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MICROBIAL ENHANCEMENT OF OIL RECOVERY - RECENT ADVANCES

Proceedings of the 1992 International Conference on Microbial Enhancement of Oil Recovery

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PREFACE

During recent years, systematic, scientific, and engineering effort by researchers in the United States and abroad, has established the scientific basis for Microbial Enhanced Oil Recovery (MEOR) technology. In the past, basic research was left to the laboratory, and field use was generally limited to an uninformed oil producer using microbes without knowing what to expect. The merger of these two groups--researchers and producers--is fostering acceptance of MEOR technology through the petroleum community. The applicability of MEOR technologies, its economic feasibility, and indications for future directions have become essential elements of current MEOR research programs.

The successful application of MEOR technology as an oil recovery process is a goal of the Department of Energy (DOE). Research efforts involving aspects of MEOR in the microbiological, biochemical, and engineering fields led DOE to sponsor an International Conference at Brookhaven National Laboratory in 1992, to facilitate the exchange of information and a discussion of ideas for the future research emphasis.

At this, The Fourth International MEOR Conference, where international attendees from 12 countries presented a total of 35 papers, participants saw an equal distribution between "research" and "field applications." In addition, several modeling and "state-of-the-art" presentations summed up the present status of MEOR science and engineering. Spinoff technologies resulting from this research and development effort are finding applications in bioremediation and biochemical processing of oil-related problems at wellsite and downstream operations. The presentations clearly indicated the utility of an emerging, technically and economically viable enhanced oil recovery technology, and an equally important environmental remediation technology.

MEOR researchers have advanced the early ideas and theories from tentative initial investigations to the level of a distinct scientific discipline with a clear mission. The interaction of the international community is essential to this continuing development of the technology.

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Introduction to the Fourth International MEOR Conference

Fred Burtch

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I am pleased to present the introduction to the conference this morning. It is great to see so many familiar faces here today, an indication that microbial enhanced oil recovery is still a very active field of research. The large number of participants from around the world shows that improving oil recovery is a global concern, and that other countries regard MEOR as one possible answer to that concern.

As many of you know, this is a presidential election year in the United States, and all candidates seem to emphasize the word "change" in their campaigns. This is nothing new to many of you from other parts of the globe. You have experienced enormous change; the formation of the European Economic Union, the break up of the Soviet Union, the uniting of East and West Germany, conversions to democratic forms of government in many countries, and daily changes as conflicts erupt in adjacent lands.

We, in the Office of Fossil Energy, Department of Energy (DOE), are hearing the word "change" a great deal also. We are being asked to evaluate our progress and change directions, if necessary. Economic recession is felt by government agencies as well as by individuals. Our financial resources are becoming limited, considering the many challenges that lie ahead. We are being asked to choose between equally deserving programs and direct our efforts to programs with the greatest potential for improving industry's ability to recover more oil from domestic reservoirs.

For a number of years, microbial enhanced oil recovery, or MEOR, research has been supported by the Department of Energy as a potential method of recovery from some of the most critical producing wells, those in danger of abandonment in the near future. In fiscal years 1988 through 1991, as DOE was implementing its National Energy Strategy - Advanced Oil Recovery Program, Congress supported MEOR with special "add on" appropriations. Our Oil Research Program Implementation Plan, a prioritized, balanced plan of enhanced oil recovery research, was initiated in response to the goals of the National Energy Strategy. There are three key elements in this plan: Analysis and Planning, which involves the Tertiary Oil Recovery Information System (TORIS); Field R&D, which involves reservoir class demonstrations; and Supporting Research. Supporting Research is a key element in DOE's Oil Research Plan, and MEOR is applicable in both targeted and disciplinary branches of Supporting Research. Targeted Supporting Research involves specific reservoir problems, i.e., reservoir class related, whereas disciplinary Supporting Research involves cross-cutting, fundamental research, i.e., university support projects.

Because MEOR has the potential for contributing to these goals of increased economic production and reduced well abandonment, it has been a component of DOE's EOR program. But now we have come to a fork in the road--a decision point --it is time to decide whether or not we will continue to support this research and, if we are, what aspects of MEOR show the most near-term promise, and what will the funding level be.

MEOR as a science is relatively young. Although the first suggestion that microorganisms might be used to increase oil production was made in 1926, and

laboratory investigations were conducted by Claude ZoBell in the 1940s, the majority of the MEOR work leading to field trials has been completed in about the past 15 years. Researchers are still sorting out the characteristics of various organisms, and determining what they require to flourish in the subsurface environment. To many in the oil industry, the MEOR approach still looks like a "hit or miss" proposition.

In the early years of oil exploration, there were many theories used to justify a drilling location. Thus, Oklahoman Harry Sinclair, the founder of Sinclair Oil, did not like geologists. He believed that you must drill only where there were blackjack bushes. We would have to assume that, based on his inflexible requirements, he would have never drilled offshore.

The pseudoscience of "creekology" was pursued by many early drillers. The theory, briefly stated, was that since oil often coated the surface of streams or springs, creekologists claimed petroleum could be found beneath the riverbeds or in the curve of rivers or creeks.

Another successful group claimed that oil would be found near cemeteries and recommended that you never drill near a sawmill. Historically, in America, cemeteries have been generally located on high ground to be away from the seepage of groundwater or streams. Sawmills were nearly always near the lowlands where water transportation was easy and a ready market was located. If you can imagine an area where the surface topography was controlled by a series of anticlines and synclines, you can understand why sporadic successes would lead drillers to this axiom. As geologists began studying drilling prospects, they would later come to understand why there was some merit to these axioms, but this occurred only after the science of petroleum geology had matured.

In the first few decades of drilling, geologists' recommendations were regarded very skeptically by drillers. Engineers often, with humor, suggest they should still be regarded with skepticism today; the science and practice of MEOR is regarded in the same manner by the majority of the petroleum industry.

Today, much of industry views MEOR as just another version of "creekology." They have heard of the cases where the application of MEOR techniques coincided with an increase in oil production, but industry is not convinced the success is due to the microorganisms. While laboratory success is important, success in a field demonstration is essential. In this day of disappearing financial resources and dwindling reserve bases, the average operator is unwilling to test laboratory success on his reserves. That is why the government was willing to make these demonstrations more attractive and less risky by supporting them financially.

There were successes in MEOR that captured people's attention, but it was unclear as to why they were successful, particularly when methods used in one location did not work as expected in other locations where conditions appeared similar. Operators no longer want to take the risk that the technique will not work, and have returned to their traditional, and sometimes equally unsuccessful, methods. I might point out here that this is an area where reservoir modeling with tools such as our Personal Computer reservoir simulation programs, validated with field data, can improve performance prediction and reduce risk--a must for independent operators.

With the opportunity to review the results to date, researchers were reminded of something they probably suspected all along: The approach to MEOR must be site-specific. The hope that one variety of microorganism would be found that is inexpensive, easy to maintain, flourishes on any nutrient supplied, and releases more oil to the wellbore is a dream. The reality, as we know it, is that each site has different problems and needs different approaches. Just as drillers approach each new well with its own particular well plan, a specific

plan must be devised for each particular MEOR field application. Unfortunately, many in industry believed in the dream, and when they were disappointed, they turned away from the whole concept.

If every site is different, most operators will never take the time and money needed to make a site suitable for a specific formulation. Instead, researchers must take the time to identify the organisms that are problem solvers under specified conditions. Then, rather than studying the site to find the right microbe or microbial combination, let the operator describe the problem or problems that must be addressed, and the researcher will prescribe the appropriate MEOR approach. It will be a collection of technical choices, with each choice catalogued and described, waiting to be selected for the proper situation.

We are making progress toward goals such as these, but it will take a concerted effort on the part of all of us for some time in the future. We have something to build on, however, for much has been done in the past. The Department of Energy has sponsored MEOR conferences and workshops since 1982. It is our mission to encourage research in areas of science that show promise for improving the recovery of oil from our domestic reservoirs. Part of that mission is now to transfer the technology to those in the industry who can use it to improve the efficiency of their oil recovery processes.

During our initial reviews of MEOR research and usage, one fact became very clear. Field trials are an essential part of the effort to educate and convince the operators of a technique's value. In regard to MEOR, operators had been spending a lot of time and money trying to rid their wells and production systems of microbes. It seemed ludicrous to tell them to put them back in. It was something that had to be demonstrated, so the DOE recognized the need to sponsor fundamental laboratory research leading to field tests of MEOR processes.

The public also had numerous concerns that the use of microorganisms in the oil patch would somehow create a hazard to the handlers or the near-well surface environment. Generally, this concern has been successfully dispelled by specific research and education in the field.

Unfortunately, some of the early field tests were not rigorously performed, and, in many cases, the only wells available for tests were those in advanced stages of decline, and the operators were willing to try anything as long as it was inexpensive or free. Many of these wells were so depleted or damaged that positive results were unlikely. After all, ten percent of zero is still zero.

The DOE has funded several field demonstrations using, if not prolific producers, at least sound wells and fields. Financial support for MEOR research in the last six years started in Fiscal Year 1986 with $600K, (Table 1). It peaked in Fiscal Year 1990 at $3,600K and dropped to $2,200K in Fiscal Year 1991. By 1992, support for the MEOR projects had decreased to $1,100K. Reduction in FY 1991 was mainly due to the termination of several MEOR contracts with no new MEOR starts. In FY 1992, the reduction was mainly due to the redirection of funds to the prioritized Class 1 Reservoir demonstrations. Over this entire period, 1986 to 1992, a total of $12,500K was funded by DOE for MEOR research by National Laboratories, National Institute for Petroleum and Energy Research (NIPER), universities, and small businesses (Table 2).

Three DOE-sponsored field demonstration projects are coming to an end, and final reports are being prepared. The field-wide flood of the Phoenix field in Oklahoma, with injected microorganisms and nutrients, was conducted by Rebecca Bryant and Anita Stepp of NIPER. Roy Knapp of Oklahoma University injected nutrients to encourage growth of indigenous microorganisms in the Vassar-Vertz field in Oklahoma. The third test was conducted by Franz Hiebert of Alpha

Table 1
MEOR History FY86 - FY92 ($000)

	FY86	FY87	FY88	FY89	FY90	FY91	FY92	Project Total
Alpha Envirnmental - MEOR Field Demo	249	75	350	193	0	0	0	867
BNL - Thermophilic	0	0	155	210	222	224	225	1,036
EG&G Idaho - MEOR & Wettability	0	0	1000	1000	1480	1492	600	5,572
NIPER - MEOR Waterflood Experiment	0	120	160	160	99	0	0	539
NIPER - Improved MEOR Flood	100	100	300	300	300	300	295	1,695
Injetech - New Processes for MEOR	0	0	0	50	175	0	0	225
Mississippi State U - MEOR of Indigenous Microorganisms	0	0	0	186	377	0	0	563
Oklahoma State U - Isolation Screening of Clostridia	64	0	0	0	0	0	0	64
State of Texas - MEOR Research (Annex V)	0	0	0	81	99	1	0	181
U of Oklahoma - Microbial Field Study	131	0	200	260	197	3	0	791
U of Michigan	0	0	0	0	155	0	0	155
BNL - Conference	0	0	0	0	0	60	0	60
U of Oklahoma - Conference	0	0	0	0	57	0	0	57
Farleigh Dickenson	0	0	0	0	49	0	0	49
U of Oklahoma - Novel/ Advanced MEOR Processes	0	0	0	0	154	0	0	154
Hardin Simmons U - Workshop	45	0	0	0	0	0	0	45
U of Oklahoma - Quantification of Microbial Products	0	0	0	123	219	103	0	445
TOTAL PER YEAR	589	295	2,165	2,563	3,583	2,183	1,120	12,498

Projects @ Stanford, U of Oklahoma, and U of Georgia were funded before 1986.
Two MEOR Workshops with U of Oklahoma were funded - in FY 81 and in FY 83.

Environmental in National Petroleum Reserve 3 (Teapot Dome) in Wyoming. Information on all of these projects will be presented during the conference.

Recently, the Hughes-Eastern Corporation, working with researchers from Mississippi State University, was selected for award under the recent Class I procurement. The project will test the ability of indigenous microorganisms to preferentially plug the more porous zones of previously waterswept areas of the reservoir. The project differs from other DOE-supported MEOR projects by using inorganic nutrients to stimulate the microbes to use oil as their carbon source, rather than an injected source, such as molasses.

The other area of recent interest to the MEOR program at the DOE is the process of single-well treatments to control or remediate wellbore or near-well problems. Research by private companies in the specific area of paraffin control has led to the development of microbial products and the formation of profit-making companies to apply these products in wells to reduce costs as well as to improve recovery rates.

Another aspect of MEOR that has recently developed is the modeling of microbial transport in porous media. Computer simulation has become a way of life for the petroleum engineer, and modeling programs are needed for industry to access the effect MEOR treatments or flooding would have in a site-specific case. Several contractors are developing and refining suitable models to accurately predict the microbial activity. The Bartlesville Project Office has developed user-friendly Personal Computer programs for reservoir simulation that are in wide use throughout industry, academia, and government.

All of this takes money, and money seems to be perennially difficult to obtain. As the DOE project budgets for Fiscal Year 1993 are undergoing approval, efforts are being made to maintain MEOR funding. At Bartlesville, we are developing programs that, if funded, will stimulate focused MEOR research leading to field testing and support the creation of a reference database.

Many researchers are looking at the President's National Technology Initiative recommendation for creating Cooperative Research and Development Agreements, known as CRADAs. The guidelines for these agreements were revised substantially in 1991 in response to industry and National Laboratory suggestions to speed negotiations and management of the projects.

CRADAs are created by three parties: the researcher, a private company, and the government. The private interest shares the cost of funding the research with the government, and then benefits from any new technology that evolves from the research. These agreements will leverage existing government allocations and ensure that there is an industry interest in the research being supported. Both Brookhaven National Laboratory and Idaho National Energy Laboratory are working to complete CRADAs with the Department of Energy and private companies.

Research in MEOR has led the researchers to ponder the uses of microorganisms for purposes other than enhanced oil recovery. As an example, next year in September 1993, the DOE's Office of Energy Research, an office separate from Fossil Energy, is sponsoring an international symposium on subsurface microbiology in Bath, England. The symposium will not be confined to MEOR, but will include some of the offshoot areas of interest, such as microbiology of nuclear and other hazardous waste disposal, microbial processes relevant to groundwater protection, and strategies for *in situ* bioremediation of subsurface contaminants.

In July 1992, that same office issued a call for grant applications for support of research on the origins of microorganisms in deep subsurface geological formations. This coordinated multidisciplinary program, with emphasis on field investigations, is designed to aid in the development of bioremediation strategies.

Table 2
MEOR History FY 86 - FY 92 ($000)

	FY86	FY87	FY88	FY89	FY90	FY91	FY92	Project Total
Labs	0	0	1,155	1,210	1,702	1,776	825	6,668
University	240	0	200	650	1,258	107	0	2,455
Industry	249	75	350	243	224	0	0	1,141
NIPER	100	220	460	460	399	300	295	2,234
TOTAL PER YEAR	589	295	2,165	2,563	3,583	2,183	1,120	12,498

Already, some of the MEOR research experiments sponsored by the Department of Energy have given results that point to very important ideas about bioremediation of surface sites near oil wells, such as oil tanks, mud pits, ponds, and oil-soaked soils.

Although seemingly simple, the actual complexity of MEOR research and development requires a multidisciplinary approach. Engineering principles, geologic descriptions, chemical assays, computer programming, and microbiological techniques are all necessary to sort out the riddles hidden in the science of MEOR. In addition, it is clear that, to survive, the science needs continued international exchange. Attendance at this conference includes representatives from Australia, Canada, China, Japan, Romania, Russia, Saudi Arabia, Trinidad, The United Kingdom, Venezuela, Norway, and the United States.

MEOR research is being conducted in all of these countries. Now, as we are being asked to examine our successes and failures and chart our future areas of interest, we need global input to those decisions. Our meeting between DOE, the Venezuelan representatives of the U.S./Venezuelan Cooperative Agreement on MEOR, and Fossil Energy MEOR contractors was very useful. The participants gave the Department of Energy significant input and their comments will help us to formulate our research strategy and subsequent plans for implementation.

The U.S. Congress will be deciding the Fiscal Year 1993 budget appropriations soon, and the Department of Energy will be faced with that fork in the road. Which projects show the most promise and should be funded? How shall we best proceed? During this conference, I urge all of us to participate in defining the best choices for the direction of our future research. By clearly defining our tasks we can meet the challenge to push MEOR science into maturity and dispel some of the industry's notion that MEOR is just updated version of "creekology."

M.O.R.E. to M.E.O.R.: An Overview of Microbially Enhanced Oil Recovery

T.R. Jack

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Abstract

For more than four decades, petroleum microbiologists have endeavored to develop technologies to enhance the production of hydrocarbon resources. Efforts have spanned a wide range of targets and met with variable success. This overview will attempt to organize the field in terms of the microbial mechanisms involved and to identify some of the factors and constraints which ultimately control the success of individual applications. The need for other disciplines and the implications of microbially enhanced oil recovery (MEOR) results in other areas continues to generate interesting opportunities.

1. EARLY HISTORY

The concept of using microorganisms to promote oil recovery from underground formations can be traced back more than sixty years [1]; however, the first practical demonstration that such a concept might be feasible did not occur until the 1940s. Research supported by American Petroleum Institute Research Project 43A, headed by C.E. Zobell at Scripps Oceanographic Institute, showed that anaerobic sulfate-reducing bacteria could release bitumen from Athabasca oil sands as well as conventional oil from laboratory test columns. On March 17, 1944, a U.S. patent application was filed describing a microbial process whereby oil could be released by bacterial activity in an oil reservoir [2]. Six potential mechanisms of oil release were identified:

- acid production to dissolve carbonate rocks
- dissolution of sulfate minerals
- production of gases to repressurize the reservoir and push oil out of pore spaces
- biofilm development on solid surfaces physically displacing oil
- production of biosurfactants
- viscosity reduction related to oil modification or gas dissolution effects

Several American oil companies became interested in these early ideas, and the first serious field test was carried out by Socony Mobil, in the Lisbon field, Union County, Arkansas, in 1954. By this time, sulfate-reducing bacteria had been dismissed as being ineffectual and potentially dangerous agents due to their ability to produce hydrogen sulfide, sour reservoirs, create plugging through iron sulfide formation and induce corrosion. Reconsideration of the originally proposed mechanisms led to the use of a *Clostridium acetobutylicum* species able to ferment molasses to give copious amounts of gas and organic acids along with solvents and surfactants under reservoir conditions. A U.S. patent was granted [3]. This was the first example of one of the primary strategies for microbial oil recovery enhancement, the use of injected organisms as factories underground to convert a cheap injected substrate (molasses) into agents of oil recovery (gases, surfactants, solvents, and acids) *in situ*. The field test showed that this strategy could work as a production enhancement tool within the context of an ongoing waterflood operation in an appropriate target reservoir.

