterium lividum*
	GMS-2	Crude Oil	Kualasimpang	*Neisseria sp.*

Table 2 Results of Identification and Determination of Isolates Obtained at 55°C

Area	Code	Source	Field	Genus/Species
CEPU	GAN/T-1	Form. water	Nglobo	*Bacillus alvei*
CIREBON	GAJ/T-1	Form. water	Jatibarang	*Bacillus licheniformis*
	GAK-2	Form. water	Jatibarang	*Corynebacterium pseudodiphtheriticum*
	GAK-1	Form. water	Cemara	*Bacillus alvei*
	GMN-1	Form. water	Tugubarat	*Bacillus coagulans*
RANTAU	GAR/T-1	Form. water	Rantau	*Bacillus coagulans*
	GAR/T-2	Form. water	Rantau	*Bacillus coagulans*
	GAS/T-1	Form. water	Kualasimpang	*Bacillus licheniformis*

Table 3 Results of Microbe Activity Test

Species	Code	Growth Rate Coefficient	
		Non-Hydrocarbon Medium	Hydrocarbon Medium
At 30°C			
Staphylococcus epidermidis	GAL-1	0.081	0.127
Staphylococcus sp.	GAN-1	0.133	0.205
Staphylococcus sp.	GAK-2	0.130	0.175
Staphylococcus sp.	GAC-1	0.061	0.068
Staphylococcus sp.	GMP-1	0.139	0.186
Corynebacterium pseudodiptheriticum	GAJ-1	0.308	0.391
Corynebacterium sp.	GAK-1	0.174	0.211
Pseudomonas sp.	GTJ-1	0.155	0.273
Flavobacterium sp.	GMN-1	0.064	0.116
Flavobacterium sp.	GAS-2	0.130	0.175
Bacillus latherosporus	GAR-1	0.189	0.214
Bacillus coagulans	GAR-2	0.425	0.438
Bacillus sp.	GMN-2	0.127	0.141
Neisseria sp.	GAS-1	0.255	0.313
Neisseria sp.	GMC-1	0.217	0.358
Neisseria sp.	GMT-1	0.256	0.372
Chromobacterium lividum	GMS-1	0.102	0.233
Alcaligenes faecalis	GAT-1	0.191	0.202
At 55°C			
B. coagulans	GAR/T-2	0.123	0.157
B. licheniformis	GAJ/T-1	0.030	0.150

Table 4 Test Results on the Effect of Activity on pH on Media

Species	Code	Δ pH after 24 hr	
		Nutrient Medium	PPGAS Medium
At 30°C			
Staphylococcus sp.	GAN-1	+1.08	-1.31
Corynebacterium pseudodiphteriticum	GAJ-1	+0.35	-1.36
Corynebacterium sp.	GAK-1	+0.93	-1.08
Flavobacterium sp.	GMN-1	+0.59	-0.75
Flavobacterium sp.	GAR-3	+1.02	-1.75
Bacillus coagulans	GAR-2	-0.24	+0.12
At 55°C			
Bacillus alvei	GAN/T-1	–	-1.12
Bacillus coagulans	GAT/T-1	–	-0.88
Bacillus licheniformis	GAJ/T-1	–	-0.72

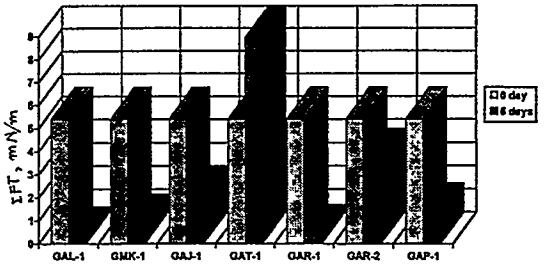

Figure 1 Test Results on the Effect of Activity on Oil-Nutrient Medium Interfacial Tension at 30°C

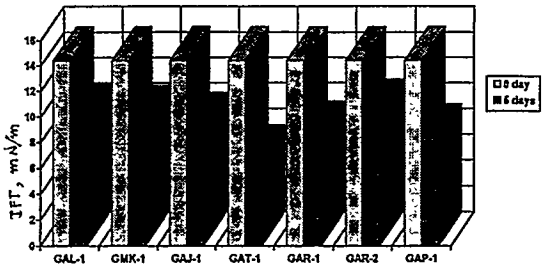

Figure 2 Test Results on the Effect of Activity on Oil-PPGAS Medium Interfacial Tension at 30°C

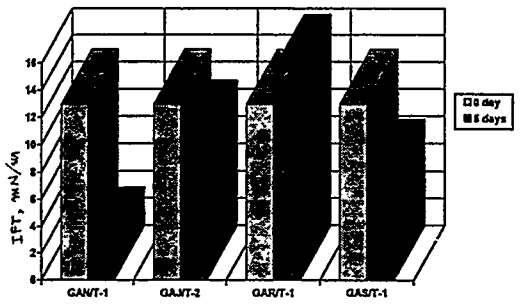

Figure 3 Test Results on the Effect of Activity on Oil-Tryptone Dextrose Medium Interfacial Tension at 55°C

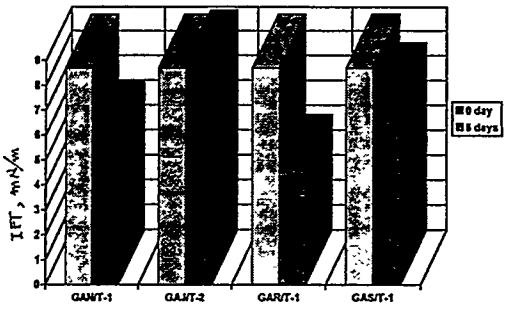

Figure 4 Test Results on the Effect of Activity on Oil-PPGAS Medium Interfacial Tension at 55°C

Mechanisms of Microbial Oil Recovery by *Clostridium acetobutylicum* and *Bacillus* Strain *JF-2*

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Abstract

Core displacement experiments at elevated pressures were conducted to determine whether microbial processes are effective under conditions that simulate those found in an actual oil reservoir. The in-situ growth of *Clostridium acetobutylicum* and *Bacillus* strain JF-2 resulted in the recovery of residual oil. About 21 and 23% of the residual oil was recovered by *C. acetobutylicum* and *Bacillus* strain JF-2, respectively. Flooding cores with cell-free culture fluids of *C. acetobutylicum* with and without the addition of 50 mM acetone and 100 mM butanol did not result in the recovery of residual oil. Mathematical simulations showed that the amount of gas produced by the clostridial fermentation was not sufficient to recover residual oil. Oil recovery by *Bacillus* strain JF-2 was highly correlated to surfactant production. A biosurfactant-deficient mutant of strain JF-2 was not capable of recovering residual oil. These data show that surfactant production is an important mechanism for microbially enhanced oil recovery. The mechanism for oil recovery by *C. acetobutylicum* is not understood at this time, but the production of acids, solvents, or gases alone cannot explain the observed increases in oil recovery by this organism.