While the Lisbon field test was successful, interest in exotic EOR technologies rapidly faded in the United States in the face of sustained supplies of cheap oil.

2. THE 1970s - FIELD DEVELOPMENT

For the following two decades, microbially enhanced oil recovery was actively pursued in the U.S.S.R. and in several countries in Eastern Europe.

In 1955, La Riviere correlated oil release with reduced surface tension in laboratory experiments using rapidly growing cultures of sulfate-reducing bacteria [4]. Although this work has been criticized [5], similar observations were reported subsequently from Czechoslovakia. Here, rapid growth of injected sulfate reducers with incremental oil release was seen in field tests using mixed cultures and injected molasses [6]. In the absence of other mechanisms, surfactant facilitated release of oil was assumed. These treatments often led to an ultimate decline in production possibly due to plugging effects.

Elsewhere in Hungary, Poland, U.S.S.R., and Romania, field tests through the 1960s and 1970s proceeded based on the injection of mixed anaerobic cultures including *Clostridia*. These cultures were selected on their ability to produce the types of agents identified in the earlier work by Socony Mobil from the fermentation of injected molasses. Many of these tests, especially those undertaken in Poland, were single-well stimulations by Karaskiewicz involving the injection of the microbial system, a period of shut-in, followed by back production from the same well. Analysis of results concluded that the gases, acids, solvents and surfactants produced by fermentation in the well bore region stimulated production through a cleaning action [7]. Use of small injections to stimulate single-well production proceeds commercially today.

Van Heiningen et al. 1958, suggested another target for microbial enhanced oil recovery [8]. This group proposed to improve the recovery from waterfloods by producing polysaccharide slimes *in situ* from an injected microbial system based on molasses. It was reasoned that the injected aqueous slurry of bacteria and nutrients would enter more permeable water zones already swept clear of oil preferentially and produce slime there. This would result in a localized loss of permeability and divert flood water to previously unswept zones. Substantial improvement in waterflood recoveries was reported. Selective plugging has now been recognized as an important additional mechanism of enhanced oil displacement. No further work in this area ensued in the decade that followed, although substantial effort was put into producing polysaccharides, such as xanthan or scleroglucan as viscosifying agents for EOR. These shear-thinning polymers improve the sweep efficiency of flooding operations by matching the viscosity of the "pusher" fluid to the target oil viscosity, thus reducing local fingering effects and oil bypass. The focus, however, became the manufacture and isolation of these agents in relatively pure form in surface facilities for subsequent injection as a chemical component in various flooding schemes

By the end of the 1970s, there was a substantial body of laboratory and field data on the mechanisms, strategies, and performance of various approaches to microbially enhanced oil recovery. This early work was critically reviewed in a comprehensive text by Davis in 1967 [9] and more recently by Updegraff [5]. Hitzman compiled a comprehensive summary of field tests done in this period [10].

By 1979, when more global interest in this area revived, a great deal had been achieved. The strategy of using mixed or pure bacterial populations as factories underground to convert cheap injected substrates into agents of oil release had been identified. The inability of petroleum to act as an efficient food source

for bacterial activity had been shown. The basic nature and existence of indigenous microbial populations in oil reservoirs had been established. Reservoir characteristics essential to a successful MEOR application had been deduced. The undesirable nature of sulfate-reducing bacteria had earmarked them as a potential problem in further development. Single-well stimulation, enhanced performance in waterflooding and selective plugging had been demonstrated as feasible field applications. At this point, unstable oil prices and an exploding interest in biotechnology generally prompted a new round of creative activity.

3. BACK TO BASICS

Activity through the 1980s took place in many countries on many themes with varying degrees of secrecy and success. In general, target selection and systems development became increasingly explicit. The era began with a review of fundamentals. Several reviews of early work appeared, including one for existing patents [11]. In Oklahoma, an extensive database of American reservoirs was reviewed to assess the potential target for microbial processes limited by temperature (<75° C), salinity (<10%), pH (4 - 9), oil gravity (>17 degrees API), and the permeability of the formation (>75 mD). Of nine states considered, all but Mississippi showed that 20% or more of known reservoirs were eligible candidates for a microbial technology, while California showed that more than 50% of known reservoirs could be targets [12]. This optimistic assessment spurred interest.

The anaerobic use of oil by microorganisms was revisited in two independent studies [13,14]. Both concluded that oil degradation occurred in the virtual absence of oxygen but that the process was exceedingly slow. Paraffinic or alkane rich crudes were found most susceptible consistent with earlier observations by Muller in 1957 [15]. Radioisotope studies confirmed the presence of labelled carbon in the methane and carbon dioxide produced. The conclusion was that injected nutrients were essential to foster significant bacterial activity *in situ* in the time frame of a practical EOR scheme. This entails the necessary constraint that microbial action in the reservoir is limited by the amount of nutrient that can be introduced. Recently, publications on anaerobic hydrocarbon degradation [16] have begun to appear. These will have an immediate effect on the bioremediation of subsurface hydrocarbon contamination and may have long-term implications for MEOR.

3.1. Practical challenges

A complete MEOR system consists of microorganisms, a nutrient package, and such other amendments as may be needed to promote a desired effect *in situ*. Several common problems face any such system:

- avoidance or repair of lost injectivity due to well bore plugging
- successful dispersion/transport of all necessary components to the target location in the reservoir
- promotion of desired metabolic activity *in situ*
- preclusion of competition or undesirable secondary activity by indigenous organisms, including sulfate-reducing bacteria.

The 1980s saw systematic work in all these areas.

3.1.1. Injectivity

Injection of microbial systems can result in plugging of the formation face in the injection well, resulting in a compromised ability to introduce further material into the reservoir. Successful injection [17] requires the following:

- removing particulates in nutrient solutions by filtration before injection
- selecting microorganisms of appropriate size (preferably as small as possible and singly dispersed if indigenous organisms cannot be used)
- an absence of polymer production (even soluble polymer production) during injection
- an absence of microbial gas formation during injection
- control of microbial absorption to rock surfaces in the injection well.

Sacrificial agents consisting of cheap, treated pulp-mill effluents were used to control adsorption of *Leuconostoc* cells on clays [18]. Use of dormant cell forms such as spores and ultramicrobacteria, were suggested as a means of minimizing adsorption problems [11, 19]. Elf Aquitaine patented a complex injection sequence involving surfactants, polymers, and hydrocarbonaceous compounds for the same purpose [20] and supported research which led to the development of a model for bacterial transport in porous media based on adsorption phenomena [21].

Where surface fouling or filter cake formation does lead to a loss of injectivity or production, reperforation has proven to be an effective remedial action in the field [18]. Use of bleach to remove biomass plugs [11, 22] has been successfully shown although chlorine dioxide proved less corrosive [23]. Strong chelating agents also may be used [11].

3.1.2. Transport

Once inside the formation, all components of an injected system must travel through the reservoir to arrive at the target site at the same time [24]. For near-well applications, such as paraffin removal, this is relatively trivial, but for deep reservoir treatments, variable absorption losses and rates of travel for nutrients requires carefully timed injection sequences. Complex injection protocols were recently patented by Phillips Petroleum Company [25]. Patents also were granted on specific forms of phosphate for injection with bacterial systems by Chevron [26]. Microencapsulation of nutrients for controlled reservoir release is being investigated [27].

Early studies on growth and diffusion of bacteria through rocks suggested that rock of surprisingly low permeability can be penetrated, but rates are too low to provide a practical means of placement for MEOR schemes [24]. Even motile organisms travel at rates less than 0.5 cm/hour, which decreases logarithmically with permeability in sandstone cores below 100 mD permeability [28].

Active transport of bacterial cells through porous media remains poorly understood. Viable organisms tend not to behave as simple particulates. Multiple mechanisms of cell retention appear to occur [29]. At least one of these mechanisms may be a "log jam" effect by clusters of cells caught in pore throats because trapped cells can be remobilized simply by shutting off and restoring flow [30]. This suggests that pulsed injection protocols may be beneficial in deep placement of cells in a reservoir.

In general, transport of cells through formations of less than 75 mD is regarded as being impractical [12]. On this basis, concerns about low permeability constrain application of MEOR in fifteen of thirty-five of the largest oil reservoirs in the United States [24]. Transport limitations are one of the biggest constraints on the potential of MEOR, but singly dispersed cocci [30], spores [11], ultramicrobacteria [19], and indigenous organisms [31] provided feasible MEOR agents for targets of increasing difficulty in terms of decreasing permeability or the need for extensive penetration.

In the early 1980s, laboratory studies on microbial transport were modelled using a deep filtration model. This model showed discouraging prospects for deep placement of MEOR systems [32]. Recently, more sophisticated simulators were fit

to laboratory data [31-35], but predictions remain a function of empirical correlations. Simulation at this point provides an interesting tool for considering possible MEOR schemes, identifying key parameters in injection plans, and refining our understanding of a target reservoir based on observation of bacterial travel in the field. Further development of simulators, in conjunction with clarification of mechanisms involved in viable cell transport, promises to lead to much more efficient design of MEOR systems with a significantly increased probability of success. As for any EOR application, the availability of accurate, detailed information on reservoirs will always limit the precise prediction of field performance.

It is interesting that field test results suggest much more facile transport of injected bacteria through the reservoir than laboratory studies and dependent modelling would suggest [10, 36]. This may be in part due to a poor understanding of the specific reservoirs involved.

3.1.3. Metabolism *in situ*

Past oilfield sampling [28] as well as deep drilling programs, such as the U.S. D.O.E.'s Deep Microbiology Program, suggest that there are organisms deep underground.

Temperature, pH, and salinity are usually cited as constraints for MEOR applications. Both pressure and temperature increase with depth in the earth's mantle but limiting temperatures are reached for microbial metabolism before prohibitive pressures. Nevertheless, accessible pressures can alter microbial growth characteristics and toxicity effects [37]. In thirty-five large U.S. oil reservoirs examined as targets for MEOR, temperature was identified as a limiting factor in five cases [24]. Development of useful thermophiles could significantly extend the accessible target range for MEOR [38]. Injection of huge volumes of surface water can reduce temperatures of reservoirs significantly near injector wells. This was noted in North Sea waterfloods. In this circumstance, thermal limitations on microbial activity may be decreased over time.

Salinity and pH are less restricting. Freshwater slugs injected into a reservoir mix poorly with connate brines in the formation and tend to travel as discrete slugs [30]. Thus, sensitive organisms, such as *Clostridia*, can be used in MEOR schemes in saline fields provided they are injected in a freshwater slug.

3.1.4. Competition and secondary activity

Analysis of produced fluids and gases from field tests suggests that for MEOR tests involving injected nutrients like molasses, competition by indigenous organisms has not been an overwhelming concern [30].

Secondary activity, especially by sulfate-reducing bacteria, is more of a problem. Original field tests done in Czechoslovakia where *Desulfovibrio* were intentionally injected into the reservoir failed to note significant hydrogen sulfide production, possibly due to the low sulfate content of the reservoir [9]. Similarly, excess barium in the Standard Hill field precipitates sulfate and probably precluded use of the lactic acid produced by injected *Leuconostoc* in other field applications [30].

The observation that nitrate at low levels suppresses hydrogen sulfide production [13, 39] has prompted its inclusion in nutrient packages for field tests [31]. Recently, the injection of a sulfide tolerant *Thiobacillus denitrificans* strain was patented as a means to control the net production of sulfide [40]. In the presence of nitrate, sulfide is oxidized to sulfate.

3.2. Field results and new ideas

Work on the logistical challenges provided a context for developing new ideas and refined approaches in the field. Field tests undertaken in the 1980s generally became more ambitious and better documented [36, 41].

3.2.1. Enhanced oil recovery

In the United States, emphasis was placed on assisting marginal production operations important to sustaining domestic production and reservoir access. The field test run by the National Institute for Petroleum and Energy Research (NIPER) at the Mink Unit in the Delaware-Chivers field in Nowata County, Oklahoma, showed incremental production from a waterflood treated with a proprietary microbial system [42, 43] at minimal cost. Hundreds of single well treatments aimed at control of paraffin deposition were undertaken commercially [44, 45]. While the percentage of successes is encouraging, more work is needed to perfect this approach. Mechanistic studies [J. Boivin, personal communication] suggest that biosurfactants are major agents of paraffin removal. These systems have been extended with success to resuspension of sludges in tank bottoms and may provide a broadly applicable technology.

Ivanov and Belyaev reported successful stimulation of indigenous bacteria by introducing oxygen into a reservoir, which resulted in the restoration of oil production at the Bondyug field in Tatar [46]. Using indigenous organisms avoids many application problems and costs.

Wagner described the successful enhancement of oil production from a carbonate reservoir in the Zechstein formation in Germany [47]. Both "huff and puff" and flooding treatments were tried using a *Clostridial* species. Increases in oil production from 50 to 150 tons per month were observed along with a substantial decrease in water cut in the flooding operation. The "huff and puff" treatment resulted in a twofold increase in fluid production presumably due to carbonate dissolution in the well bore region.

A patent was issued on the use of *Thiobacillus denitrificans* to generate acids and dissolve formation carbonates through the oxidation of reduced sulfur species in the presence of nitrate [48].

Another new concept for enhanced production was developed in Australia [49, 50] and was successfully demonstrated to increase oil production by 40% over eighteen months in the Alton field in Queensland. The approach is called Biological Stimulation of Oil production (BOS). The process generates ultramicrobacteria from the indigenous bacteria in the reservoir through nutrient manipulation. The wettability of these cells changes, resulting in oil release and formation of emulsion in the reservoir prompted by the cells themselves.

Lazar reported continuing efforts in Romania injecting nutrients and bacteria for a variety of applications related to well-bore cleaning and stimulation [41]. Yulbarisov [51] reported similar field operations.

Successful MEOR applications remain focused on struggling waterfloods where a continuous water phase enables us to introduce the technology, or on single-well stimulations, where its low cost makes it a method of choice.

3.2.2. Selective plugging

A field test of a *Leuconostoc*-based plugging system, based on sugar beet molasses, was reported for a heavy oil field in Saskatchewan, Canada [30]. Insoluble dextran production tripped *in situ* by the introduction of sucrose has been shown to plug unconsolidated sand formations of the type found in the target reservoir. The objective was to plug water channels from an edge aquifer responsible for the watering out of many wells on primary production deep in the field. Results showed that the organisms carried out the planned metabolism

underground, but the nature of the channel system proved surprising. A low-volume, extensive web of high-velocity water channels resulted in the injected microbes being carried more than a kilometer from the injection point.

A new concept for selective plugging was reported by Cusak et al. at the University of Calgary, Canada [19]. Ultramicrobacteria (UMB) formed by selective starvation was shown to be very effective at pervading sand formations with little or no plugging or loss of injectivity. Subsequent feeding results in regrowth and plugging. The system offers the chance to treat tight formations to form deep plugs. Because the ultramicrobacteria are a dormant stage, field inocula would be easily shipped and handled and would have a long shelf-life.

Another new concept in selective plugging is the use of biomineralization to form calcite cements capable of sand consolidation and perhaps fracture closure in carbonate formations [52]. Feasibility was shown and the process was patented. The action of a urease-producing bacterial species causes the hydrolysis of injected urea to shift the pH of a saturated calcium bicarbonate solution from neutral to 9. This results in rapid calcite deposition to give thermally stable, durable cements or plugs depending on the degree of application.

Selective plugging strategies remain the most promising in terms of significantly changing the economics of oil production. While stimulations, cleaning treatments, and enhanced waterflooding can improve economics and extend production life, flow diversion and control of coning problems have the potential to radically alter producible reserves in flawed reservoirs.

4. SPIN OFFS

Research on microbially enhanced oil recovery has many implications for associated areas. Petroleum microbiology and recovery have much to offer in designing and performing bioremediation operations for contaminated industrial sites. Oil field problems arising from related detrimental microbial activities result in corrosion, souring, well-bore plugging, and chemical additive degradation. Water-well fouling, transport of viruses and pathogens through soil, and loss of permeability in agricultural soils are related issues of concern.

Products and processes arising from MEOR spin off into other applications. The use of biosurfactants to pipeline heavy oil as an oil in water emulsion, facilitate bitumen separation from oil sands, enhance oil recovery, and clean out tank sludges have been spawned as independent activities. Use of bacterial cells as de-emulsifiers is the inverse of the BOS process. Desulfurization and other forms of upgrading are related as is the commercial production of biopolymers for a host of purposes, including enhanced oil recovery.

Microbially enhanced oil recovery has provided a challenging and stimulating field of study for the further definition and refinement of a range of technologies and matters related to both oil production and ecology.

5. CONCLUSION

Many uncertainties related to the application of MEOR systems have been resolved. Field projects continue to build a record of successful applications. The low capital and operating costs and environmentally safe nature of the technology prompt its consideration. Continuing innovations, improved simulation tools, and more subtle yet more practical approaches increase appeal. Patents

by some major companies and profitable exploitation of clean out technologies by minor players suggests sustained activity will be seen through the 1990s. Synergy with environmentally driven research on bioremediation will increase the effort on MEOR. All this bodes well for the future.

Hurdles of familiarity and predictability remain. Low oil prices and an enduring recession in North America have produced a conservative environment. The persistence of focus on MEOR as a late life strategy suited to extending or improving marginal production operations suggests that it will be the selective plugging technologies that may see the next significant step in the scale and profit of application.