Introduction

Enhanced oil recovery due to microbial activity is a commercially viable technology in the petroleum industry with thousands of wells treated on a yearly basis in the United States.[1,2] Several well-controlled field trials have shown that additional oil is recovered and that the economics of microbially enhanced oil recovery (MEOR) are very attractive.[3,4] Although these results are promising, the implementation of MEOR still suffers from a lack of understanding of how microorganisms can recover oil. In order to develop MEOR as a commercially viable technology more fully, a greater understanding of the mechanisms of MEOR processes is needed.

After a reservoir has been waterflooded, brine, gas, and small droplets of oil remain trapped within pores of the reservoir medium. Many tertiary processes currently being employed can increase the recovery of this trapped oil. These processes include in-situ combustion, CO_2 and chemical flooding, and selective plugging. In MEOR, the growth and metabolism of indigenous bacteria are stimulated. These bacteria produce products (such as carbon dioxide, solvents, surfactants, and polymers) similar to those used in chemical flooding. Thus, MEOR provides a cost-effective alternative to the above methods. Laboratory studies conducted by Bryant and Douglas[5] suggested that the production of gas, solvents, organic acids, and surfactants were important mechanisms for microbially enhanced oil recovery in sandstone cores. However, it is not clear whether all of these products need to be produced, and whether some of these activities, such as gas production, would be important at elevated pressures.

Fluids in an oil reservoir are directly affected by the chemical changes which can occur as a result of microbial processes. The end products of microbial metabolism, especially gases, could result in changes in the relative permeability of the reservoir. Microbial production of gases such as carbon dioxide, hydrogen, nitrogen, and possibly methane could create a free gas phase if the reservoir pressure is low enough. The presence of a free gas phase would increase the nonwetting phase saturation which would mobilize residual oil.[6] Holmgten and Motse found that increasing the initial free-gas saturation caused a decrease in the residual oil saturation by 6% to 10% of the pore volume.[7] In fact, the generation of a free gas in a core accounted for a fifth to a third of the residual oil recovered after waterflooding.[7] However, since most oil reservoirs have high pressures, it is not certain whether the amount of gas produced by microbial processes would be sufficient for the formation of a free gas phase.

One of the main mechanisms that limit the ultimate recovery of oil is the capillary forces that entrap the oil in microscopic pores. Only when the interfacial tension between oil and water is lowered will residual oil be recovered. Biosurfactants are a

potentially important MEOR mechanism since these chemicals decrease the interfacial tension between the oil and brine. In chemical surfactant flooding, much of the surfactant adheres to the surface of the reservoir rock near the wellbore. This decreases the concentration of the surfactant to levels below that needed to recover residual oil. A potential advantage of microbial processes is that the surfactant is produced in-situ, near the site where it is needed to recover residual oil. However, since biosurfactant-producing organisms have other activities that could also result in oil recovery, it is difficult to determine whether surfactant production alone is a major mechanism for oil recovery by microorganisms.

The objectives of this work were twofold. First, core displacement studies were done to determine whether microbial processes could recover residual oil at elevated pressures. Second, the mechanisms of oil recovery by two potentially useful bacteria, *Clostridium acetobutylicum* and *Bacillus* strain JF-2, were studied. *C. acetobutylicum* produces acids, solvents, and gases during growth with carbohydrate substrates. *Bacillus* strain JF-2 produces a biosurfactant that can significantly reduce the interfacial tension between oil and brine.[8] The use of a mutant deficient in surfactant production and a mathematical MEOR simulator were used to determine the major mechanisms of oil recovery by these two strains.

Materials and Methods

Organisms. *Clostridium acetobutylicum* and *Bacillus* strain JF-2 were obtained from our culture collection.

Media and Conditions of Cultivation. Compositions of the media used to grow *C. acetobutylicum* and *Bacillus* strain JF-2 in liquid culture and in the sandstone cores are shown in Table 1. The media were similar to each other in that both had the same inorganic minerals and organic growth factors, the same buffer, and the same energy source, glucose. The media differed in that a cysteine-sulfide reducing solution was needed to grow the strict anaerobe *C. acetobutylicum.*[9] Anaerobic growth of *Bacillus* strain JF-2 required the addition of sodium nitrate as an electron acceptor, and maximal surfactant production by this strain required higher amounts of sodium chloride.

Bacillus strain JF-2 and a nonsurfactant-producing mutant of this strain were also grown in medium E modified by the addition of 0.5 g/l each of yeast extract and sodium nitrate.[10]

Procedures used for the preparation and use of anaerobic media and solutions were those of Bryant as modified.[11,12]

Isolation of Nonsurfactant-Producing Mutant. A spontaneous mutant of *Bacillus* strain JF-2 that no longer lowered the surface tension of the medium was obtained by selection for nonhemolytic colonies on blood agar plates.[13] Strain JF-2 was grown aerobically in modified medium E at 37°C, then transferred to sterile medium when the culture reached the stationary phase of growth. The culture was serially transferred in this manner 20 times to enrich for nonsurfactant-producing cells. The nonsurfactant-producing mutant was isolated by serial dilution and inoculation of blood agar plates. Nonhemolytic colonies were picked and restreaked to obtain a pure culture.

Core Flow Apparatus. Berea sandstone cores were steam cleaned for two weeks to remove humic acids and other organic materials. After steam cleaning, the cores were dried at 125°C for 24 hr, then placed in a vacuum desiccator to cool. Each core was wrapped with Teflon tape, then inserted into a rubber sleeve. Liquid gasket material was applied to the ends of the rubber sleeve to prevent leaks. The rubber sleeve with the core was placed in a stainless steel cylindrical core holder. The core holder was connected to the core flow apparatus using stainless steel tubing and compression fittings (see Fig. 1). The components of the system were rated to operating pressures of 5,000 psig.

The core holder was placed inside of a constant temperature oven to maintain the temperature at 36°C. Pressure gauges were attached to monitor the confining pressure and the pore pressure. A back-pressure regulator was used to control the pore pressure. A stainless steel transfer vessel operated at a pressure equal to that of the pore pressure of the core was used to inject fluids into the core. A piston-driven pump was used to displace the fluid from the transfer vessel into the core. A plastic syringe located downstream of the back-pressure regulator was used to collect liquid and gas samples. Prior to use, the core flow was pressurized with nitrogen for several days to check for leaks.