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Use of Natural Microflora, Electron Acceptors and Energy Sources for Enhanced Oil Recovery

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Abstract

Oil reservoirs naturally contain inorganic and organic materials which can be exploited through simple supplementation to support the growth of microorganisms, which aid in releasing oil from the rock matrix. Other compounds, which may serve as nutritional sources for microorganisms, are added to reservoirs during production and operation of oil fields. These materials include sulfate, nitrate, carbonate, volatile fatty acids, nitrogen-containing corrosion inhibitors, phosphorous-containing scale inhibitors, and trace elements. Our experiments show that, with minimal supplementation, growth of naturally occurring microorganisms can be used to produce viscosifying agents to aid oil recovery. This natural microflora is also capable of removing sulfide from oil reservoirs and preventing the formation of new sulfide leading to more oil recovery and increased value of the produced oil. The metabolic products of these microorganisms are CO_2, water, N_2, and oxidized forms of sulfur, all of which are environmentally innocuous. Laboratory experiments with defined mixtures of microorganisms as well as mixed populations release more oil from sand pack columns.

1. INTRODUCTION

The ability to produce more oil from aging and played out oil fields may have a great effect on the future energy dependence of the United States [1,2]. Classically, inexpensive, carbohydrate-containing carbon substances with some mineral nutrients are injected into wells to stimulate naturally occurring or introduced microorganisms. These organisms are designed to play various potential roles, including selective plugging, solvent and organic-acid formation, surfactant production, gas formation, and possibly, oil degradation. The growth of these microorganisms necessarily causes a change in the environment of the formation, especially near the well bore, and clogging often occurs.

The economical and technical potential for microorganisms to help in the release and mobilization of oil is great. We believe that instead of overwhelming the reservoir environment, the potential is great for making small changes, which could significantly increase oil production. The anaerobic environment of virgin oil reservoirs is not usually conducive to the growth of microorganisms because of the lack of vital nutrients. Otherwise, oil would never be formed. However, small changes brought about by the penetration of the reservoir and production of the oil can cause rapid growth of microorganisms with a concomitant change in the reservoir ecosystem. Some of these changes can be detrimental, such as souring due to hydrogen sulfide formation. This process is underway in some major oil fields, including some Alaskan fields. The subtle change in Alaska is probably due primarily to the use of seawater in waterflooding operations as well as the addition of chemicals to aid in surface production operations. With seawater comes high levels of sulfate, completing the necessary mineral balance required for sulfate-reducing bacteria (SRB) to flourish. This then leads to souring of the field and results in lower quality

crudes and higher operating costs. Thus, this subtle change has brought about an enormous change in the reservoir ecology and field operations. Our approach to MEOR seeks such subtle changes in the reservoir environment, and equivalent, but positive, changes in field characteristics, which will lead to the increased production of better quality oil.

2. MATERIALS AND METHODS

2.1. Microorganisms and culture methods

Mixed cultures of microorganisms were isolated from produced water from various oil fields, including primarily carbonate reservoirs and sandstone reservoirs. Water from the Kuparuk and Prudhoe Bay oil fields in Alaska were sources of both water for analysis and microorganisms. Strains of *Thiobacillus denitrificans* were obtained from the American Type Culture Collection and isolated from local soil. These strains also were obtained from the Soda Dam thermal area in New Mexico and were cultivated in the following medium (g/l): $Na_2S_2O_3 \cdot 5H_2O$ (5.0), NH_4Cl (1.0), KNO_3 (2.0), KH_2PO_4 (2.0), $NaHCO_3$ (2.0), $MgSO_4 \cdot 7H_2O$ (0.8), $FeSO_4 \cdot 7H_2O$ (0.02), Trace Metal Solution SL-4 (1 ml) [3] (final pH 6.5). For the dissolution of limestone, a medium with the following constituents was used (g/l tap water): reduced sulfur source (may be Na_2S, $Na_2S_2O_3$, S^0, or $Na_2S_4O_6$) (5.0), NH_4NO_3 (2.5), KH_2PO_4 (0.1) and crushed limestone (>100 mesh) (2.5). For the growth of sulfate-reducing bacteria the following medium was used (g/l): $MgSO_4 \cdot 7H_2O$ (2.0), Na citrate (5.0), $CaSO_4$ (0.5), NH_4Cl (1.0), K_2HPO_4 (0.5), Na acetate (2.5), Na propionate (0.5), Na butyrate (0.1), yeast extract (0.1) and a 5% solution of $Fe(NH_4)_2(SO_4)$ (20 ml) (final pH 7.5). The medium was reduced in an anaerobic chamber, dispensed in serum bottles, capped and sterilized. The head space contained 5% CO_2, 10% H_2 and 85% N_2. Samples were injected with a syringe. Acid-washed mill creek sand was used for sand pack experiments and Kuparuk (Alaskan) crude oil was used in experiments where crude oil was required.

3. RESULTS

The inorganic constituents in oil field ecosystems may be manipulated to achieve certain results. When examining produced waters from various oil fields, it was found that many fields contained sufficient quantities of most mineral and other nutrients for the growth of various classes of microorganisms. Table 1 shows an average composition of produced water from an Alaskan oil field after flooding with seawater. This water will support the growth of SRB without the addition of other constituents, although the addition of more N and P greatly enhances growth.

A study of this environment revealed that a microbial balance might be achieved through the cooperation of two common microbial processes. The first process is the ongoing, natural souring due to hydrogen sulfide production mentioned above. This is defined by the following equation:

$$\text{Sulfate} + \text{Volatile Fatty Acids} \dashrightarrow \text{Cells} + CO_2 + H_2S \qquad (1)$$

This reaction operates in some fields in the process of souring. The SRB, which are responsible for these reactions, are capable of using a wide variety of soluble organic substances as carbon and energy sources [4-10]. The volatile

Table 1
A typical composition of produced water from an Alaskan field

Component	Concentration (ppm)
Chloride	11400
Sodium	7100
Bicarbonate	2600
Sulfate	240
Calcium	94
Magnesium	56
Carbonate	50
Iron	6
Nitrogen	0.5
Phosphorous	0.1
Total Dissolved Solids	2.2%
Acetate	1000
Propionate	80
Butyrate, isobutyrate, C_5 acids	10
Other dissolved organic substances	300
pH	8.1

fatty acids, which are quite common in oil reservoirs [11], are good substrates for SRB to drive this process.

Thiobacilli are not normally considered anaerobic bacteria and would not be considered as potential organisms for MEOR processes because of their typically aerobic nature. However, *T. denitrificans* is capable of anaerobic growth if nitrate is supplied as an electron acceptor. The overall growth is defined by the following equation:

$$CO_2 + NO_3^- + \text{reduced S} \dashrightarrow \text{Cells} + N_2 + SO_4^{=} + H^+ \quad (2)$$

Combining equations 1 and 2 yields:

$$\text{Volatile Fatty Acids} + NO_3^- \dashrightarrow \text{Cells} + N_2 + H^+ \quad (3)$$

Equation 3 also describes heterotrophic denitrification, with the addition of acid production. The formation of acid may then dissolve carbonates leading to the formation of dissolved CO_2. In addition, this dissolution of carbonate buffers the environment at approximately a pH of 6.5, which is optimal for the growth of *T. denitrificans*. Thus, an oil field with some carbonate and a reduced S source (sulfide) may only require the addition of nitrate and minerals for growth and oil release.

To test these ideas, initial laboratory studies followed the sequence below:

1. Isolation and characterization of sulfate-reducing bacteria from oil-field-produced water.

2. Isolation and characterization of denitrifying strains of *Thiobacillus* and their reaction with limestone.

3. Sequential feeding studies.

4. Mixing and oil-release experiments.

5. Viscosifying agents and heterotrophic denitrifiers.

3.1. Isolation and characterization of sulfate-reducing bacteria from oil-field-produced water

Desulfovibrio desulfuricans and *Desulfotomaculum* were obtained from the American Type Culture Collection. These organisms were cultured on a minimal medium containing acetate, propionate, and butyrate as carbon sources. We also isolated SRB strains from material obtained from Alaskan oil field pipelines and other pipeline fouling materials. These organisms were very common in these samples and could use a wide variety of organic substances including the common volatile fatty acids normally found in produced waters. These organisms were active from 30-75°C and could use acetate and propionate individually or together. When both acetate and propionate were present, the propionate was used preferentially over the acetate.

3.2. Isolation and characterization of denitrifying strains of *Thiobacillus* and their reaction with limestone

All denitrifying *Thiobacillus* sp. that we tested will dissolve limestone, using it as their sole source of carbon (Tables 2 and 3). The examples cited below use *T. denitrificans* ATCC 25259 and ATCC 23642, as well as some wild isolates from our laboratory experiments and enrichment studies, including moderately thermophilic isolates (40-45° C). There are no known true thermophilic strains of denitrifying *Thiobacilli*. We also isolated proprietary strains from produced waters and other natural oil field related sites and geothermal areas.

These results directly reflect the ability of *T. denitrificans* to use the reduced sulfur source. Thiosulfate is the best energy source for *T. denitrificans* and, although sulfur also is a very good energy source, it presents physical problems for the organism, which makes it more difficult for them to derive all available energy from this energy source. Sulfide is potentially a very good energy source because of the available chemical energy, but its relatively high toxicity impairs its use as an energy source.

Gaseous products produced by denitrifying bacteria under conditions which might occur in an oil reservoir were determined, i.e., reducing conditions [redox potential about -100 mv, sulfide as an energy source, high nitrate concentrations]. We tested three sulfur sources as sources of energy: thiosulfate, sulfide, and elemental sulfur. The cultures were sparged with He before sealing. The produced gases were then collected and subjected to gas chromatographic separations and quantitations. A key indicator of active denitrification is the production of nitrous oxide (N_2O) and nitrogen by the organisms. Copious N_2O was formed by most strains of *T. denitrificans* when using thiosulfate as an energy source. ATCC strains 25259 and 23644 both produced N_2O while using elemental sulfur (S°). ATCC strains 29685, 23642, and 25259 produced nitrous oxide with sulfide (S^-) as an energy source although growth appeared to be much less when compared with growth with thiosulfate. In addition, large amounts of nitrogen were produced by all cultures tested with each of these sulfur energy sources.

Table 2
Dissolution of crushed (≥ 100 mesh) and solid limestone with thiosulfate as energy source

	Crushed Limestone	Solid Limestone
Strain	% Dissolved	mg/l Dissolved
ATCC 25259	88.3	740
ATCC 23642	82.9	760
Buck Tail	85.0	723
Meadow Creek	86.1	635
1 N HCl	87.4	nt[1]
No Microorganisms	11.6	nt

[1]not tested

It appears from the data in Table 2 that there is approximately 12-17% acid insoluble material (probably silicates) in this limestone sample.

Table 3
Dissolution of solid limestone with sulfur and sulfide as energy sources

	Sulfur	Sulfide
Strain	mg/l Dissolved	mg/l Dissolved
ATCC 25259	380	60
ATCC 23642	320	160
Buck Tail	370	30
Meadow Creek	330	35

Sulfide is inhibitory (>80 ppm) and must be kept at low concentrations. Resistant strains, which can tolerate up to 200 ppm sulfide, are readily obtained. We tested the profiles of sulfate-reducing bacteria grown in the presence of the same medium as *T. denitrificans*, but with added iron, organic acids (acetate, lactate, propionate, butyratem, and formate), and reducing power to about -200 mv. The gases produced under these conditions are primarily CO_2 and H_2S. Traces of ammonia also are formed in these cultures. We have not been able to show significant methane production in any of our cultures although low levels of methanogens are present in many of our oil field samples.

The amount of limestone dissolved could open new channels for water flow and have significant effects. In addition to the effect of limestone dissolution, *T. denitrificans* could also have the effect of the typical MEOR microorganisms, i.e., selective plugging by the microbe bodies of well washed channels, production of considerable N_2 gas for well pressurization (enough N_2 gas is

produced in some cultures, that if the vessel is sealed tightly then the gas pressure can actually cause a glass tube or bottle to rupture), and production of increased CO_2 pressure through the dissolution of carbonate, which is in equilibrium with CO_2, and then may dissolve into the oil, making it more mobile. With optimization, about 1 g of limestone can be dissolved by *T. denitrificans* per liter of culture in the laboratory. A waterflood, which injects 15,000 liters of water per day, could dissolve about 15 kg of limestone per day or 2 to 3 tons of carbonate could be dissolved in a year, if the microorganisms are continuously and actively growing. This is not likely to happen. However, these statistics show the great potential of using *T. denitrificans* in MEOR processes. The limestone supplies the CO_2 necessary for the growth of *T. denitrificans* by the dissolution of carbonate, which is in equilibrium with dissolved CO_2. Ordinarily, CO_2 is supplied to *Thiobacilli in vitro* in the form of dissolved carbonate or bicarbonate. The dissolution of carbonate buffers the growth medium of the culture to a constant pH of 6.5, which is optimum for the growth of *T. denitrificans*. All acid produced during growth from the oxidation of reduced sulfur compounds is thus neutralized by the dissolution of limestone. The necessary added nutrients are ammonia and nitrate in the form of ammonium nitrate (supplying both the alternate electron acceptor and a nitrogen source for the organisms), a small amount of phosphate (only just enough to satisfy the nutritional requirements of the microbes; otherwise, the buffering capacity of the phosphate may interfere with the dissolution of the limestone), a reduced sulfur source necessary for the energy reactions of the cultures (this may, theoretically, be any of the following: sulfur, thiosulfate, tetrathionate, or sulfide), and trace metals (most brines contain high enough concentrations of B, Zn, Cu, Co, Mn, Fe, Mo, W, Ni, and Se to satisfy the requirements of typical denitrifying microbes for trace elements). As can be seen, under the best conditions, the only necessary materials, which would need to be injected into a well for MEOR processes, would be ammonium nitrate and low concentrations of phosphate. Under the least ideal conditions this list would include ammonium nitrate, phosphate, a reduced sulfur source, the microbes and possibly some trace metal nutrients in very low concentrations.

3.3. Sequential feeding studies

Under normal laboratory conditions, both the SRB and the denitrifying *Thiobacilli* grow at roughly the same rates. Therefore, the organisms might be capable of coexisting and supporting each other. If *T. denitrificans* and *Desulfovibrio* can be mixed in a single culture then the naturally occurring fatty acids will drive sulfate reduction, which will serve as the energy source for the denitrifying *Thiobacilli*. These organisms exist mixed in nature, but it is difficult to know whether or not they can be artificially mixed in a non-typical environment and both be active at the same time.

These experiments were performed with the following results. We grew various species of *Desulfovibrio* in media containing acetate and propionate (25:1 on a molar basis, which is the typical ratio of these volatile fatty acids found in oil field waters, see Table 1). This medium was then filtered through a .22 μM filter, and sodium bicarbonate and ammonium nitrate were added. The medium was then inoculated with *T. denitrificans*, which grew well as long as the sulfide content was not too high (<80 ppm). *T. denitrificans* did not grow in the medium if no reduced sulfur was present.

The cultures were then linked directly by a two-stage culture. This culture was designed to supply the necessary reduced sulfur sources as sulfide from the growth of the sulfate-reducing bacteria to the denitrifying bacteria in the

second vessel. The first vessel contained all of the necessary nutrients for sulfate-reducing bacteria, and after 12 days, good growth of the latter was evident. Helium was passed through the first vessel to scrub the sulfide as hydrogen sulfide out of the culture and introduce it into the second vessel, where the medium was complete for the growth of the denitrifying bacteria, except that it lacked a reduced sulfur source. Growth commenced 3 days after the first vessel began to turn black but was not very vigorous. The first vessel supported the growth of $5x10^8$ sulfate-reducing bacteria/ml, and the second vessel contained about $2x10^8$ denitrifying *Thiobacilli* per ml culture.

3.4. Mixing and oil release experiments: sand pack experiments

Sand was saturated with oil over 1 week exposure, and was then washed very slowly with a reverse gravity flow of water, until very little was released over 24 hr. This washing took approximately 2 weeks. This sand was then drained of free water and introduced into graduated cylinders. A liquid, which contained artificial produced water (Table 1), as well as different carbon and energy sources and ammonium nitrate was added. Air was removed by sparging with He. Released oil was measured in the graduated cylinder as oil released and found on the surface of the liquid. Table 4 shows the results of this experiment.

These data are very revealing. The addition of a non-fermentable carbon source, such as acetate, by itself does not increase oil release, which would be expected because that is the case in many oil fields. The introduction of a fermentable carbon source (sugars), such as found in molasses, increased oil release. This is the "old" MEOR technology which was shown to have some applicability. However, the additional additive of nitrate with not only fermentable carbon substances, but also those which cannot be readily fermented (acetate and propionate) also significantly increases oil release.

Sand packs also were used to simulate cores. A 2.5 x 70 cm column was packed with acid washed sand and 0.1% w/w crushed limestone. The column was fed from the bottom using a hydrostatic head. The feed was the nutrient solution at a redox <-300 mv. One hundred mesh iron filings were included in a layer to detect the formation of FeS. After two weeks, the column became slightly more blackened at the layer of the iron filings and much gas was produced in the sand matrix above the iron layer. This gas was shown to be nitrogen and some carbon dioxide, consistent with what would have been expected from the process of denitrification. In addition, the effluent water contained no acetate or propionate. *T. denitrificans* was found in the effluent at approximately 10^9/ml, and sulfate-reducing bacteria also were found in the effluent at about 10^4/ml.

However, once the systems were not controlled for pure cultures, heterotrophic denitrifiers became dominant and used the acetate, propionate, and nitrate for growth. They did not prevent the growth of sulfate-reducing bacteria nor the *T. denitrificans*, but they became more than 90% of the total microbial population. Simply put, the addition of nitrate to oil field waters prevents the growth of sulfate-reducing bacteria because denitrifiers are more efficient and more rapidly growing organisms. They also metabolically remove sulfide. The major result of this experiment is the demonstration that sulfate-reducing bacteria are completely inhibited, but not killed, by the introduction of nitrate into their environment.

3.5. Viscosifying agents and heterotrophic denitrifiers

We have shown that the heterotrophic denitrifying bacteria found in produced water of oil fields are uniformly able to produce cultures of high viscosity when starved for ammonia. Many denitrifying bacteria are not capable of operating both dissimilatory and assimilatory-nitrate reducing processes simultaneously.

Table 4
Sand pack oil release studies

Additions to Water	Oil Released ml	% Increase over Control
None (control)	.75	0
Nitrate	.8	6
1000 ppm acetate	.75	0
100 ppm propionate	.85	13
Acetate + nitrate	1.64	119
Propionate + nitrate	1.55	107
Acetate + propionate + nitrate	1.45	93
Molasses (.2%)	1.25	67
Molasses + nitrate	1.38	84

Therefore, the addition of the Na or K salt of nitrate causes the cultures to become nitrogen starved; under these conditions, excess carbon is transformed into viscous extracellular polysaccharides. Cultures grown with acetate and propionate and acetate:propionate mixtures readily showed the formation of viscosifying agents (150-500 centipoise).