Core Flow Experiments. Petrophysical data for the cores used in this study are given in Table 2. To determine whether the in-situ generation of microbial products at reservoir pressures enhances oil recovery, cores were incubated at an initial pore pressure of 1,000 psig, except for core 1. The initial pore pressure of core 1 was varied to determine its effect on microbial activity. For cores inoculated with *C. acetobutylicum,* a 5 g/l sodium chloride solution was used to waterflood the core after the incubation period. A 50 g/l sodium chloride solution was used for cores inoculated with *Bacillus* strain JF-2.

Each core was vacuum-saturated with the respective brine solution. The saturated core was then inserted into the rubber sleeve which was placed in the core holder. The core holder was connected to the flow system, and the core was flooded with 10 PV of the respective brine solution containing 0.1 M $CaCl_2$ to stabilize clay

particles, and 50 ml/l of methanol to disinfect the core apparatus. The core apparatus was incubated for 24 hr with the brine solution containing calcium chloride and methanol. The apparatus was then flushed with 10 PV of the respective brine solution without methanol and calcium chloride. The core was flooded with oil to connate water saturation, then flooded with the respective brine to residual oil saturation.

To determine whether the disinfection procedure was effective, an inoculated core was aseptically flooded with sterile clostridial medium and incubated. Neither the presence of viable cells in the effluent nor an increase in pore pressure was observed after five days of incubation.

A series of nutrient treatments were performed on each core after the core was flooded to residual oil saturation. Each core received from 3 to 5 PV of the respective culture medium containing a 5% (vol/vol) inoculum of a culture of *C. acetobutylicum* or *Bacillus* strain JF-2. The core was incubated without fluid flow until no further change in pore pressure was observed. The core was then flooded with about 5 PV of the respective brine solution. The amounts of oil, gas, and brine collected after each treatment were measured volumetrically. Table 3 shows the actual amounts of culture medium and brine that each core received, and the incubation times of each treatment.

Analyses. Absolute permeability, porosity, pore volume, connate water saturation, residual oil saturation, and effective permeabilities to oil and water were determined as described.[14,15,16] Permeability reduction factor (in percent) was calculated from the change in permeability after each treatment.[15] Pore pressure was measured using a calibrated gauge.

The amount of gas produced was estimated volumetrically, using a plastic syringe connected to the effluent flow line. Organic acids and alcohols were quantitated using high pressure liquid chromatography (HPLC) and gas chromatography, respectively.[17,18] Carbon dioxide was measured by gas chromatography, and hydrogen was measured using a gas chromatograph equipped with a mercury reduction detector.[19,20] Glucose was measured using the glucose oxidase method (Sigma). Since the HPLC method did not separate lactate and succinate, the succinate dehydrogenase assay (Sigma) was used to check for the presence of succinate. No succinate was detected.

The amounts of products produced by liquid cultures were corrected for the small amounts of these products present in the medium at the start of incubation, and for the amounts of these products produced in control cultures that lacked glucose. The amounts of fermentation products produced during in-situ growth in cores were corrected for the small amounts of the products in the influent medium.

Growth of liquid culture was measured spectrophotometrically by following the change in absorbance with time. Cell concentration was determined by quantitating the amount of whole cell protein. Samples were microfuged for 2 minutes to collect the cells. The cell pellet was washed twice by resuspending the pellet in a 10 mM sodium/potassium phosphate buffer (pH 7.2) and recentrifuging. The final cell pellet was resuspended in 0.1 N NaOH and incubated at 70°C. Protein was determined colorimetrically using bovine serum albumin as the standard.[21]

Surface tension of cultures and core effluents was measured using a DeNoy ring and an automated tensiometer.[8] The relative amount of surfactant produced was estimated from the number of units of surfactant activity as described previously.[8]

Results and Discussion

Fermentation Studies. When grown in liquid culture, *Clostridium acetobutylicum* produced 39 mM of acetate, 43.2 mM of butyrate, 5.7 mM of ethanol, 16.8 mM of butanol, and 164.3 mM of CO_2 (calculated) from 100 mM of glucose. The carbon recovery was 82%. These products are similar to those previously reported for this organism.[22] The cell yield was 18.2 g per mole of glucose. It is not known whether isopropanol was produced because our gas chromatographic method did not separate isopropanol. Hydrogen was also produced, but the amount of hydrogen was not quantitated. The pH of the medium decreased from its initial value of 7.0 to a final value of 3.8.

The fermentation balance for *Bacillus* strain JF-2 is incomplete because this strain produced a large amount of an unknown metabolite. Of the identified products, 47.2 mM of lactate, 21.8 mM of acetate, 13 mM of propionate, and 21.8 mM of CO_2 (calculated) were produced from 100 mM of glucose. The carbon recovery was 41%. The unidentified product migrated between isobutyrate and butryate on both gas chromatography and HPLC. Assuming that the unidentified peak has four carbons and has a similar detector response as butyrate, about 80 mM were produced per 100 mM of glucose. This would give a carbon recovery of about 95%.

A conversion factor that relates the units of surfactant activity to the moles of biosurfactant can be calculated. M. Javaheri found that the most purified fraction of the biosurfactant contained 1,090 units of activity per mg (dry weight).[8] Sharma and Georgiou reported a molecular weight of the JF-2 biosurfactant of 1,035 g/M.[23] Assuming that Javaheri's most pure fraction contains only the JF-2 biosurfactant, then one mole of the biosurfactant would have 1.1×10^9 units of activity. This conversion factor can be used to estimate the molar concentration of

the JF-2 biosurfactant produced in core experiments by determining the number of units of surfactant activity present.

Isolation of a Biosurfactant Mutant. Cultures of strain JF-2 lost the ability to reduce the surface tension of the medium when they were repeatedly transferred in liquid medium. We noticed that the cultures that had been repetitively transferred had a large number of smooth colonies, whereas those that had been inoculated directly from an agar colony had rough colonies, with only a very small percentage of smooth colonies (<0.2%). We hypothesized that the ability to produce the surfactant was not a stable trait of JF-2, and that smooth colonies were mutants of JF-2 that did not produce the biosurfactant.[24] We tested this hypothesis by determining whether smooth colonies were hemolytic and whether they had surfactant activity.

The number of rough and smooth colonies in cultures that had been transferred 15 times was compared to that found in cultures that were inoculated directly from a rough colony. Cultures that had been transferred 15 times in liquid medium had about 1.5×10^9 smooth colonies per milliliter. No rough colonies were observed ($<10^7$ colonies/ml). The surface tension of these cultures was greater than 40 mN/m. With some of the cultures, the surface tension was as high as 60 mN/m. Cultures that were inoculated directly from a rough colony had predominantly rough colonies (about 2.4×10^9 colonies/ml) and very few smooth colonies (about 1×10^7 colonies/ml). These cultures always had surface tensions below 30 mN/m. One culture that had been repetitively transferred and one that had been inoculated directly from a rough colony were plated onto blood agar medium to determine the number of hemolytic and nonhemolytic colonies. All of the colonies that grew on plates inoculated with culture that had not been transferred were hemolytic. Blood agar plates inoculated with cultures that had been transferred 15 times had a large number of nonhemolytic colonies; about 69% of all of the colonies were nonhemolytic. These data suggest that clonal selection for rough colonies is required to maintain biosurfactant production by strain JF-2, and that repetitive transfer in liquid medium is not recommended for the maintenance of the strain.

One of these nonhemolytic colonies was picked and restreaked onto blood agar medium to obtain a pure culture of a biosurfactant-deficient mutant of JF-2. When grown in liquid culture, the JF-2 mutant did not lower the surface tension of the medium below 60 mN/m, which was close to the surface tension of uninoculated medium E. As a comparison, the wild-type strain of JF-2 consistently lowered the surface tension of medium E below 30 mN/m and had a critical micelle dilution of 16. The biosurfactant-deficient mutant of JF-2 was nonhemolytic and formed smooth colonies on modified medium E. Otherwise, it had the same physiological properties as the wild-type strains. The mutant grew anaerobically at 45°C in modified medium E which contains 50 g/l NaCl. Fermentation end-products of the

mutant were similar to those of the wild-type strain of JF-2 as were the morphology and gram reaction. These data show that a spontaneous, biosurfactant-deficient mutant of strain JF-2 was obtained. The mutant strain will be useful as a negative control to determine the importance of surfactant production in MEOR.

Oil Recovery by Clostridial Fermentation. Oil recovery due to the in-situ growth and metabolism of *C. acetobutylicum* was sporadic, and large amounts of oil were recovered only after several treatments (see Table 4). However, after several treatments, significant oil recoveries were observed, even at elevated pressures. About 16% and 26% of the residual oil were recovered in cores 1 and 2, respectively. For core 1, pore pressure was increased with each transfer to acclimate the organism to higher pressures. For the last treatment, the pore pressure was decreased to 50 psig (see Table 4). With each increase in pressure, microbial activity also increased, as evidenced by increases in the amounts of fermentation products (see Table 4 and Fig. 2). Less acids and solvents were produced during the fifth treatment, when the initial pore pressure was 50 psig, than in the fourth treatment when the initial pore pressure was 1,000 psig. Thus, high pressures did not drastically inhibit the metabolism of this organism.

There are several possible mechanisms by which *C. acetobutylicum* could enhance oil recovery. The production of gases may swell the oil or create a large nonwetting phase.[6, 14] The production of acids and solvents may change the wettability of the rock surface, or the solvents may act as surface active molecules to reduce interfacial forces. Finally, the in-situ growth may have plugged the larger pores in the core and diverted fluid flow to smaller pores that have high residual oil saturations.[25] Analysis of the data on product formation and permeability reduction suggests that oil recovery occurred when the microbial activity was high in the cores (see Table 4; Figs. 2 and 3). However, it is difficult to see any direct relationship between the production of one product and oil recovery. For core 1, most of the oil was recovered after the fifth treatment (see Table 4). During this treatment, a large amount of gas and a large reduction in the permeability occurred, suggesting that these two factors may be involved in oil recovery (see Table 4, Fig. 2). For core 2, the large amount of gas was produced in the fifth treatment, but the amount of oil recovered was less than other treatments where the amount of gas produced was much less (see Table 4, Fig. 3). After the second treatment, a large reduction in permeability occurred and a large amount of oil was recovered in core 2. However, oil was recovered in the third and fourth treatments even though no further reductions in permeability were observed.

The data in Figures 2 and 3 do not indicate a strong relationship between acid and solvent production and oil recovery. To test whether these products alone could recover residual oil, cell-free culture fluids were injected into a core that had been flooded to residual oil saturation. No oil was produced when the culture fluid was

used as the displacing fluid. Also, no oil was produced when cell-free culture supplemented with 50 mM acetone and 100 mM butanol was used. Thus, the fermentation products alone are not the mechanism of oil recovery by *C. acetobutylicum.* This experiment also excludes the possibility that the *C. acetobutylicum* produces a biosurfactant. The data do suggest that in-situ microbial activity is needed for oil recovery by this organism.

Mathematical modeling was used to further study the role of gas production in oil recovery by *C. acetobutylicum.* A three-phase, multicomponent, MEOR simulator was developed.[26] This simulator was used to determine whether the amount of gas produced by *C. acetobutylicum* was sufficient to recover residual oil. In the simulation, 111 mM glucose was fermented with the production of 200 mM of CO_2 per liter of medium. This caused a 250 psig increase in the pore pressure. During the post-incubation waterflood, 80% of the CO_2 was produced, but no oil was produced.[28] A solution gas-oil ratio of 10 scc/scc was estimated. At this solution gas-oil ratio, the oil swelling factor was about 1.04. Thus, it appears that the amount of gas produced by the clostridial fermentation was not sufficient to recover residual oil. In an actual oil reservoir, the indigenous bacteria may be able to completely mineralize the carbohydrate substrate, producing 6 moles of carbon dioxide for every mole of glucose rather than just 2 moles as occurs with the clostridial fermentation. Further studies are needed to determine whether the amount of gas produced by the complete mineralization of the substrate is sufficient for the recovery of residual oil.

Oil Recovery by* Bacillus *Strain JF-2. The biosurfactant-deficient mutant strain of JF-2 was used to determine the importance of biosurfactant production for oil recovery. Two cores (cores 3 and 4) were inoculated with the wild-type strain of JF-2 that had not been repetitively transferred (see Table 5). Also, one core was inoculated with a culture of JF-2 that had been transferred about 15 times (core 5), and another core was inoculated with a culture of the biosurfactant-deficient mutant strain (core 6). When the wild-type strain of JF-2 was used, 23% and 21% of residual oil were recovered from cores 3 and 4, respectively. Analysis of the core effluents showed that small amounts of acetate (0.1 mM), butyrate (1.1 mM), and lactate (0.8 mM) were produced. Surface tensions of the effluents were below 30 mN/m. Microscopic analysis of the effluent showed that most of the turbidity in the aqueous phase was due to very small drops of oil, about the size of a bacterial cell. Effluent samples from the core inoculated with the serially transferred culture of JF-2 had a thin film of oil. The amount of oil was much less than the gradations on the 50-ml syringe. When the JF-2 mutant was used, a small amount of oil was produced after the first treatment. In the second treatment, only a thin film of oil was detected. Less than 6% of the residual oil was recovered when the mutant strain was used. These data show that the main mechanism for oil recovery by *Bacillus* strain JF-2 is biosurfactant production. Oil recovery is lost when the ability

to produce the biosurfactant is lost. Figure 4 shows that cumulative oil recovery was highly correlated ($r^2 = 0.979$) to cumulative surfactant production for core 4. Thus, the reduction in interfacial tension by biosurfactant production is an important mechanism for oil recovery.