4. DISCUSSION

Several conclusions can be drawn from these experiments. These are outlined below.

1. In reservoirs undergoing souring, significant populations of sulfate-reducing bacteria occur. These microorganisms may be supported by minerals native to the reservoir and simple dissolved organic material in the formation water. Even without significant dissolved organic material, SRB are capable of autotrophic growth in oil reservoir environments where sulfate and other mineral nutrients are available [12-14]. They also are capable of coupling sulfate reduction to the ammonification of nitrate [15]. Often sulfate is absent from formation waters, and therefore, the growth of sulfate-reducing bacteria is not favored. However, when waterflooding operations begin, new substances, such as sulfate, are introduced into the reservoir, and SRB then become active and powerful geochemical agents.

2. In sour or souring carbonate-containing reservoirs, which have negligible levels of simple dissolved organic materials, active populations of denitrifying *Thiobacilli* can be supported by the introduction of nitrate and low concentrations of other mineral nutrients. Under these conditions, SRB and the denitrifying *Thiobacilli* form a mutually supporting microbial population. This work led to US patent 5,044,435 [16]. There are almost always metal sulfides present in oil formations, and we showed that denitrifying bacteria are capable of solubilizing these, releasing the sulfide for metabolic purposes. Other *Thiobacilli* are well known for this property. These microorganisms may be very

beneficial for MEOR processes, both for oil release and sweetening of the reservoir.

3. In typical reservoirs, which have complicated mixtures of available nutrients, such as simple dissolved organic substances (volatile fatty acids) and reduced sulfur compounds, the introduction of nitrate will produce several effects. First, heterotrophic denitrifying bacteria will rapidly become predominant and will slowly remove sulfide (both dissolved sulfide as hydrogen sulfide and some crystalline metal sulfides through solubilization). The growth of these organisms rapidly inhibits the growth of SRB and inhibits new sulfide formation. This sulfide metabolism is not involved in energy metabolism. Second, these microorganisms, given enough nutrients, will completely oxidize the simple dissolved organic compounds to CO_2 with concomitant production of nitrogen gas. The contribution of CO_2 produced by these organisms directly to oil mobilization may be great. CO_2 floods are an accepted and valuable EOR technique. The possibility of the completely removing dissolved organic materials from the reservoir is quite real and, after that removal, organisms such as *T. denitrificans*, may then become established. Third, they may form highly viscous solutions, if the available nitrogen is limited. All of these effects could be positive factors for MEOR processes. An additional possible advantage is that, because the alterations to the natural environment are minimal and simple, it may be possible to avoid well bore clogging by microorganisms since the nutrients could more readily penetrate into the rock formations.

5. ACKNOWLEDGEMENTS

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Bug Rock: Bacteriogenic Mineral Precipitation Systems for Oil Patch Use

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Abstract

Bacteriogenic mineral precipitation is a well documented natural phenomenon. Several mechanisms have been explained, but the commercial use of bacteria to precipitate minerals has not been seriously pursued. Through the investigation of several candidate systems, a practical approach to the application of bacteriogenic mineral precipitation was developed. The research program leading to this innovative new technology will be described and potential oil field applications identified.

1. BIOMINERALIZATION

Biomineralization is a well known geological phenomenon in which microorganisms induce the formation of mineral precipitates [1].

Such a process has been invoked to explain unusual drops in pressure developed in at least one oil reservoir. Studies on carbonate oil reservoirs in the Ural-Volga region of Russia revealed that certain zones near oil-water contacts were made impermeable by accumulations of calcite [2]. Lab studies [3] showed that sulfate-reducing bacteria in the presence of oil and calcium sulfate promoted calcite precipitation at appreciable rates.

Stable isotope analysis also supports the involvement of biomineralization in the formation of mineral cements in oil reservoirs in the Gulf of Alaska, Oklahoma, and Western Australia [4], as well as Alberta [5].

While substantial mineral deposits have been generated by bacterial mineralization over geological time, a practical rate of precipitation for oil field applications requires mineral formation in a matter of weeks. Rapid rates of mineralization were reported. For example, cultures of bacteria (circa 10^6 cells/mL) isolated from marine beach rock precipitated up to 4.7 mg/L of calcium carbonate per hour [6], which is not trivial. Given an increase in cell density to 10^9 cells/mL (a feasible target for application), rates of 4.7 g/L hour are attainable on this basis. At this rate, a 50% reduction in porosity would be achieved in a high permeability formation in a week or two.

Based on this background, a project was begun to investigate possible commercial systems and targets for biomineralization as a tool for the oil industry.

2. PRECIPITATION OF AUTHIGENIC MINERALS

Minerals that develop from connate fluids in sediments are authigenic. The overall precipitation process can be divided into two stages, nucleation and crystal growth.

If concentrations of ions in solution gradually increase and exceed the solubility product for a solid mineral phase, precipitation will not occur until

a certain degree of supersaturation is achieved [7]. The process during which the maximum free energy is attained is known as nucleation and involves the growth of critical crystal nuclei. Once formed, these nuclei grow as more ions or atoms are added to the nascent crystal. This process is accompanied by a decrease in free energy and is referred to as crystal growth. The presence of bacteria can promote either process.

Passive biomineralization involves induced nucleation. Here, the surface of the microbial cell provides a template on which nucleation can occur. This reduces the free energy required during the nucleation step and focuses crystal growth because nucleation on the biological template occurs before nucleation in homogeneous solution. Most types of bacteria are capable of acting as nucleation templates [8].

Active biomineralization fosters accelerated crystal growth as well as nucleation. Active metabolism can change solution conditions by shifting pH, reducing or oxidizing ions, or producing new chemical species in solution. This can result in oversaturation of the solution with respect to mineral deposition and thereby promote nucleation. Similar processes can provide ions necessary to sustain crystal growth especially near the microbial cells. Crystals nucleated on cell surfaces and associated with biomass may particularly benefit. In the absence of microorganisms, rapid crystal growth can become transport limited as concentrations of relevant ions are depleted near the crystal surface. Where saturation conditions are sustained by microbial action, associated crystal growth may be accelerated.

Active biomineralization strategies offer the best prospect for commercial application because sustained oversaturation and crystal growth in passive systems would depend on natural processes in the target environment.

3. POTENTIAL MINERAL SYSTEMS

There is considerable evidence for the biologically induced formation of a variety of minerals.

3.1. Phosphorites

Phosphorites are solid mineral phases containing more than 10% (by volume) individual phosphate grains. Phosphorite deposition is associated with biological activity, especially in shallow near-shore marine environments [9-12]. In modern marine environments, upwelling of deep ocean waters rich in phosphates precipitates metal ions; however, metallic ion binding by bacteria appears to enhance precipitation [13]. A similar mechanism was advanced for the formation of phosphate minerals in geochemical modelling studies conducted with metal loaded bacterial cells in the laboratory [14]. These concepts are consistent with biomineralization being a significant agent of phosphorite formation in modern and ancient sediments.

3.2. Carbonates

Bacteria were implicated in the precipitation of carbonates in several laboratory studies [15-17]. In these experiments, the cells function as nucleating elements for aragonite and high magnesium calcite. Similar mineralizations are noted in modern sediments in intertidal zones, quiet water lagoons, and in reefs [18-23]. Carbonate precipitating bacteria were isolated from beach rock formations [6], and fossilized bacteria are associated with many ancient carbonates [24-26].

3.3. Sulfides

The formation of sulfide minerals proceeds as a direct result of bacterial sulfate reduction. Patterns of sulfide isotope fractionation in many sedimentary sulfide deposits support the bacteriogenic origin of reduced sulfur [27]. In laboratory experiments, metal sulfides are commonly precipitated on the surfaces of bacterial cells probably due to the concentration of metallic ions there [28-31]. Incipient mackinawite formation was clearly documented in biofilms associated with the corrosion of oilfield piping [32].

3.4. Silicates

Until recently, remarkably little was known about the influence of bacteria on silica deposition, despite the presence of fossilized bacteria commonly found in siliceous deposits [24-25]. Extensive accumulation of complex iron aluminum silicates have now been observed on the surfaces of bacterial cells growing in a lake sediment contaminated with metal [29]. Direct examination of acidic hot spring sediments by electron microscopy revealed individual bacterial cells in successive stages of mineralization by iron silica crystallites [33]. Similar bacterial accumulations were reported in the laboratory [34-35].

3.5. Selection of candidates

Brief consideration of the above prospects led to the exclusion of sulfide systems based on the action of sulfate reducing bacteria. These bacteria are notorious for reservoir souring and corrosion problems. Similarly, formation of metal oxides, while feasible by biomineralization were excluded as being impractical in anaerobic environments. Thus, laboratory evaluations were restricted to phosphorite, carbonate, and silicate precipitation by active biomineralization.

4. DEMONSTRATION OF THE CONCEPT

The concept of a bacteriogenic mineralization system is to use injected bacteria or indigenous organisms to induce precipitation of authigenic minerals in water permeable zones in an oil reservoir. A complete system consists of viable active bacteria, nutrients to sustain the desired metabolic activity, and a mineralization solution saturated with respect to the proposed precipitation. The mineralization solution may or may not be the connate brine in the reservoir depending on the situation. Regardless of origin, placement of all elements in the target location within a reservoir is required for mineralization to proceed.

To demonstrate the concept, a simple metabolic activity was chosen as the basis for mineralization; pH changes. Many organisms are known to alter the pH of a medium through acid or base production associated with specific anaerobic metabolic activities. Carbonates, phosphates, and silicates are known to undergo precipitation in the biologically accessible range of pH alteration with ubiquitous cations like calcium. Laboratory screening trials were set up on this basis.

Leuconostoc mesenteroides B523 was used to produce acidic conditions for silica precipitation. This organism is able to reduce pH to 4 by the anaerobic production of lactic acid and was shown to survive and function in oil reservoirs in another context [36]. *Bacillus pasteurii* NRS 673 was used to produce basic conditions for carbonate and phosphate precipitation. This is a facultative anaerobe which secretes urease, an enzyme able to hydrolyze urea to form ammonia. An initial round of tests eliminated phosphate-based mineralization. Solutions of potassium phosphate and calcium chloride were made up at 1 mM and 10 mM at pH

6.0 in a biological nutrient medium made up of 3 g/L nutrient broth, 20 g/L urea, and 10 g/L NH_4Cl. Precipitation proceeded spontaneously at the higher concentration. Growth of *B. pasteurii* increased pH to 9, but failed to yield significant quantities of calcium phosphate at 1 mM. This and other considerations led to the dismissal of the phosphate based approach.

Silicate and carbonate systems proceeded to a second round of trials in simple unconsolidated sand columns under gravity drainage. Experimental protocols were described in detail elsewhere [37]. Briefly, four columns were run simultaneously; a control pretreated with two pore volumes of 10% (w/v) glutaraldehyde, a biological control consisting of septic reservoir sand from Lloydminster and duplicate test columns.

Test columns were prepared in a cell suspension of *B. pasteurii* or *L. mesenteroides* such that organisms were distributed throughout these columns before influx of a mineralization medium. This medium consisted of a nutrient solution saturated with respect to calcium carbonate or silica deposition.

In these tests, the silica system proved uncontrollable, spontaneously forming precipitates in the stock solution of mineralization fluid. This system was therefore abandoned in favor of the calcite system which gave reproducible results.

For the carbonate system, a mineralization solution consisted of 3 g/L nutrient broth, 20 g/L urea and 20 g/L ammonium chloride along with 2.1 g/L sodium bicarbonate and 2.8 g/L anhydrous calcium chloride. No loss of permeability in either the disinfected control or for the septic reservoir sand was evident after more than 100 pore volumes of mineralization solution had been injected through these columns. Only cases inoculated with *B. pasteurii* showed permeability loss. This exceeded 90% reduction after injection of 50 to 100 pore volumes (Figure 1).

Sectioned cores were inspected for biological and mineral content. The disinfected control showed <50 viable bacteria/g of sand in most probable number counts while the Lloydminster sand case showed 10^6 to 10^8 cells/g sand. Test

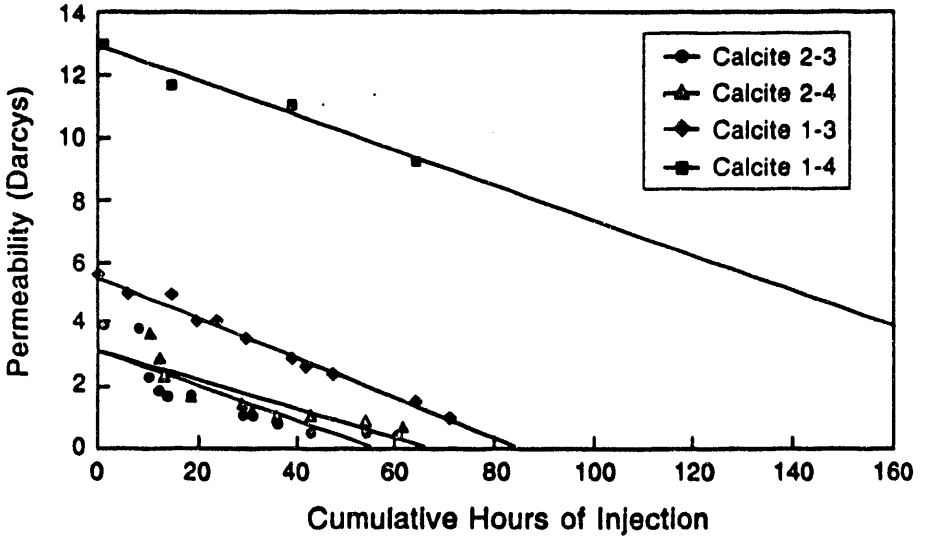


Figure 1. Decrease in core permeability from calcite precipitation by *B. pasteurii* in the first 80 hours for four different sandpacks preloaded with bacterial cells.

cases gave cell counts in excess of 10^9 cells/g sand. This represented considerable culture growth during the test run. The mineralization medium minus precipitants was used as the assay medium.

Control cores and cores made from reservoir sand showed no consolidation. In contrast, test cores had been consolidated for a length of about 10 cm at the leading end of the core, about 3 cm from the face of the core, which was apparently free of precipitation effects.

The consolidated core had to be dissembled and sectioned by hammer and chisel. Once removed from the core holder, consolidated sections were unaffected by running water or organic solvents. Acid caused effervescence and disintegration consistent with cementation of the sand grains by precipitated calcium carbonate. Energy dispersive x-ray analysis on sections of consolidated core (Figure 2) showed the presence of a calcite cement between sand grains (Figures 3 to 5). Bacteria were intimately associated with the calcite precipitate (Figure 2).

On the basis of these tests, a patent application was filed and granted (U.S. patent No. 664769).

Further test runs were carried out using a glass bead pack as well as sandpacks [38]. In these runs, bacterial suspensions were injected through the core to inoculate the test matrix. Following a controlled injection of mineralization solution, consolidation and permeability reduction were observed. Figure 6 shows similar precipitation effects could be achieved in smooth glass bead (60-100 mesh) and coarse sandpacks. This Figure is based on images from computer assisted tomographic scans of cores using an EMI 5005 x-ray CAT scanner.



Figure 2. Scanning electron microscope micrograph for calcite cemented sandpack.

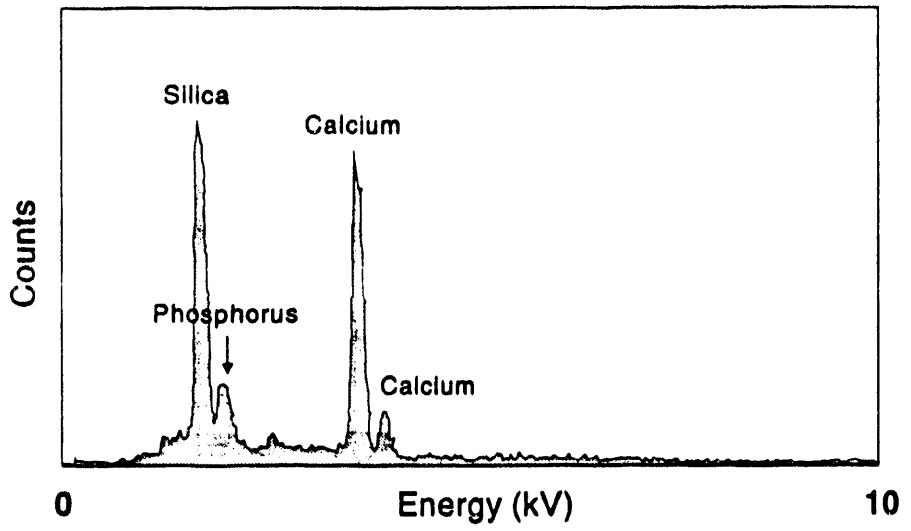


Figure 3. Energy dispersive x-ray analysis of bulk surface.

Porosity values calculated using in-house software were used to compare porosity losses due to biomineralization along each core. Again a lack of precipitation is evident at the inlet face [38].

Including tetrazolium blue dye in the mineralization solution made the biomass distribution in a sandpack visible. A strong correlation was evident between biomass concentration and sand consolidation. This suggests that the shift in pH (due to urea hydrolysis) and the consequent calcite precipitation occurs in the locale of the bacterial cells despite the fact that the urease produced is released as an extracellular enzyme. It appears that this process while perhaps

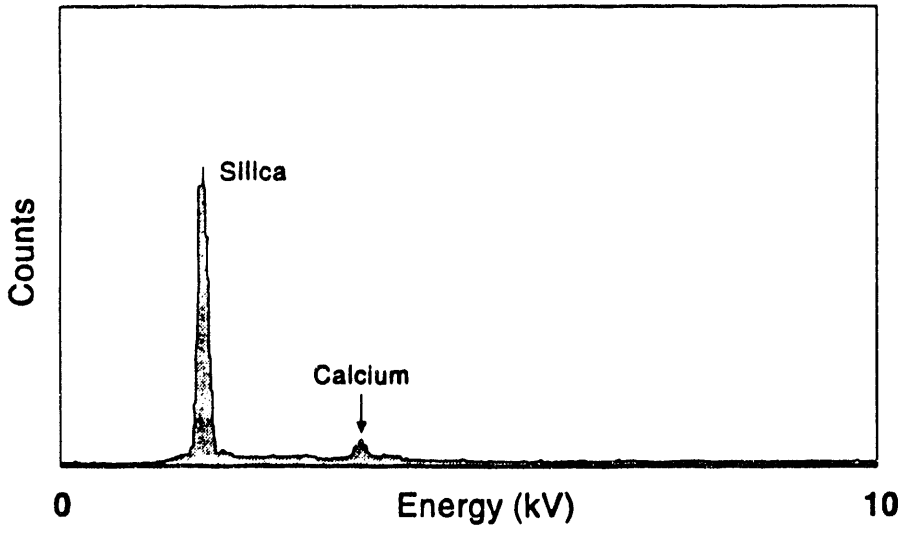


Figure 4. Energy dispersive x-ray analysis of exposed sand grain surface.

free of serious facial plugging constraints, still is dependent on the successful injection of bacterial agents to the target site for mineralization.