Acknowledgments

This work was supported by contract DE-AC22-9OBC14662 from the U.S. Department of Energy.

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Table 1 Medium Components Used for Growth of *Clostridium acetobutylicum* and *Bacillus* Strain JF-2 in Liquid Culture and in Sandstone Cores

Component	*C. acetobutylicum* (g or ml/l)	JF-2 (g or ml/l)
Tanner's Minerals[a]	20.0 ml	20.0 ml
Tanner's Metals[a]	10.0 ml	10.0 ml
Glucose	20.0 g	1.8 g
Yeast Extract	5.0 g	5.0 g
NaCl	5.0 g	50.0 g
PIPES	4.0 g	4.0 g
Balch Vitamins[a]	5.0 ml	5.0 ml
Cysteine Sulfide Solution[a]	20.0 ml	None
$NaNO_3$	None	1.0 g

[a] Tanner et al., 1989

Table 2 Petrophysical Properties of the Berea Sandstone Cores

Core No. a	1	2	3	4	5	6
Porosity (%)	18.4	18.0	19.0	14.0	17.0	17.5
Pore Volume (ml)	106	107	113	85	103	105
Absolute Permeability (md)	178	373	181	106	291	256
Oil Permeability (md)	112	270	91	74	198	154
Connate Water (ml)	33	24	41	30	33	35
(%)	31	22	36	35	32	33
Water Permeability (md)	42	66	23	22	60	65
Residual Oil (ml)	31	50	35	26	30	26
(%)	29	47	31	31	29	25

Table 3 Treatment Regimes for Microbially Enhanced Oil Recovery Experiments

Core No.	Treatment No.	Organism	Nutrient Volume (ml)	Brine Flood Volume (ml)	Incubation Time (days)
1	1	*C. acetobutylicum*	550	200	12
	2		550	250	8
	3		500	300	5
	4		440	300	8
	5		500	300	7
2	1	*C. acetobutylicum*	350	350	5
	2		250	350	4
	3		400	300	3
	4		400	400	4
	5		400	400	4
3	1	JF-2 (Wild-type)	300	400	8
	2		300	400	9
	3		300	400	4
4	1	JF-2 (Wild-type)	300	400	4
	2		300	400	5
	3		300	400	4
	4		300	400	5
	5		300	400	5
5	1	JF-2 (Serially Transferred)	250	400	5
	2		250	400	6
	3		250	320	2
6	1	JF-2 (Mutant)	300	350	4
	2		300	350	5
	3		300	350	4

Table 4 Experimental Results Obtained for Cores Incubated with *Clostridium acetobutylicum*

Core No.	Treatment No.	Initial Pore Pressure (psig)	Maximum Pore Pressure (psig)	Oil Recovery (ml)	Gas Produced (ml)	PRF (%)	pH	
							Influent	Effluent
1	1	500	575	1	27	95	ND[a]	ND
	2	700	880	1	62	136	ND	ND
	3	750	1,050	0	51	131	ND	ND
	4	1,000	1,090	0	83	136	ND	ND
	5	50	178	3	126	29	ND	ND
2	1	1,000	1,100	0	0	89	6.5	6.1
	2	1,000	1,040	0	22	89	6.6	4.9
	3	1,000	1,075	4	28	41	6.5	5.3
	4	1,000	1,100	6	79	50	6.5	5.1
	5	1,000	1,170	3	184	44	6.7	5.0

[a]ND=not determined

Table 5 Experimental Results Obtained for Cores Incubated with *Bacillus* Strain JF-2

Core No.	Treatment No.	Culture	Initial Pore Pressure (psig)	Maximum Pore Pressure (psig)	Oil Recovery (ml)	Oil Recovery % Resid. Oil	Gas Produced (ml)	Surfactant (units)	PRF (%)	pH Influent	pH Effluent
3	1	JF-2 Wild-Type	1,000	1,110	3		0	180	79	6.8	7.3
	2		1,000	1,120	2		1	120	90	6.9	7.3
	3		1,000	1,070	3	23	0	60	0.7	6.8	7.0
4	1	JF-2 Wild-Type	1,000	1,050	1		0	132	105	7.0	7.1
	2		1,000	1,070	2		0	116	118	6.8	7.2
	3		1,000	1,050	2		0	79	109	6.8	7.2
	4		1,000	1,050	0.5		0	57	96	6.9	7.2
	5		1,000		<0.5	24	0	46	91	7.1	7.4
5	1	JF-2 Serially Transferred	1,000	1,050	<0.5		0	ND	262	6.8	7.5
	2		1,000	1,050	<0.5		0	165	257	6.9	7.4
	3		1,000	1,050	0	<3	0	6	245	6.9	7.0
6	1	JF-2 Mutant	1,000	1,050	1		0	53	131	6.5	7.0
	2		1,000	1,050	<0.5		0	ND	140	6.8	7.0
	3		1,000	1,060	0	<6	0	ND	140	6.9	7.1

ND = not determined

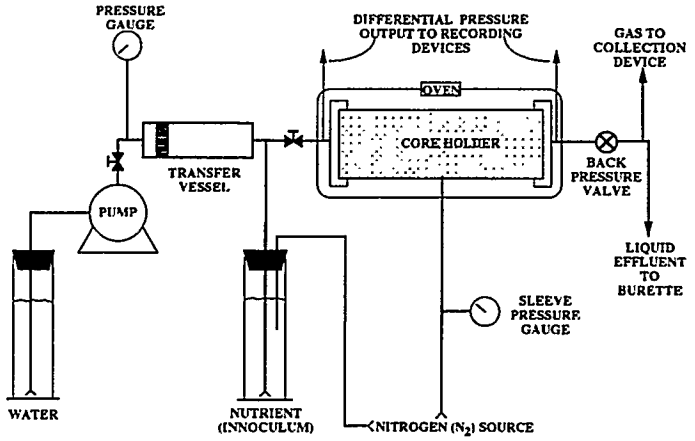

Figure 1 High-Pressure Core Apparatus

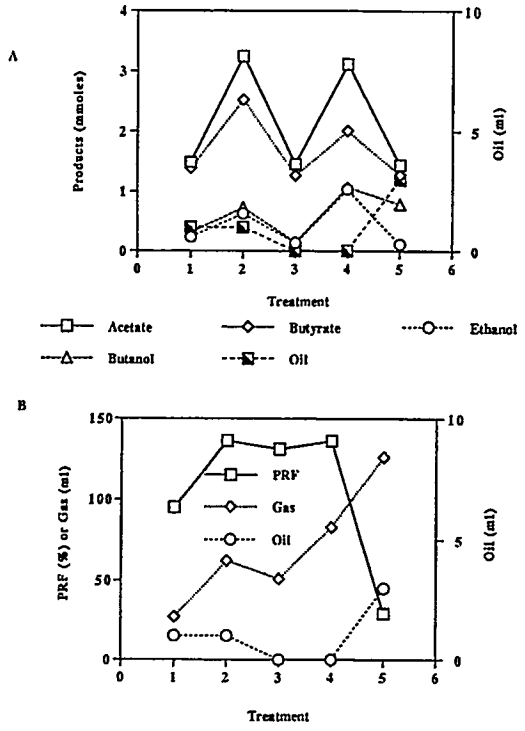

Figure 2 The Relationship to Oil Recovery for Core 1 of (A) Acid and Solvent Production and (B) Gas and Permeability Reduction.