5. TARGETS AND OPPORTUNITIES

Two obvious applications of the biomineralization system are selective plugging of high water permeability zones and sand consolidation.

Unlike previous plugging systems, this biomineralization system will give a thermally stable, durable plug, particularly in reservoirs saturated by limestone dissolution. Use of urease offers a relatively inexpensive, widely available substrate, urea, to support the shift in pH needed for the process. Urease itself is an enzyme widely distributed among bacteria allowing a wide range of choices for the organism to be used. Its occurrence in the *Bacilli*, for example, suggests that spore formers might be discovered for deep reservoir applications.

A significant target might be the plugging of fractures in carbonate reservoirs which presently thwart late life strategies for gas and oil recovery by fostering gas and water breakthrough to production wells. If this approach can encourage biomineralization on the carbonate rock faces in such a fracture, it may be possible to "grow" fractures closed. While this objective requires considerable further development, the target is economically worthwhile. The high contrast in permeability offered by the fracture with respect to its host matrix would ensure that most of the mineralization solution injected would pass through the fracture system focusing transport of injected calcium and bicarbonate ions to the target site. Even the formation of limited plugs would succeed because the opportunity for bypass of the closed fracture would be negligible.

Sand consolidation is a near well bore application designed to retard movement of sand or fines into a well bore. It avoids the challenge of injecting microbial systems to target sites deep in a reservoir and could be a very beneficial way to avoid loss of productivity, particularly in horizontal wells due to sand influx.

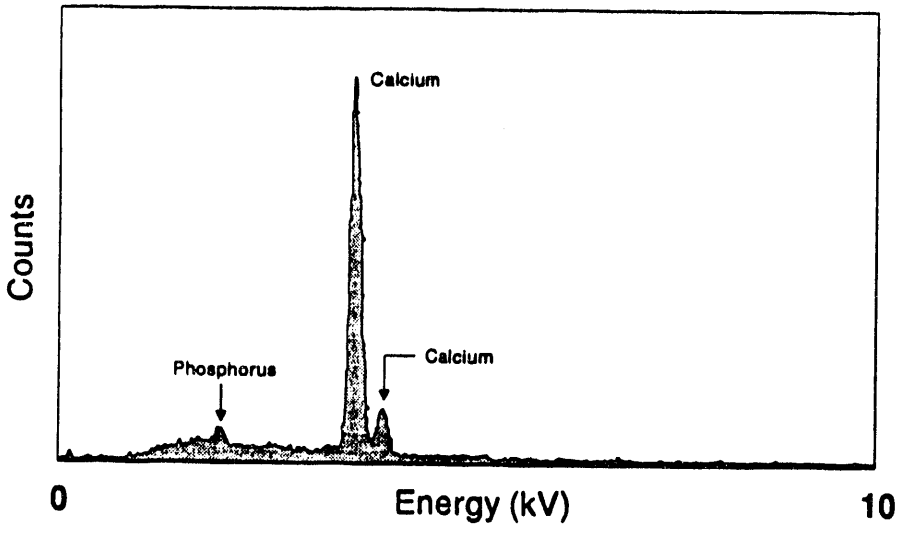


Figure 5. Energy dispersive x-ray analysis of calcite cement deposited in pore throats of sandpack.

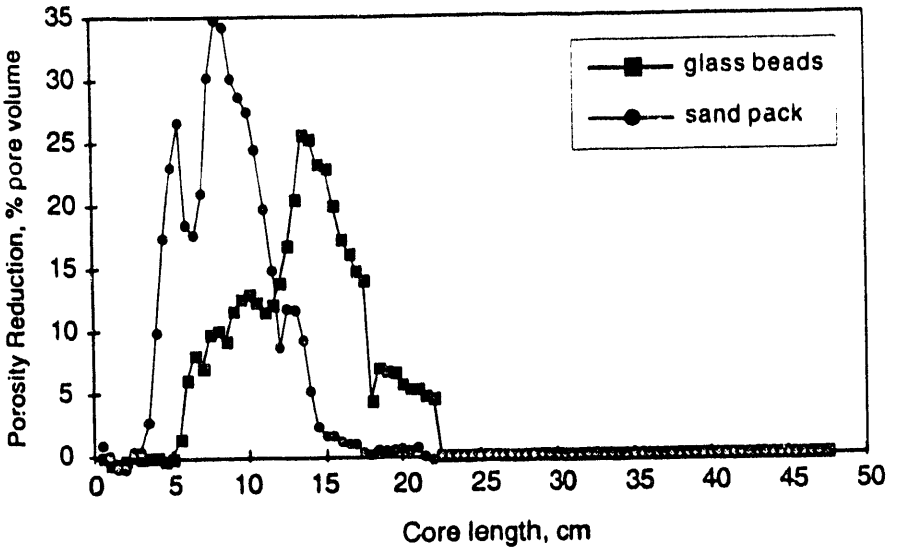


Figure 6. Comparison of porosity loss along length of core due to biomineralization in a glass bead pack and a sandpack after injection of *B. pasteurii*; followed by mineralization solution.

6. ACKNOWLEDGMENTS

This work was enabled by the participation of the Energy Research Laboratories of CANMET, Energy, Mines and Resources, Ottawa, through the cost shared Energy Conversion Program; DSS Contracts 23440-9-9258 and 23440-8-9222/01-SQ. The authors also thank Husky Oil for permission to publish this information. Inquiries respecting the licensing and use or the further development of this technology are welcome.

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Chemical Markers of Induced Microbial Transformations in Crude Oils

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Abstract

Biochemical processes associated with the interactions of acidophilic and pressure-adapted thermophilic or thermoadapted microorganisms suitable for enhanced oil recovery produce characteristic chemical markers. These markers indicate the extent and the nature of chemical alterations of crude oils by microorganisms which have been experimentally introduced and grown in crude oils. The chemical markers include high and low molecular-weight species, organic sulfur compounds, trace metals, and the extent of emulsification. Most recent results suggest that the distribution of major groups of compounds, i.e., the asphaltenes, maltenes, and saturates are also biochemically altered. Experimental evidence indicates that multiple biochemical reactions are involved in the microbial interactions with crude oils. The chemical markers associated with these biochemical reactions are described and discussed in terms of their significance and applicability to the biotreatment of crude oils and enhanced oil recovery.

1. INTRODUCTION

Systematic investigations and analyses of the effects of different microorganisms on crude oils have shown that the biochemical interactions which occur during the biotreatment of oils depend on the microbial species and the types of oils used [1-3]. These microorganisms belong to several groups that can live at high temperatures and pressures, variable pH, and high salinity. Specifically, the microbes convert biochemically the crude oil which, through various pathways, lead to (1) a decrease in the C_{20} to C_{30} alkanes; (2) a breakdown of higher molecular weight hydrocarbons, including polycyclic and saturated fractions; (3) an increase in lighter ($<C_{20}$) hydrocarbons; (4) the transformation of organic sulfur compounds with a net decrease in the organosulfur content of oils; (5) a decrease in the concentration of trace metals; and (6) emulsification of crude oils with variable efficiencies. Some typical examples are shown in Tables 1 and 2. In Table 1, the Naval Petroleum Reserve (PR3) crude oil was treated with four different types of bacteria under identical conditions. The results are expressed as the extent of the emulsion (Klett Units) and the oil content. Table 2 shows the variations in the amounts of total trace metals removed from the oil during biotreatment. This apparent versatility of the biochemical pathways involved in microbial interactions with crude oils can influence the efficiency of Microbial Enhanced Oil Recovery (MEOR). Furthermore, the chemical markers which characterize the changes induced in the chemical composition of crude oils by different organisms may also serve as tools to evaluate microbial processing selected for MEOR. The significance and the applicability of the chemical markers is the subject of this paper.

Table 1
Treatment of Naval Petroleum Reserve (PR3) in Medium 2

Extent of the Emulsion (Klett Units)	Microbial Strain	Oil Content in the Emulsion (g/ℓ)
50	BNL-4-21	3.58
200	BNL-4-22	11.0
700	BNL-4-23	18.0
30	BNL-4-24	3.03

Table 2
Cerro Negro Crude treated with BNL-4-23 in Medium 2

Metal	Total Metal Concentration μg/mℓ Untreated	Treated
V	3330	2290
Ni	926	639
Mg	78	6.8
Sr	9.6	1.7
Mn	15.8	1.8

2. MATERIALS

2.1. Instrumentation

The instrumentation, Gas Chromatography-Mass Spectrometry (GC-MS), Gas Chromatography with sulfur specific Flame Photoemission Detector (GC-FPD), and Induced Coupled Plasma-Mass Spectrometry (ICP-MS), were described elsewhere [2], as were the culture media. Thus, Medium 1 (M_1) contains only inorganic salts, Medium 2 (M_2) contains inorganic salts and 0.08% organic carbon, and Medium 3 (M_3) is the same as Medium 1 but the sulfates are replaced with chlorides. Hewlett-Packard gas chromatograph model HP5921A, equipped with atomic emission detector, was used to analyze metal complexes. XANES (X-ray Absorption Near Edge Structure) analyses were carried out at the Brookhaven National Laboratory-National Synchrotron Light Source (BNL-NSLS) Beam X19 using procedures described elsewhere [4]. The microbial species used in the current studies [2] included thermoadapted *Acinetobacter* sp. (BNL-4-21), *Arthrobacter* sp. (BNL-4-23), *Ochrobactrum* sp. (BNL-4-24), and thermophilic microbial isolates BNL-NZ-2, BNL-NZ-3, and BNL-NZ-5. These organisms appear as positive cocci; however, they are pleomorphic and can appear as positive rods. All the isolates were derived from geothermal reservoirs containing dispersed oil seeps in boiling water and mud mixtures. Biotreatment of crude oils was carried out between 45°-65°C. The experimental test oils have been described elsewhere; namely, Wilmington [5], Teapot Naval Petroleum Reserve #3 (PR3) [6], Venezuelan [7], and Monterey Oils [8]. These oils represent a wide range of API gravity, from about 35.2 to 8, and have a sulfur content from 0.14% to 5%.

Table 3
XANES analysis of sulfides, thiophenes, and sulfoxide contents of untreated and treated crude oils

Crude Oil	Microorganisms		Relative Content Sulfide	Thiophene	Sulfoxide
Boscan		untreated	0.198	0.738	0.064
	BNL-4-22	treated	0.159	0.655	0.186
	BNL-4-23	treated	0.121	0.743	0.135
Cerro Negro		untreated	0.147	0.781	0.072
	BNL-4-22	treated	0.179	0.683	0.138
	BNL-4-23	treated	0.103	0.713	0.184

3. METHODS

3.1. Sulfur constituents of crudes

Biotreatment of crude oils from Venezuela [2,3] containing an average of 5% sulfur with BNL-4-22, BNL-4-23, and BNL-4-24 for seven days lowered the content of organic sulfur by 10-25%. The GC analyses using a FPD have shown that with the overall decrease in organic sulfur there are also significant qualitative and quantative changes in the content of benzothiophenes (C_8H_6S) and higher homologues, resulting in an overall decrease in the content of substituted organic sulfur compounds. An analytical tool exceptionally useful to follow total changes in sulfur compounds present in whole crude oils is the XANES analysis which measures and differentiates between gross sulfur species, such as inorganic and organic, inorganic sulfides, thiophenes, and sulfoxides [4]. Table 3 shows the results of comparative analyses by XANES of Boscan and Cerro Negro crude oils. Biotreatment decreases the sulfide and thiophene contents of the crudes, and increases the sulfoxide contents. Since volatile products containing sulfur have not been detected, and there is definite decrease in sulfur content, a possible implication is that some products are soluble in the water phase which is always present in the culture medium. An investigation of the "sulfoxides" and other products which may be present in the aqueous phase has begun. Some of the products may be soluble sulfonated emulsifying agents. For example, a sulfone-type, water-soluble compound may be produced which would contribute to the concurrent emulsification of the oils.

3.2. Organometallic compounds

Earlier observations showed [2] that under our experimental conditions, biotreatment of crude oils causes changes in the composition of nickel porphyrin complexes. These studies were extended to a comparison of the effects of several different microbial strains on the content of nickel and vanadium complexes in Cerro Negro crude oil.

Porphyrin complexes were analyzed by gas chromatography using an atomic emission detector; the results are given in Table 4. The concentrations given represent the metal content of the vanadium and nickel porphyrin complexes and do not represent the total metal concentration of the crude; therefore, the values are, by definition, different from those given in Table 2, which represent the total metal content of the same oils. The chemical treatment and handling

Table 4
Gas chromatographic analyses of the nickel and vanadium porphyrin complexes in Cerro Negro crude oil treated with different microorganisms in Medium 2

Microorganisms	Metal	Complex Concentrations Exposed As Integrated Peak Areas		Decrease in Complex Concentration
		Treated	Untreated	%
BNL-4-24	Ni	186,016	246,579	25
	V	276,882	493,497	38
BNL-4-22	Ni	12,484	246,579	95
	V	6,328	493,497	99
BNL-4-23	Ni	160,786	246,579	35
	V	204,903	493,497	58
BNL-TH-29 + BNL-TH-31	Ni	168,343	246,579	32
	V	212,204	493,497	57
BNL-2-45 + BNL-3-26	Ni	122,018	246,579	51
	V	157,401	493,497	68

of the treated and untreated samples for a series of experiments was always identical. For example, for the determination of total metals, identical quantities of untreated crude oil were chemically digested in the same manner as the biotreated oil, before ICP-MS analyses. This correspondence compensates, to a certain extent, for metal losses due to chemical manipulation and allows the results to be used as chemical markers of compositional changes in crude oils due to biotreatment. Such analyses have consistently shown that the biotreatment of crude oils alters the chemical composition, which appears to depend on both microbial species and the type of oil used. The results also indicate that there is a decrease in the total metal concentration as well as degradation of the complexes. At present, the most plausible mechanism is that the speciation of metals present in the organic phase (i.e., oil) is changed, resulting in solubilization and transfer of metals from the organic to the aqueous phase (i.e., the culture medium and brine). We note that such reactions also will depend on the type of organometallic compounds and the chemical composition of the crude oils, particularly in terms of the relative abundance of saturates, aromatics, and polar fractions.

Table 5
Microbial treatment of Wilmington crude in Medium 2

Microorganisms	Days incubated	Viscosity in cps	Emulsion in Kletts units
BNL-4-21	20	3.6	180
BNL-4-22	20	3.0	400
BNL-4-23	23	3.0	180
BNL-4-24	30	3.6	55
Control	55	3.3	7.5

Table 6
BNL-4-24 Treatment of pre-emulsified Wilmington Crude Oil in Medium 2

	Days Incubated	Emulsion in Klett units
BNL-4-24 + 0.5% oil + 0075% Tergitol	7	8000
Control: 0.5% oil + 0.0075% Tergitol + Media	6	1500

3.3. Enhancement

Biochemical reactions which occur during microbial treatment of crude oils show trends in their dependence on the chemical composition of the crudes and the microbial species. Some of these trends may be useful in developing MEOR processes. Thus, treatment of Wilmington, CA, crude (compare Table 5 and Figure 1) has shown that BNL-4-24 is highly efficient in terms of its action on organic sulfur species, although not the best emulsion producer. To allow direct comparisons, all the chromatographic data were obtained under identical experimental conditions. Similar effects were observed for other oils, for example, Wyoming and Venezuelan oils [2,3,9]. The opposite effect also has been observed, i.e., a high degree of emulsification with a relatively small decrease in organic sulfur. This information implies that different emulsifying agents may be produced, and further, that the yields of naturally produced emulsifying agents may vary with the microbial species and the chemical composition of the oils. If so, then some microbial species may be more suitable as producers of surface active agents while others may be better converters of heavy to lighter oils. A consortium of such organisms may enhance the overall effect. Some preliminary experiments have tested the "enhancement effect." Thus, a sample of the Wilmington crude oil was emulsified with tergitol and then treated with BNL-4-24. Tergitol is a commercial C-21 emulsifying agent whose chemical structure is given below:

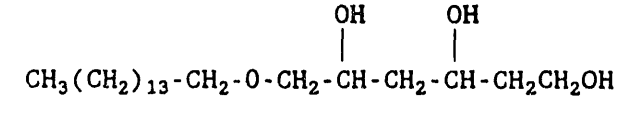


Table 7
Biotreatment of pre-emulsified Crude Oils

Oil	Microorganisms		Medium	40-Day Treatment*	Emulsion in Klett Units
Boscan	BNL-4-22	+	M_2 + oil	0	210
	BNL-4-33	+	M_2 + oil	0	140
			M_2 + oil only	0	5
Cerro Negro	BNL-4-22	+	M_2 + oil	0	120
	BNL-4-23	+	M_2 + oil	0	145
			M_2 + oil only	0	24
Boscan	BNL-4-22	+	M_1 + oil	0	50
	BNL-4-33	+	M_1 + oil	0	10
			M_1 + oil only	0	10
Cerro Negro	BNL-4-22	+	M_1 + oil	0	0.5
	BNL-4-23	+	M_1 + oil	0	4.5
			M_1 + oil only	0	3.5
	BNL-4-22,23		Bacteria + medium only	0	
Boscan	BNL-4-22		RM_1 + oil	Emulsified	6000
	BNL-4-23		RM_1 + oil	Emulsified	5500
			Medium + oil only	Emulsified	2800
	BNL-4-22,23		Bacteria + medium only	Emulsified	2600
Cerro Negro	BNL-4-22		RM_1 + oil	Emulsified	3800
	BNL-4-23		RM_1 + oil	Emulsified	4800
			Medium + oil only	Emulsified	2800
	BNL-4-22,23		Bacteria + medium only	Emulsified	2600

*Treatment: "0" means that the organisms were grown under conditions in which the organism generated the emulsification process in the medium; Emulsified means that tergitol was added to the medium containing the oil and bacteria. The result is the cumulative effect due to the added emulsion and emulsification generated by the microorganisms.

Tergitol was chosen because it is non-toxic to the microbial species under our experimental conditions. Furthermore, a benzothiophene standard of known concentration was added in each case immediately before the analyses. Therefore, the measured concentration of benzothiophene represents the total benzothiophene concentration, i.e., the concentration of added spike plus that present in the untreated oil or remaining after biotreatment. The results are shown in Table 6 and Figure 2. A five-fold enhancement in the extent of emulsification and removal of organic sulfur compounds was achieved (Figure 2) in a much shorter period of time (seven days, compare Table 5). Similar results were obtained with Boscan and Cerro Negro oils (Table 7).

Table 8
Reaction mixtures

Reaction Mixture, Type I	
	(a) oil + medium 1
	(b) oil + medium 2
	(c) oil + medium 1 + emulsifier
	(d) oil + medium 2 + emulsifier
Reaction Mixture, Type II	
	(a) medium 1 + bacteria*
	(b) medium 2 + bacteria
	(c) medium 1 + bacteria + emulsifier
	(d) medium 2 + bacteria + emulsifier
	(e) medium 1 + bacteria + oil
	(f) medium 2 + bacteria + oil
	(g) medium 1 + bacteria + oil + emulsifier
	(h) medium 2 + bacteria + oil + emulsifier

*Deliberately introduced bacteria, e.g., BNL strains.

3.4. Comparison of induced bioconversion of crude oils

In continuing studies of the induced bioconversion of crude oils, the effects of "indigenous" vs. "introduced" microorganisms must be addressed. In this study, the "indigenous" microorganisms are considered as those which were either present in the original crude or introduced during shipping and handling, or a combination of both possibilities, which may occur under our experimental conditions. To better understand the effects due to introduced microorganisms, several studies were conducted in different media and in the presence and absence of an added emulsifying agent. Thus, at the beginning of treatment, at a given temperature and pressure, several potential "reaction mixtures" must be considered which are defined in Table 8. Preliminary results presented in Figures 3 and 4 show that after a forty- or a twenty-day treatment, there are significant differences in the end effects; these differences were determined as the extent of the resulting emulsions. Some important consequences due to the reaction mixture type (I or II) and duration of the treatment are evident. In the absence of oil, and therefore, no carbon source, there is no growth of microorganisms in Medium 1. In the presence of oil, there is some growth in Medium 1, which must be due to indigenous microorganisms. In Medium 2, which contains inorganic salts plus added carbon (0.08%), there is a considerable growth of all organisms tested, including the indigenous ones (Figures 3, 1, M_2). In addition to Boscan crude, a significant enhancement in growth is observed in all cases except that of BNL-NZ-3 in Medium 1 (Figures 3, 5a, M_1). Furthermore, marked variations in gross effects due to different microbial species in either Medium 1 or 2 also are clearly evident. Further results were obtained from experiments in which crude oil and tergitol were biotreated in Medium 1 (inorganic salts only) over a period of forty and twenty-days. Addition of emulsifier to oil in Medium 1 results in a better emulsion than that due to oil in Medium 1 only (compare Figures 4, 1 and Figures 3, 1M). There are also variations in the interactions between different microorganisms and the emulsifying agent, as shown in Figure 4, Experiments 2 to 7. Since all the experiments were carried out under identical conditions and with the same

concentration of tergitol, the observed variations probably are due to a combination of processes by which the indigenous and introduced microorganisms interact with the added emulsifying agent in a medium without other sources of organic carbon, possibly using it as another source of carbon. The addition of oil, however, causes several major changes. First, a twenty- rather than a forty-day treatment produces a significant effect as shown by comparing Figure 4, 2a to 7a, to Figures 3, 2a-6a in Medium 1. The results shown in Figures 4

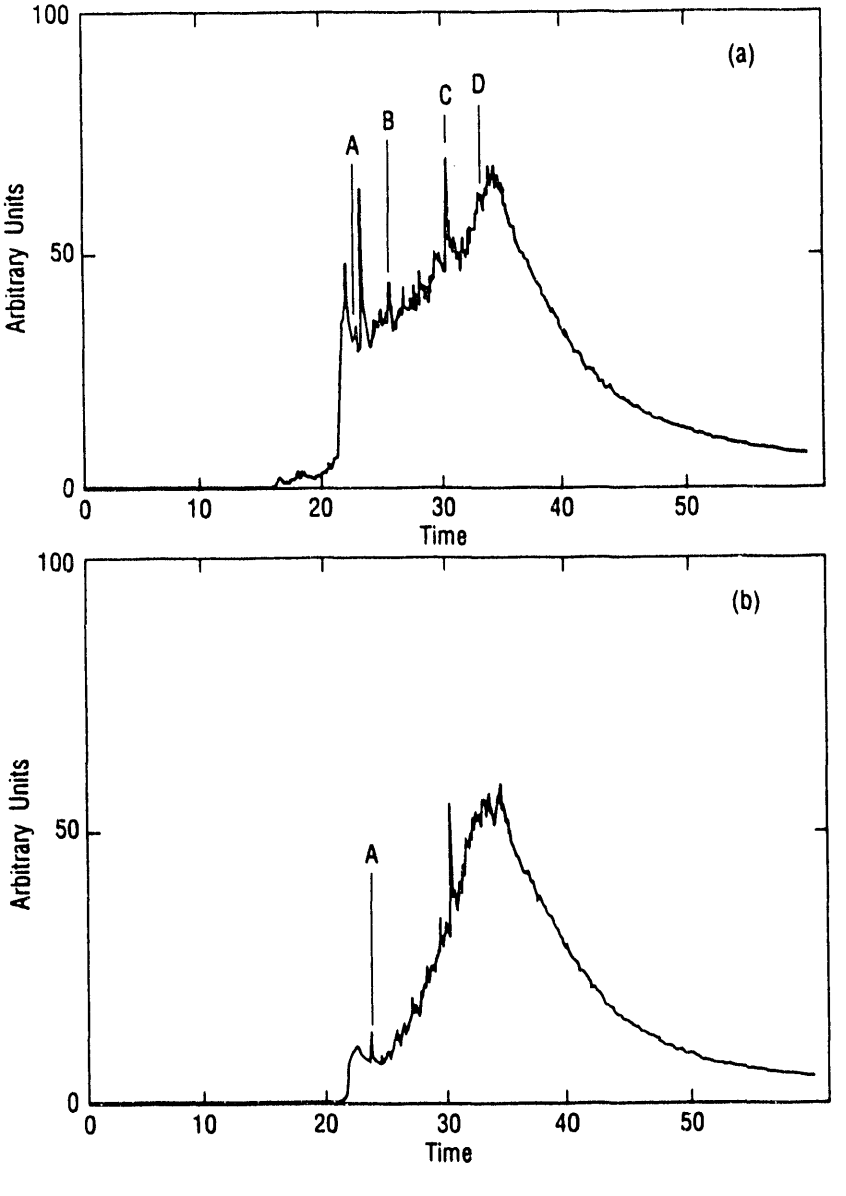


Figure 1. (a) Gas Chromatography-Flame Photoemission Detector (GC-FPD) trace of untreated Wilmington, CA, Crude (sulfur specific trace); and (b) treated with BNL-4-24. Molecular Markers A: Benzothiophene + 5.2 mg benzothiophene standard added prior to analyses; B: Phenyl Sulfide; C: Dibenzothiophene; D: C-1 Dibenzothiophene.

Table 9
Comparison of heavy ends of crudes

Sample	A836	A837	A851	Cerro Negro	Boscan
wt% asphaltene	56	51	6	25	40
wt% maltene:	44	49	94	75	60
% saturate fraction	10.3	12.8	22.0	11.7	10.7
% aromatic fraction	8.7	5.8	20.5	18.3	14.4
% NSO (polar fraction)	25.0	30.4	51.5	45.0	34.8
wt% S	4.0	3.3	2.0	3.7	4.8

reflect the net result due to the interaction(s) between introduced and indigenous microorganisms in the presence of an organic hydrocarbon-based emulsifying agent. In Figure 3 and Medium 2, the same microorganisms have acted on oil and the added 0.08% nutrient. Although in both situations there is a large excess of oil, the microorganisms, nevertheless, have two sources of organic matter; one is oil and the other is added either as a nutrient or as an emulsifying agent. These sources may be used by microorganisms in different ways. For example, under one set of circumstances, the added nutrient may be used faster because it may be biochemically more liable, and in the other case, the emulsifying agent may serve predominantly as a reagent which facilitates emulsification, and therefore, allows a much better contact between the

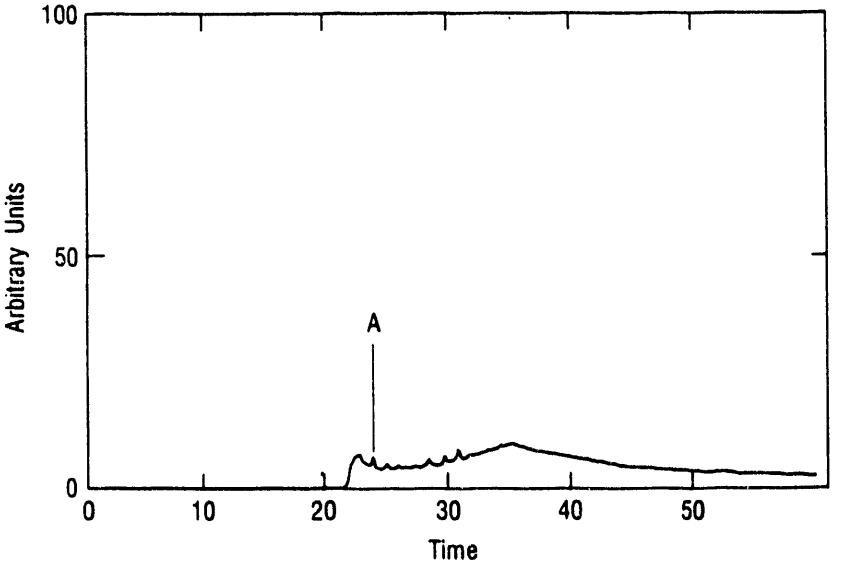


Figure 2. GC-FPD trace of Wilmington, CA, Crude Pre-emulsified with 0.0075% tergitol and biotreated with BNL-4-24. 5.2 ng benzothiophene spike added immediately before analysis.

biochemically reactive species. Therefore, the observed gross effects are a consequence of microbial action on all sources of carbon which may well influence the biochemical pathways involved, and may cause the observed differences in measured parameters which occur during biotreatment.

3.5. Relative abundance of major fractions and the nature of end products

Although the chemical compositions of crude oils vary, they fall within well-defined categories, based on the relative abundance of major fractions containing hydrocarbons within certain molecular-weight ranges [10]. These fractions include C_4 - C_{10} (gasoline), C_{11} - C_{13} (kerosine), C_{14} - C_{18} (diesel fuel), C_{19} - C_{25} (heavy gas oil), C_{26} - C_{40} (lubricating oil) and $>C_{40}$ (residuum) hydrocarbons. Terms like light oil, waxes, and heavy crude oils refer to mixtures of these hydrocarbons with an increasing content of hydrocarbons possessing higher carbon numbers.

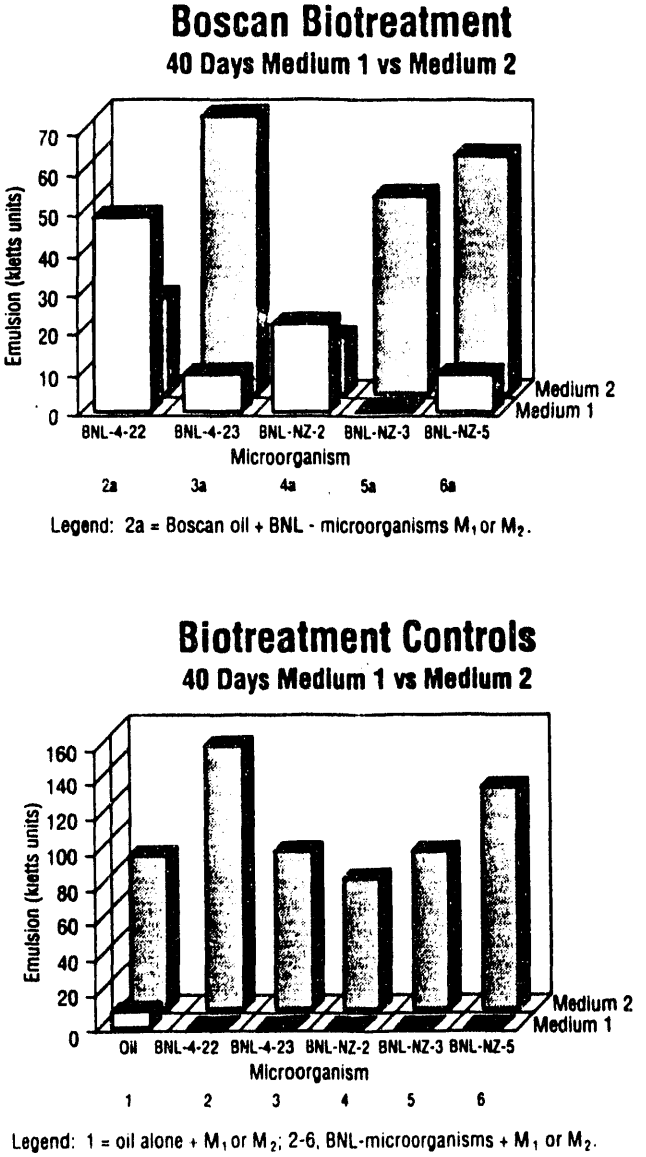


Figure 3. Biodegradation of Venezuelan Boscan Crude Oil.

In addition to this classification, the role of heteroatoms is very important, particularly in describing heavy crude oils. Such oils are often heavy because of cross-linking, for example, of sulfur species, as well as other heteroatoms and metals. A chemical or biochemical process capable of degrading higher molecular weight hydrocarbons and breaking cross-links will have obvious advantages in enhanced oil recovery. If such process(es) were cheap, the success of their application would be secured. MEOR falls into this category. Therefore, an understanding of the biochemical reactions with major fractions of heavy crudes will allow better production strategies. This knowledge may be gained by analyzing the crudes for key chemical markers which characterize the biochemical process(es).

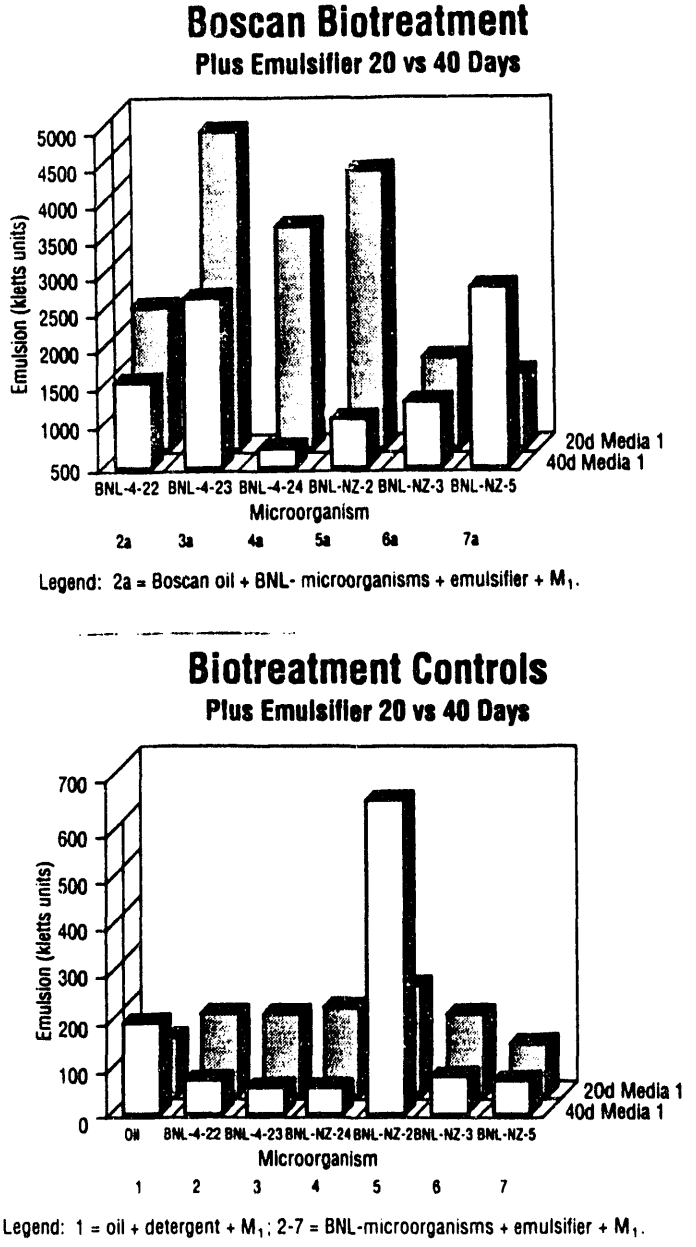


Figure 4. Biodegradation of Venezuelan Boscan Crude Oil in presence of an emulsifying agent (e.g, tergitol).

To test this concept, several heavy crude oils were chosen for comparisons. The oils varied from oils which are heavy because they are immature, to those which are heavy due to biodegradation under reservoir conditions over a geological period, due to indigenous bacteria. Further, the biochemical processes which occur during the biotreatment of crude oils appear to be influenced by other factors discussed earlier, which include (i) the oil is the sole source of carbon; (ii) a carbon source other then oil has been added to the medium; and (iii) the presence of microbially produced or added detergents.