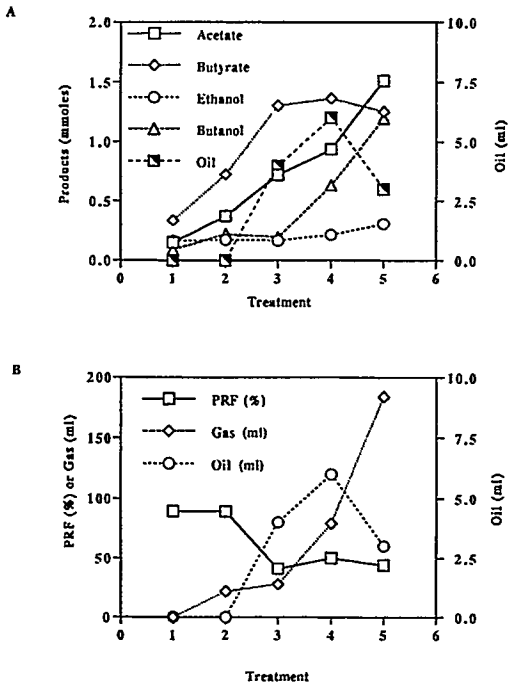

Figure 3 The Relationship of (A) Acid and Solvent Production and (B) Gas and Permeability Reduction for Core 2

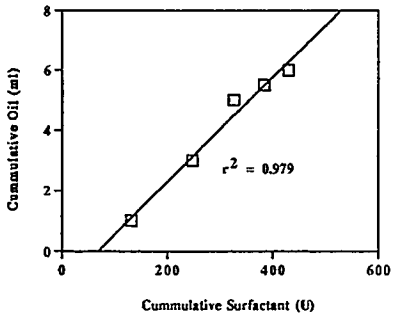

Figure 4 Correlation of Cumulative Oil Recovery to Cumulative Surfactant Production in Core 4

A Microbial Trigger for Gelled Polymers

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Abstract

A process using a microbially gelled biopolymer was developed and used to modify permeability in coreflood experiments. Alkaline-soluble curdlan biopolymer was mixed with microbial nutrients and acid-producing alkaliphilic bacteria, and injected into Berea sandstone cores. Concurrent bottle tests with the polymer solution were incubated beside the core. Polymer in the bottle tests formed rigid gel in 2–5 days at 27°C. After 7 days incubation, 25–35 psi fluid pressure was required to begin flow through the cores. Permeability of the cores was decreased from 852 md to 2.99 md and from 904 md to 4.86 md, respectively, giving residual resistance factors of 334 and 186.

Introduction

Gelled polymers have been widely used for permeability modification in petroleum reservoirs. High molecular-weight polymers are mixed with gelling agents, commonly crosslinking compounds such as multivalent metal ions in low viscosity pregel solutions which can be injected into the reservoir formation. After the pregel solution is placed in the reservoir, some triggering reaction initiates formation of a high viscosity solution or gel. Gelled polymers have the advantage of deep penetration by a low viscosity pregel solution and effective permeability control after gelation, and many systems have achieved some technical success.[1] However, gelation of these polymer systems can be dependent on many variables including critical concentrations of polymer and crosslinking ions, additional reactants, pH, and temperature. Additionally, the gelation reaction must be delayed after mixing the pregelled polymer to allow injection and placement in the reservoir. A simpler process in which polymer gelation relies on fewer variables and is under direct control would therefore be desirable.

Curdlan biopolymer is an extracellular β-1,3-D-glucan produced by the soil bacterium *Alcaligenes faecalis* var. *myxogenes*.[2] Curdlan is an attractive polymer for permeability modification because it exhibits a pH-dependent transition between a gel and a soluble state. In alkaline solutions of pH greater than about 10, curdlan is soluble and can be mixed as a low viscosity solution. Whenever the pH is lowered below about 9, curdlan forms an insoluble gel. Gelled curdlan is stable in neutral or acidic solutions to pH 2, insoluble to most organic solvents, and stable to heating to 90°C.[3] Production of similar "curdlan-type" biopolymers appears to be shared by a diverse group of bacteria. In addition to gram-positive *Alcaligenes*, strains of closely related *Agrobacterium*[4] and gram-negative *Cellulomonas*[5] have been described which produce similar glucan biopolymers.

To be useful as a gelled polymer for selective plugging, curdlan-type polymers must be mixed in the soluble form, in an alkaline solution of pH > 10, and injected with a pH modifier which can lower the pH of the pregelled polymer solution. After the polymer is placed in the high-permeability zone, pH modification will initiate gelation of the polymer. Buller and Vossoughi[6,7] developed a layered process to modify permeability using biopolymer from a *Cellulomonas* sp. The layered process consists of injecting alternating slugs of acid and alkaline-solubilized biopolymer. Injecting both acid and alkaline-soluble polymer into a porous medium should result in gelation of the polymer at any point that mixing occurs. Yet, for deep placement of the gel in a reservoir, the process would require propagating two or more slugs through the formation at close proximity without commingling, followed by mixing the slugs at a predetermined location in the reservoir.

Permeability modification with curdlan-type biopolymers could be simplified further if gelation were driven directly by a time-delayed in-situ pH shift. The pH-dependent transition makes alkaline-soluble curdlan especially amenable to gelation by acid-producing bacteria. Acid-producing bacteria which can tolerate alkaline conditions necessary to solubilize curdlan (pH > 10) can lower pH in situ and drive the gelation transition. This report describes the isolation of alkaliphilic acid-producing bacteria and the use of these bacteria to cause delayed gelation of curdlan biopolymer. Microbially gelled curdlan biopolymer was used to reduce brine permeability of Berea sandstone cores.