The microbial degradation of crude oils is routinely referred to as "biodegradation." This term may be misleading, particularly in view of the actual end-products observed. Under the experimental conditions in which selected organisms interact with crude oils, various fractions are affected in different ways. The heavy fraction may be biochemically split into smaller molecular-weight fractions by chemical reactions involving intra- and inter-

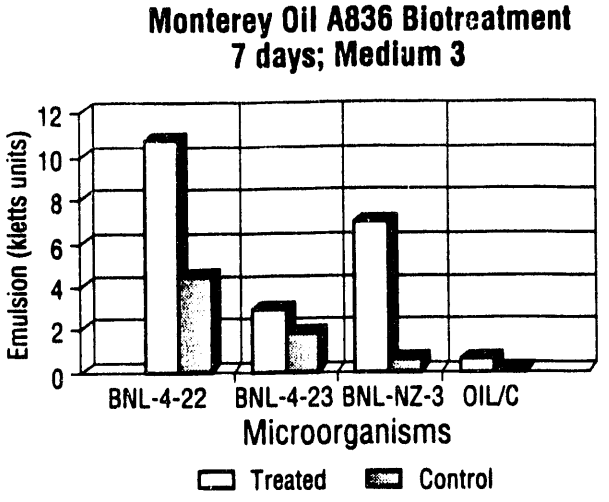


Figure 5. Biotreatment of A836.

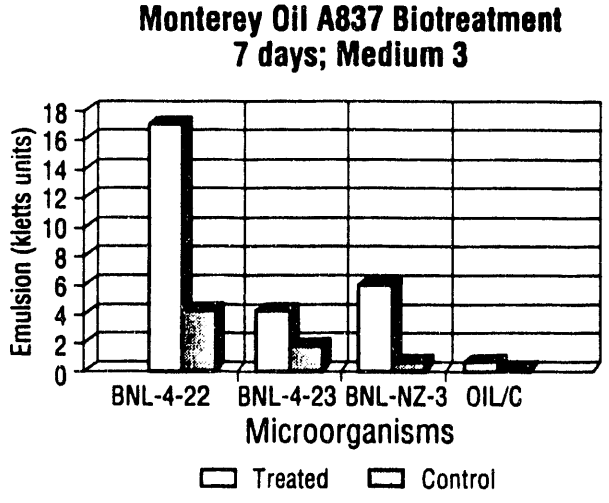


Figure 6. Biotreatment of A837.

molecular rearrangements, hydroxylation, and changes of sulfur, nitrogen and oxygen bridges. Electron transfer may occur via active group transfer and a change in organometallic complexes, followed by rearrangements yielding a variety of natural products with chemical properties significantly different from the starting complex organic matrix. These processes may occur during several phases of biotreatment with a net result of chemically altered crude oil. Therefore, the term "biotreatment" may be more appropriate, because it involves a multiplicity of biochemical and chemical reactions occurring concurrently as a direct consequence of microbial intervention.

To explore some of the reactions which may occur during the biotreatment, oils which are heavy because they are immature were compared to those which are heavy because they were "biodegraded" in the reservoir over a geological period. In Table 9, five different heavy crude oils are compared for their asphaltene, maltene, saturate, aromatic, and polar fractions, as well as their sulfur contents. Boscan oil is heavy due to immaturity and Cerro Negro is heavy due to biodegradation. Further, the three domestic oils represent immature offshore Monterey (A836, A837) and biodegraded onshore (San Joaquin Valley, A851) Monterey crudes. The fractionation of the oils was carried out by procedures described in Peters and Moldowan [8]. Briefly, the asphaltenes and maltenes were separated by adding an excess by volume (40-fold) of pentane to oil dissolved in a minimum amount of toluene. The resulting precipitate is called "asphaltene" and the soluble material is called "maltene." The latter is then separated by high-pressure liquid chromatography into saturate, aromatic, and polar fractions. Thus, A851 has the highest concentration of the maltenes and this maltene fraction has the highest concentration of NSO compounds. If the biochemical reactions proceed via the pathways we have discussed, then significant effects should be observed in the distribution of hydrocarbons before and after biotreatment. The effects of biotreatment on Venezuelan oils were described in this paper and in another [9]; therefore, this discussion will be limited to Monterey oils. All of the Monterey crude oil samples were treated under identical experimental conditions with BNL-4-22, BNL-4-23, and BNL-NZ-3, without the initial addition of an emulsifier, for seven days in Medium 3. The results of these studies are shown in Figures 5 through 7, and the corresponding GC-MS data are shown in Figures 8 through 10. For all the experiments, "control" means

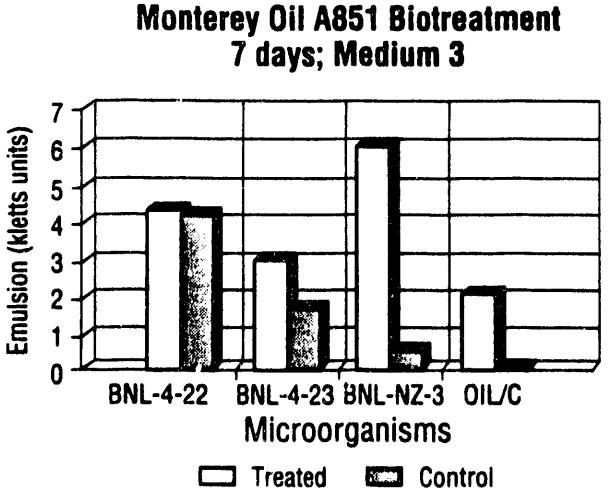


Figure 7. Biotreatment of A851.

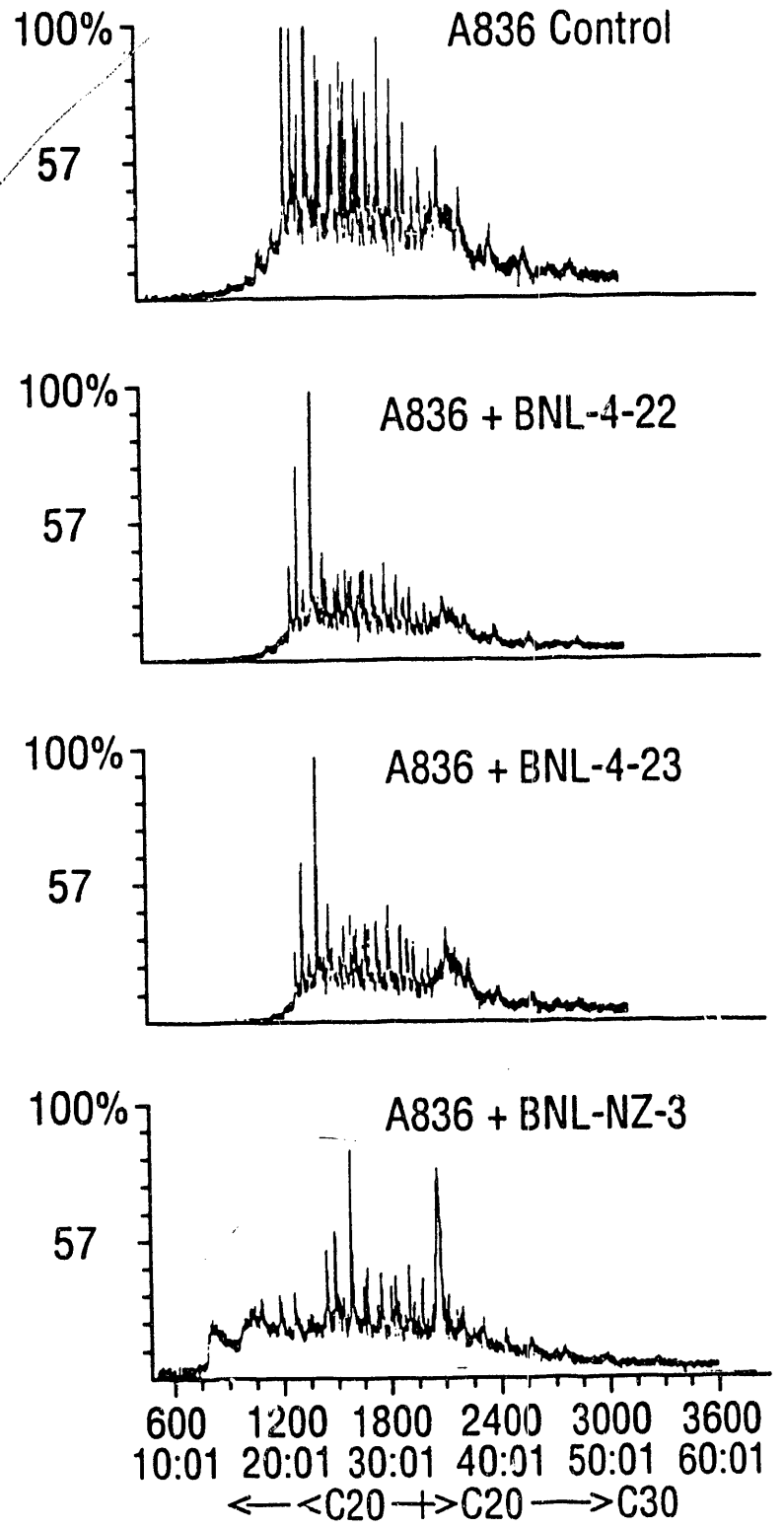


Figure 8. GC-MS Fragmentograms, m/z 57, Monterey, CA, Crude A836 treated with BNL-4-22, BNL-4-23, and BNL-NZ-3.

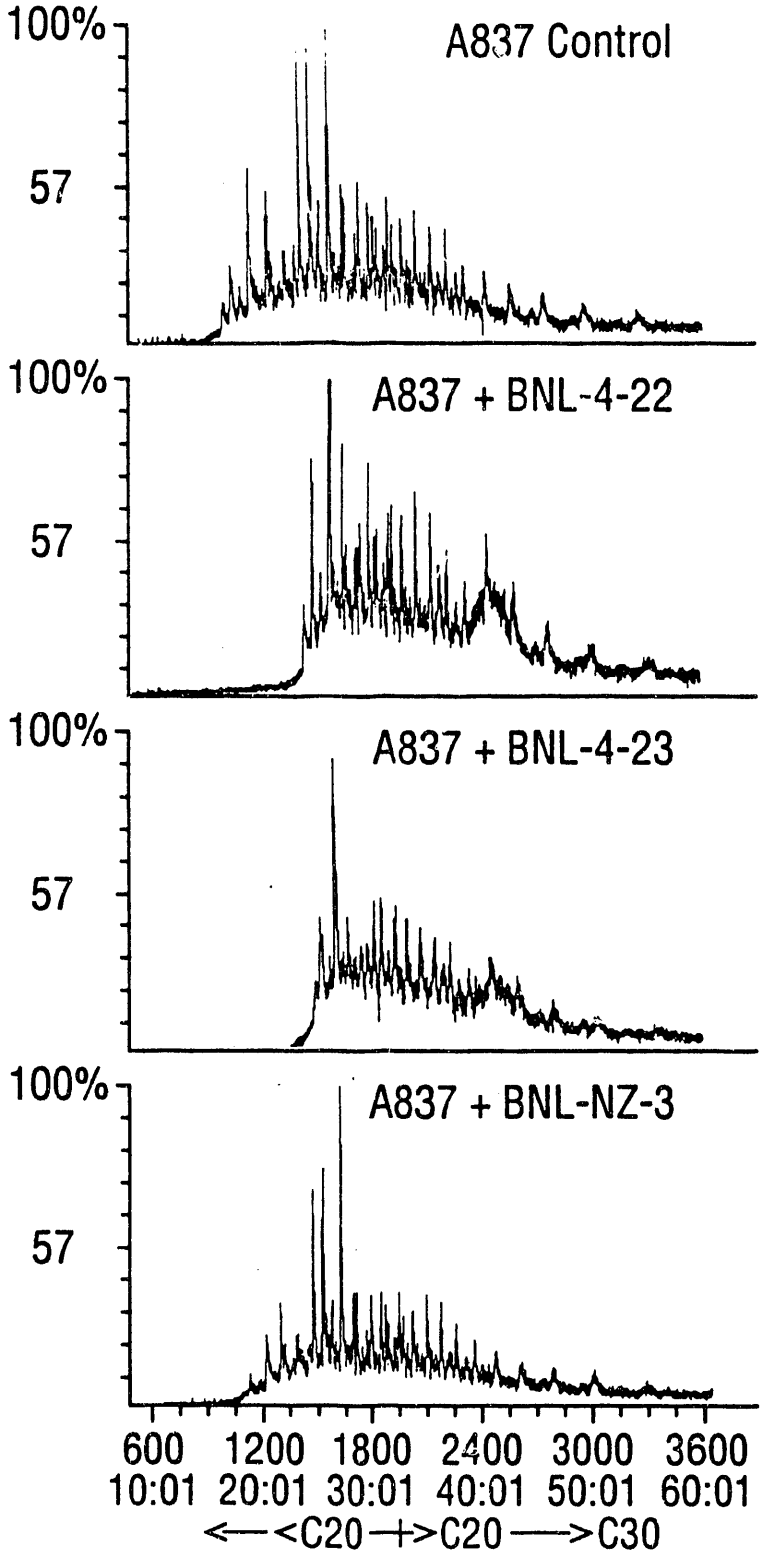


Figure 9. GC-MS Fragmentograms m/z 57, Monterey, CA, Crude A837 treated with BNL-4-22, BNL-4-23, and BNL-NZ-3.

crude oil and medium only, and 'C' stands for "medium only" control. BNL-4-22 emulsified A836 and A837 better than BNL-4-23, while BNL-NZ-3 emulsified both to about the same extent. Medium 3 with oil only (oil control) produced a negligible effect. In the absence of oil, Medium 3 supports some growth of the microbial species. For Monterey A851, BNL-NZ-3 is considerably more active than BNL-4-22 and BNL-4-23. The corresponding GC-MS fragmentograms show the significant gross and fine structural differences. Monterey A836 treated with BNL-4-22, BNL-4-23, and BNL-NZ-3 showed an overall decrease C_{20} to C_{30} compounds with major changes in the C_{20} and less than C_{20} region particularly prominent in the BNL-NZ-3 effect on A836. A similar distribution by molecular weight is observed in the effect of the same microorganisms on the Monterey crude A837. The biochemical reactions occurring during the biotreatment favor the formation of lighter hydrocarbons and emulsions. These reactions are very prominent in the treatment of Monterey A851 crude oil, which is a heavy (API gravity 11.9)

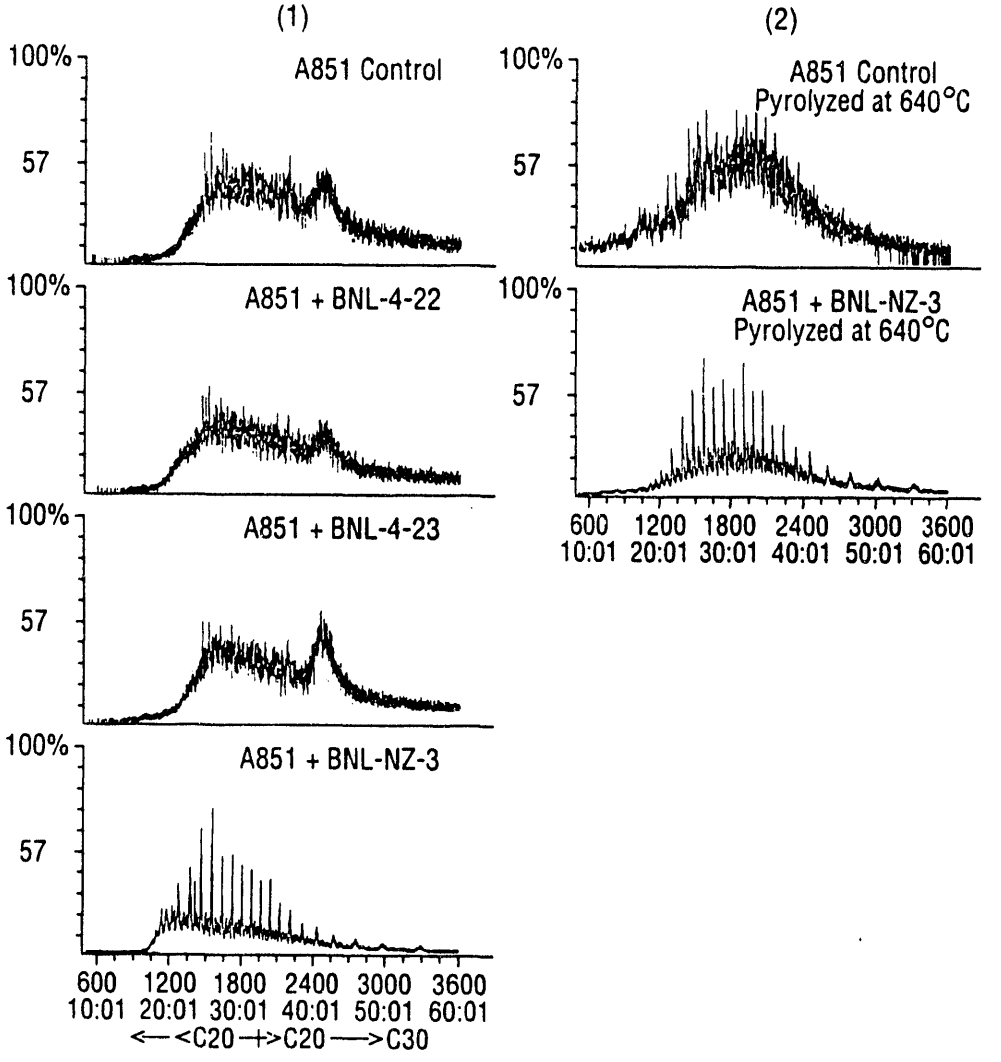


Figure 10. GC-MS Fragmentograms m/z 57 of Monterey, CA, Crude A851 (1) treated with BNL-4-22, BNL-4-23, and BNL-NZ-3 and; (2) Pyrolyzed Control and Control and BNL-NZ-3 at 640°C.

biodegraded oil. This oil is not volatile, as shown by the fragmentograms in Figure 10, even on pyrolysis at 640°C. Biotreatment of A851 with BNL-4-22 and BNL-4-23 does not significantly alter the molecular composition of the crude (compare to the effects on A836 and A837). However, the effect of BNL-NZ-3 is extensive, breaking down the heavy fractions to more volatile, lighter fractions (Figure 10), indicating that the biotreatment of A851 with BNL-NZ-3 is a mild biocracking process which may well be initiated in the polar fraction of the maltenes. Mechanistically, this may mean that the microbial action starts at metal, sulfur, and other functional group sites and then proceeds at the level of carbon-to-carbon bonds. Results of the sulfur speciation occurring during the biotreatment of Monterey and other domestic heavy crudes will be discussed elsewhere. However, the preliminary results support changes similar to those observed for the Venezuelan (Table 3) and Wilmington crudes (Figure 1), discussed in the introductory paragraphs.