Materials and Methods

Bacteria

Acid-producing alkaliphilic bacteria were enriched from lake sediment samples collected from a dry lake bed near Zzyzx, California. Sediment samples were placed in Trypticase Soy Broth (TSB) adjusted to pH 11.5 and 5% NaCl. All samples were incubated in N_2-purged Hungate tubes. Isolates SL-1A and SL-2A were picked from streak plates using Tryptic Soy Agar adjusted to pH 11.5 and 5% NaCl. Strain SL-1A is a gram-positive facultative diplococci which has an amino acid requirement. Strain SL-2A is an anaerobic, spore-forming bacillus, stains gram-variable, and has complex nutrient requirements. Both strains are able to grow anaerobically at pH 11.5 and lower the pH of the TSB growth medium to about 7.

Bottle Tests

Bottle tests were conducted to determine if acid-producing alkaliphilic microorganisms can cause gelation of alkaline-solubilized curdlan biopolymer. Pregelled polymer solution was prepared by dissolving 0.5% curdlan into 1N KOH, stirring overnight, then adding per 100 ml: Na_2CO_3, 0.225 g; TSB, 3.0 g; NaCl, 3.0 g. The pH was adjusted to 11.5 with concentrated KOH. Glucose, 1.0%, was added to some samples. Pregelled polymer was aliquoted into Hungate tubes, purged with N_2, and inoculated with undefined consortia of alkaliphilic bacteria; no pure strains were tested. All bottle tests were conducted at 25°C. Bottle tests were monitored for up to six months to determine if the bacteria noticeably degrade the polymer after gelation.

Permeability Modification

After evaluating microbial gelation of curdlan in bottle tests, coreflood experiments were performed to determine if microbially gelled polymer can modify permeability of porous media. Berea sandstone cores were evacuated and vacuum saturated with 0.5% NaCl brine. After loading cores into coreholders, 2 PV brine was pumped through each core at 2.0 ml/min. Permeability of each core was measured before treatment with 0.5% NaCl brine. To stabilize core pH prior to injecting polymer, cores were preflushed with 2 PV thymolphthalein-tagged 0.02 M $CO_3^=$ buffer at pH 11.5. Pregelled curdlan polymer solution was prepared by dissolving 0.5% curdlan into 1N KOH, stirring overnight, then adding per 100 ml: Na_2CO_3, 0.225 g; TSB, 1.5 g; glucose, 0.5 g; fructose, 0.75 g; NaCl, 3.0 g. The pH was adjusted to 11.5 with concentrated KOH. Polymer solution was inoculated with 1.0 ml each SL-1A and SL-2A from stationary phase cultures. One PV inoculated pregelled polymer was injected into each core at 2.5 ml/min. After polymer injection, cores were shut in for 7 or 9 days and incubated at 27°C. Samples of both uninoculated and inoculated pregelled polymer were collected in Hungate tubes concurrent with the polymer injection and incubated with the core. Shut-in pressure of the cores and polymer gelation in accompanying Hungate tubes were monitored during the incubation period. After the incubation period, flow was established through each core with 1 PV 0.5% NaCl brine, and post-treatment permeability was measured. Core effluent pH was monitored during preflush and polymer injection, and after treatment to assess pH alteration by the bacteria.

Results

Bottle Tests

Gel formed in all polymer solutions within 2–4 days after inoculation with alkaliphilic consortia. The gelled polymer had a pH of approximately 8.8–9.0 when measured the first day after gelation, suggesting that gelation had occurred near pH 9.0. The pH of samples containing 1% additional glucose continued to decrease to a minimum pH of 7.0–7.5, whereas the samples containing no additional glucose remained near pH 8.5–9.0. Gel that formed in the samples containing 1% added glucose appeared more rigid when pH decreased to near-neutral than gel that formed in comparable samples without added glucose. Gel forming in all inoculated samples remained firm for 6 months; no visible degradation occurred. Gelation did not occur in an uninoculated control sample during the same 6 month period.

Permeability Modification

During preflush, core C-01 pH stabilized at 11.4 after displacement of approximately 1.5 PV (see Fig. 1). Core C-02 pH exhibited the same pattern as core C-01 during preflush and polymer injection (data not shown). All inoculated polymer samples from core C-01 in accompanying Hungate tubes gelled after 2 days of incubation. Gelation of polymer samples accompanying core C-02 occurred more slowly than with core C-01 with gelation times of 5–12 days. An uninoculated control polymer sample did not form a gel after 2 months incubation. Shut-in pressure for both cores was less than 10 psi, indicating that small amounts of CO_2 may be produced. After incubation, fluid pressure of 25–35 psi ΔP was necessary to initiate flow through the cores. Core effluent pH was measured to assess pH modification by the microbial trigger (see Fig. 2). Cores had an initial pH of 11.4 at the time they were shut in. In core C-01, effluent from the core was decreased to pH 7–8 after microbial treatment. Effluent pH after treatment of core C-02 was decreased to around 8.4.

Permeability of the cores was decreased from 852 and 904 md initial permeability to 2.99 and 4.86 md, respectively, after treatment (see Table 1). Residual resistance factors, F_{RR}, were calculated using the following equation (1):

$$F_{RR} = \frac{\left\{\frac{Q}{\Delta P}\right\}_{\text{before treatment}}}{\left\{\frac{Q}{\Delta P}\right\}_{\text{after treatment}}} \quad (1)$$

where Q and ΔP are steady-state effluent rates and pressure drop across the core, respectively. When F_{RR} are plotted against frontal velocity, Figure 3 shows that gelled curdlan exhibits slight shear thinning, typical for a gelled polymer. Nonetheless, F_{RR} range from near 150 to 300 at frontal velocity up to 4–6 ft/day.

The gelled polymer in Hungate tubes accompanying core C-01 remained firm for approximately 1 month and then solubilized. A solubilized polymer sample was viewed by microscopy and found to have high-density growth of strain SL-1A, indicating that this strain may be responsible for polymer degradation. At this time, Hungate tubes accompanying core C-02 have been incubated for two months, and no visible polymer degradation has occurred.

Discussion

These experiments indicate that microorganisms are able to cause gelation of curdlan biopolymer. Acid-producing microorganisms are common to all

environments. Microbial acid production naturally complements the pH-dependent gelation of curdlan polymer and provides an in-situ mechanism to drive the gelation reaction. Specially isolated alkaliphilic bacteria are able to withstand the strong alkaline conditions necessary to solubilize curdlan and produce acids to lower the pH of the polymer solution. As a primary metabolite, acid production of a growing culture increases exponentially with time and provides a delay for the gelation reaction. Under the conditions of these experiments, gelation was delayed 2–5 days. However, acid production is proportional to the cell density and rate of metabolism, and longer or shorter gelation times can be reasonably expected by varying inoculum or fermentation substrate.

Microbially gelled curdlan biopolymer was shown to effectively modify permeability of porous media. Permeability of Berea sandstone cores was decreased by more than 99% after treatment with 0.5% microbially gelled curdlan polymer. The polymer's physical properties make it desirable for permeability modification in petroleum reservoirs. Curdlan has a low viscosity in alkaline solutions and forms a rigid gel after the microbial trigger. Formation of gel is due to hydrogen bonding[8] and does not require additional reactants. Gelled curdlan is resilient to heat, weak acids, and organic solvents. The gel has elasticity[9] and shows only slight shear thinning at flow rates up to 4–6 ft/day. Microbial metabolism provides a simple, yet effective, mechanism to cause delayed gelation of the polymer.

Acknowledgment

We thank the U.S. Department of Energy, BDM-Oklahoma, the University of Tulsa, and Associated Western Universities for supporting this research.

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Table 1 Permeability Modification in Berea Sandstone Cores by Microbially Gelled 0.5% Curdlan Biopolymer

Berea Core	Initial Permeability (md)	Permeability after Treatment (md)
C-01	852	2.99
C-02	904	4.86

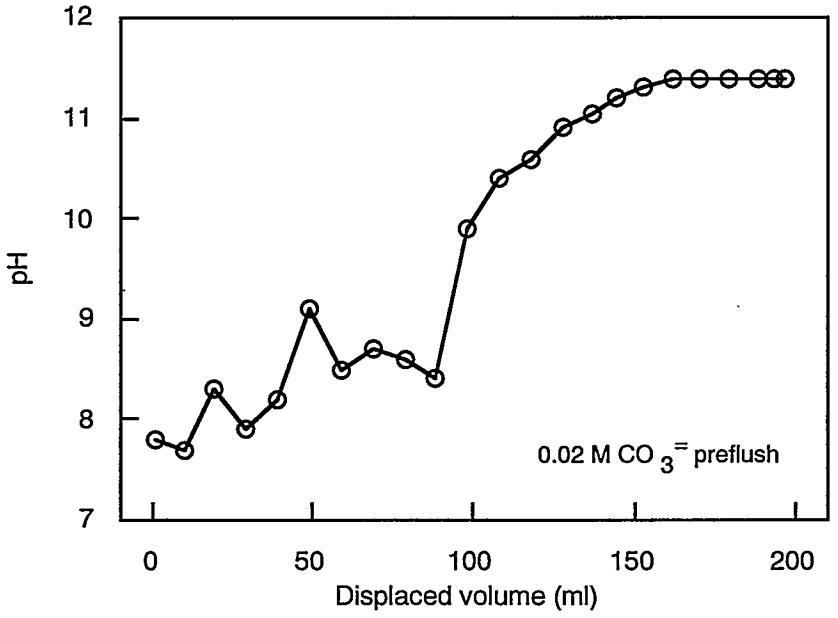

Figure 1 **Core C-01 Effluent during Preflush and Polymer Injection. Core Was Preflushed with 2 PV 0.02 M $CO_3^=$ Buffer Prior to Injection of 1 PV Pregelled Polymer.**

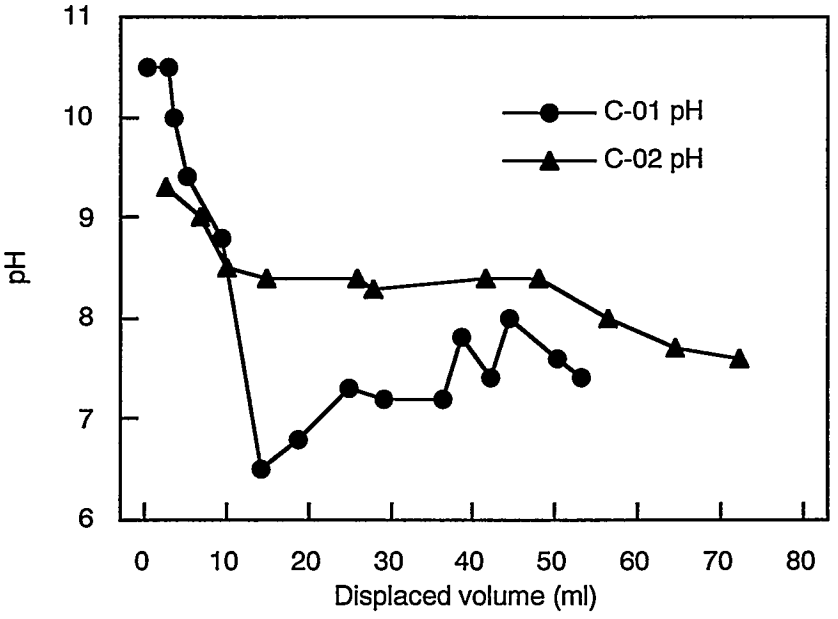

Figure 2 **Core Effluent pH after Microbial Trigger. Cores Had an Initial pH of 11.4 Prior to Microbial Trigger.**

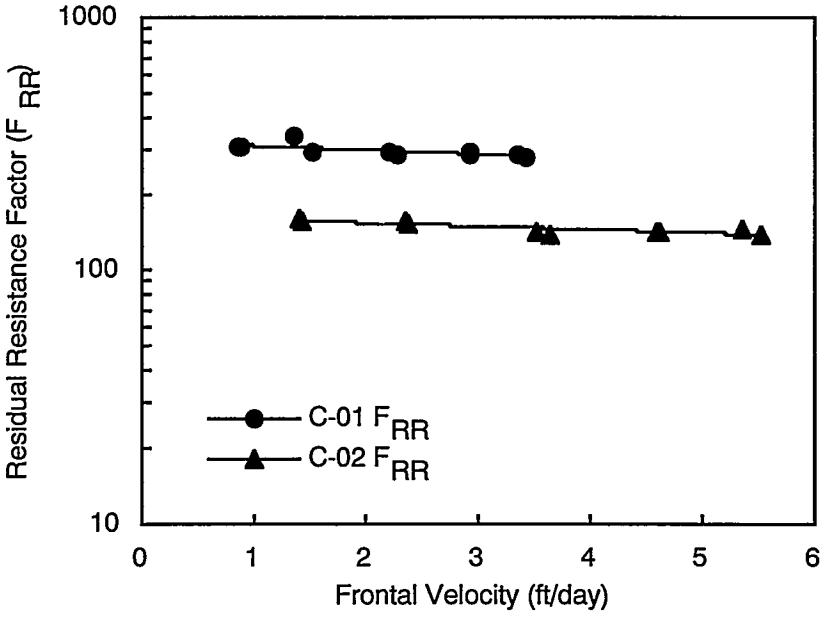

Figure 3 Permeability Modification in Berea Sandstone Cores after Treatment with Microbially Gelled 0.5% Curdlan Biopolymer