4. CONCLUSIONS

Data presented in this paper are consistent with previous observations and allow us to come to the following conclusions:

1. There are distinct trends in the biochemical interactions between microbial species and crude oils, which appear to follow a distinct pattern.

2. Shorter periods of biotreatment are possible.

3. The molecular markers used in this study are indicative of the trends and may ultimately prove to be useful diagnostic tools in choosing strategies for the biochemical production and processing of crude oils. Core flooding experiments in which the fate of molecular markers is monitored will further define their usefulness in studies of MEOR.

5. ACKNOWLEDGEMENTS

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Characterization of Xanthan Gum Degrading Enzymes from a Heat-stable, Salt-tolerant Bacterial Consortium

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Abstract

A bacterial consortium resulting from soil enrichment growth on xanthan gum has been described (NRRL B-14401)[1,2]. The enzymes involved in the degradation of xanthan gum are functional in salt-containing solutions at temperatures up to 65°C. One enzyme, xanthan lyase, has been purified and consists of a 33,000 Da. protein that specifically removes pyruvated mannose residues from the non-reducing end of the side chains of xanthan gum; unsubstituted mannose residues are not released. Hydrolysis of the linkage results in the formation of an unsaturated glucuronic acid (4,5-*ene*) terminating the side chain. Removing the pyruvated mannose from the side chain of the polysaccharide reduces the viscosity of the solution containing the gum, which depends on the degree of pyruvation of the polysaccharide. The purified enzyme was very stable at 55°C; above 55°C, stability was aided by the addition of salt (1 to 2% sodium chloride). At 65°C, the purified enzyme lost activity in less than one hour. A second enzyme, xanthan depolymerase, cleaves the backbone linkages of the polymer, which significantly lowers the solution viscosity. This enzyme also can hydrolyze carboxymethyl cellulose very efficiently.

1. INTRODUCTION

Xanthan gum is a polysaccharide produced by the bacterium *Xanthomonas campestris*. The structure of the polymer has been determined [3,4], and it consists of a cellulosic backbone with a trisaccharide side chain attached to every other glucose unit of the backbone. The side chains consist of a terminal mannose, which may be pyruvated depending upon growth conditions, followed by a glucuronic acid, and a mannose attached (α1-3) to the glucose in the backbone. Xanthan gum is produced commercially as a food additive because of its emulsifying and viscosity building properties, and has numerous industrial applications as well. It is used as a viscosifier of drilling fluids because the physical properties of this polysaccharide tolerate a variety of temperature extremes and solution conditions. It is used in drilling fluids made with brine as well as with freshwater, due to its relative stability in salt solutions. The polysaccharide demonstrates pseudoplastic behavior in solution; shearing results in a loss of viscosity, which is regained upon removal of the shear forces. These properties make xanthan gum a good candidate for a viscosifier of hydraulic fracture fluids for the recovery of natural gas, where it is used to suspend the propping agents that are forced under pressure into the fracture zone. However, once the propping agent such as sand is in place, it is desirable to reduce the viscosity of the fracture fluid to stimulate the flow of gas out of the fracture zone. Chemical degradation of the polysaccharide with hypochlorite reduces viscosity, but this method is potentially hazardous and difficult to control, and has the potential of leading to groundwater contamination.

The need for an enzymic viscosity breaker that would function at elevated temperatures and salt concentrations led to the discovery of a bacterial consortium isolated by soil enrichment growth on xanthan gum that produced the appropriate enzymes for reducing the viscosity of xanthan gum solutions [1]. The extracellular enzymes produced by this consortium were shown to retain activity up to 65°C, and were stabilized by the addition of salt. Analysis of the degradation products of xanthan gum treated with the enzyme mixture showed that pyruvated mannose was released from the side chain of the polysaccharide, and that the backbone of the polymer was cleaved in a novel manner resulting in branched oligosaccharides. Preliminary characterization of the enzymes involved in the hydrolysis of xanthan gum indicated the action of a β-(1-4)-glucanase for backbone cleavage, and a lyase, which removed the pyruvated mannose and resulted in an unsaturated 4,5-*ene*-glucuronic acid on the non-reducing end of some of the oligosaccharides. The enzymes produced by this consortium have greater heat-stability than those which have been described in earlier reports. Cadmus et al. [5] described a salt-tolerant enzyme complex that could degrade xanthan gum, which was produced by a *Bacillus* species, but this complex was not very thermostable. A salt-tolerant bacterial consortium labeled HD1 was described by Hou et al. [6], and it had somewhat greater thermal stability, but exposure for 20 minutes at 45°C reduced enzymatic activity by 66%, and it was totally inactivated by exposure to 60°C.

2. BIOSYNTHESIS OF HEAT-STABLE, SALT-TOLERANT XANTHANASE

The bacterial consortium developed by Cadmus et al. [1] can be maintained in liquid stock solutions at 4°C in a buffered growth medium containing 0.15% (w/v) xanthan gum and 2% NaCl. For the production of the xanthan-degrading enzymes, sterile media is inoculated and cultured at 45°C until the viscosity of the media is substantially reduced. A sample from this batch is then used to inoculate 1 liter cultures in 2.8 liter fernbach flasks which are incubated at 45°C with shaking. To examine the rate of bacterial growth and determine the rate of xanthanase produced when cultured under these conditions, aliquots of the growing culture were sampled at 6 hour intervals for a total of 80 hours, and the xanthanase activity measured and the viscosity of the culture determined. Enzyme activity is measured by incubating supernatants of the culture broth with buffered xanthan (0.1% w/v) at 45°C for 1 hour, and subsequently measuring the amount of reducing sugar formed by hydrolysis of the polysaccharide with a Bran and Luebbe Autoanalyzer II automated analyzer using an alkaline ferricyanide method. The results show that the viscosity of the growth media begins to decline at 16 to 18 hours (Figure 1), which is inversely proportional to the increase in cell growth, which was determined by measuring the absorbance of the media at 600 nm. To examine the amount of xanthanase produced, the sample was briefly centrifuged and the supernatant assayed for xanthanase activity. Because the glucanase (depolymerase) can also hydrolyze carboxymethyl cellulose (CMC), this compound is also tested as a substrate to discriminate between xanthan-degrading activity attributible to the lyase, and that due to the action of the depolymerase. Figure 1 shows that after an initial lag phase the amount of xanthan degrading activity rises and peaks at 36 hours, followed by a decline in total xanthanase activity over the next 48 hours. The profile of carboxymethyl cellulase activity is different; the level of CMCase activity reaches its maximum at 36 hours, but this level is maintained for the next 48 hours, after which it gradually declines. The xanthan-degrading activity is a measure of the total activity contributed by the lyase and the depolymerase enzymes.

Table 1
Effect of salt on xanthanase activity

NaCl (g/100 ml)	0	2	4	6	8	10
Activity (% of maximal)	100	75	60	50	45	40

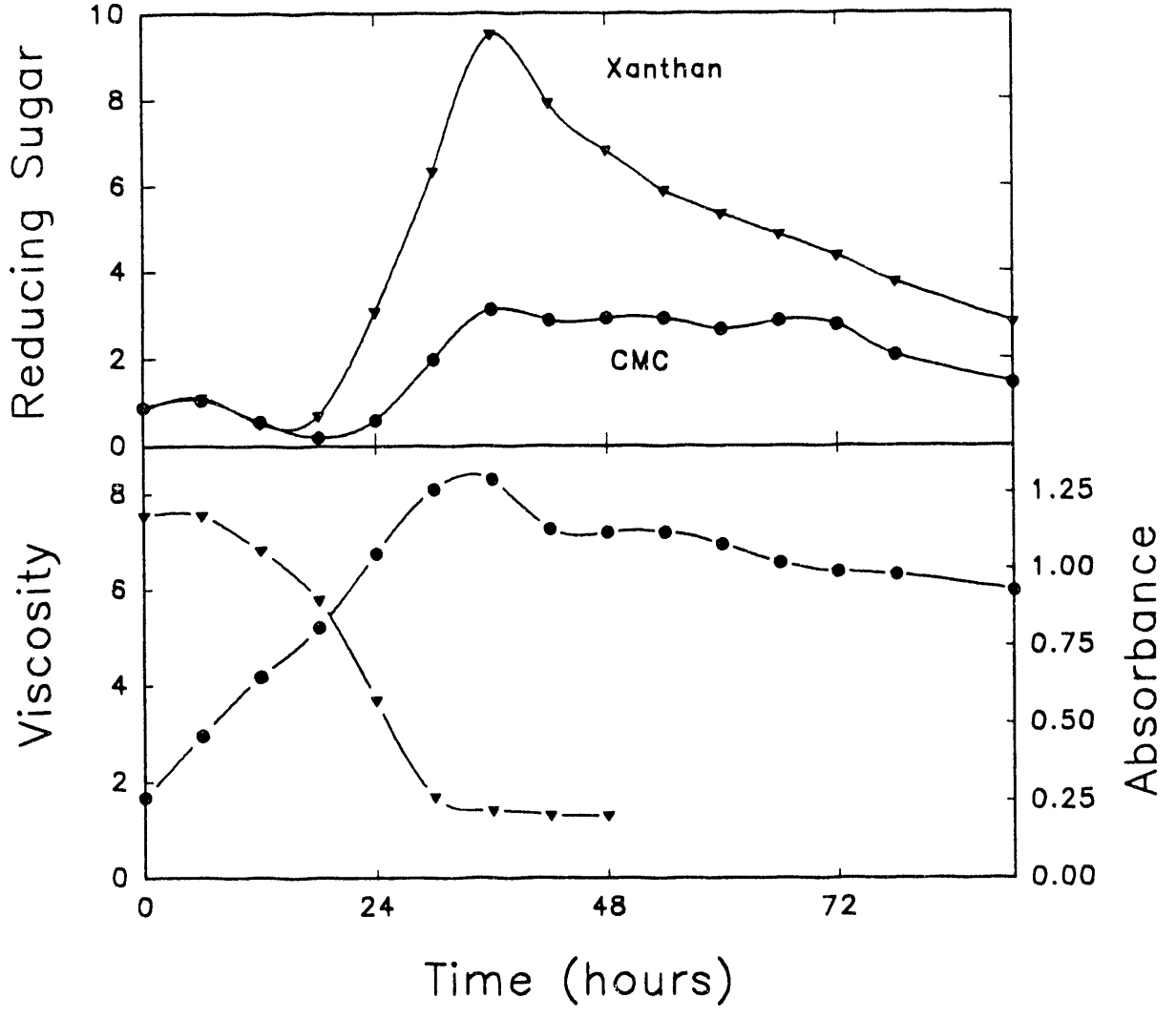


Figure 1. *Upper panel:* Rate of xanthanase enzyme production by the bacterial consortium; (▾) - reducing sugar formed in 1 hr at 45°C using xanthan gum for substrate; (•) - reducing sugar formed in 1 hr at 45°C using carboxymethyl cellulose for substrate. *Lower panel:* Change in viscosity and cell growth during 45°C culture; (▾) - viscosity of the culture medium in mPa-sec; (•) - absorbance of the culture medium at 600 nm.

Table 2
Effect of salt on thermal stability of xanthanase

Temperature (°C)	50	55	60	65	70
Activity after 20 min (% of maximum)					
- no salt	100	100	80	40	10
+ with salt	100	100	100	82	20

3. CHARACTERIZATION OF XANTHANASE ACTIVITY

The bacterial consortium is regularly cultured in 1 liter quantities contained in 2.8 L fernbach flasks, and batches up to 12 liters have been prepared. The bacteria are removed by centrifugation, and the cell-free supernatant is concentrated by ultrafiltration and then dialyzed to reduce the salt concentration. The enzymes are stable to freeze-drying, for long-term storage, or can be kept at 4°C for extended periods if an antimicrobial agent, such as sodium azide, is added to the mixture. The enzymatic properties of the xanthanase mixture have been studied to characterize the heat-tolerance, salt-tolerance, and optimal pH of xanthan-degrading activity [1]. Table 1 summarizes the salt-tolerance of this enzyme mixture; in 2% NaCl the enzyme activity is reduced 25%; at 10% NaCl the activity is reduced by 60%. The observed reduction in activity caused by the addition of salt is offset by the enhanced heat stability that salt provides to the enzyme; Table 2 shows that xanthanase supplemented with 3% NaCl will retain 82% of its original activity if incubated at 65°C for 20 minutes, while enzyme without salt retained only 40% of its activity when treated in the same fashion. The mechanism by which salt extends the thermal stability of these enzymes is not known at this time.

The xanthanase mixture was shown to be functional for at least 3 days at 50°C in a long-term assay containing enzyme and substrate, shown by the steady increase in reducing sugar formed throughout this period (Figure 2). During a 24-hour period, the amount of reducing sugar increased when the mixture was incubated at 60°C as well. These results indicate that the xanthanase mixture has the heat stability required to function in many of the underground reservoirs of moderate depth, which are in this temperature range.

The enzyme mixture has its maximal activity at pH 6; activity rapidly declines between pH 5, which is 85% of maximal, to totally inactive at pH 4. Enzyme stability to acid exposure parallels enzyme activity, with the enzymatic activity destroyed by incubation at pH 4. In alkaline conditions, the activity declines to approximately 60% of maximal rate at pH 7, and further to only 15% at pH 8, but in contrast to acid exposure, the enzyme activity is not inactivated by exposure to alkaline solutions [7].

4. PURIFICATION OF XANTHAN LYASE

Xanthan lyase, which selectively removes the pyruvated mannose residues from the terminal position of the side chain of xanthan gum, can be purified from the culture supernatant of the concentrated bacterial consortium following ion-exchange chromatography and gel filtration chromatography [8]. The culture supernatant is dialyzed to remove salts and to equilibrate the solution to 20 mM

sodium phosphate, pH 6.0, for ion-exchange chromatography. This step greatly enriches the lyase enzyme, and separates it from the depolymerase, which can be seen by the separation of xanthan-degrading activity into two peaks, of which only one also contains CMCase activity (Figure 3). The lyase is then chromatographed on a gel filtration column in 20 mM sodium phosphate buffer containing 6M urea, which helps prevent protein aggregation, and the result is an electrophoretically pure enzyme of 33,000 Da. The purified enzyme has been characterized and its properties compared to the mixture of xanthanases contained in the original culture fluid. Treatment of xanthan gum with the purified lyase partially reduces the viscosity of the solution; for a polymer with 75% of its terminal mannose residues having a pyruvate attached, the reduction in viscosity is 30 to 35% (Figure 4). The loss of viscosity under these conditions is accompanied by an increase in reducing the viscosity of the solution; for a polymer with 75% of its terminal mannose residues having a pyruvate attached, the reduction in viscosity is 30 to 35% (Figure 4). The loss of viscosity under these conditions is accompanied by an increase in reducing sugar, due to the liberation of pyruvated mannose. That only pyruvated mannose was being released was shown by recovering the low molecular weight product of hydrolysis, and subjecting it to analysis by gas chromatography/mass spectrometry (GC/MS). A single GC peak with a mass spectra consistent with pyruvated mannose was the only observed result [8]. When xanthan gum is treated with the purified lyase, the reduction in viscosity and reducing sugar values reach a constant value as the accessible pyruvated mannose residues are removed; when xanthan gum is treated

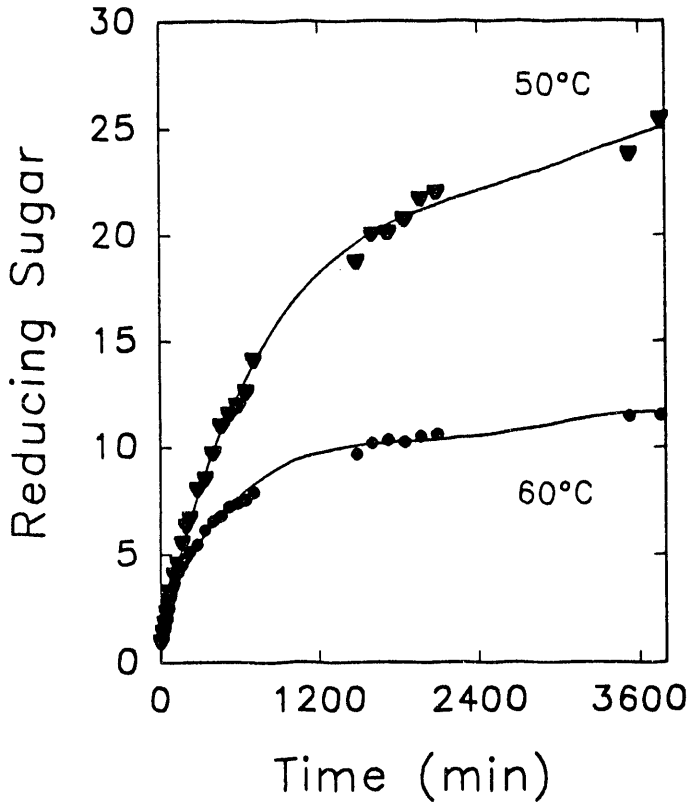


Figure 2. Time course of enzyme activity at 50 and 60°C; concentrated culture supernatant was added to 0.2% (w/v) xanthan gum and incubated at the above temperatures. At time intervals indicated samples were removed and the reducing sugar measured.

with a mixture of the lyase and the depolymerase, the viscosity continues to decline and the reducing sugar value increases.

The effect of salt, as sodium chloride, on xanthanase activity as well as on its stability to elevated temperature indicates an interesting effect. Enzymatic activity is at its highest in buffers of low ionic strength, both for the mixture and for the purified lyase. In the case of the lyase, 50 mM NaCl promoted the highest activity, slightly better than when no salt was added to the purified enzyme in 20 mM sodium phosphate. At 0.5 M NaCl (3%), activity of the lyase had declined to 40% of its optimum. The mixture of the lyase and depolymerase still had 70% of its optimum activity at 3% NaCl, indicating that the lyase may be the more salt-sensitive enzyme of the two. While the presence of salt causes a decrease in enzyme activity, it enhances the stability of the enzymes at higher temperatures. This effect can be seen by incubating the lyase in buffer of low ionic strength and also in buffer containing 0.25 M NaCl at 55°, 60°, and 65°C for extended periods, and subsequently measuring the amount of enzyme activity remaining (Figure 5). For the purified lyase, the enzyme activity was stable to exposure at 55°C for more than 6 hours, whether or not the buffer contained any

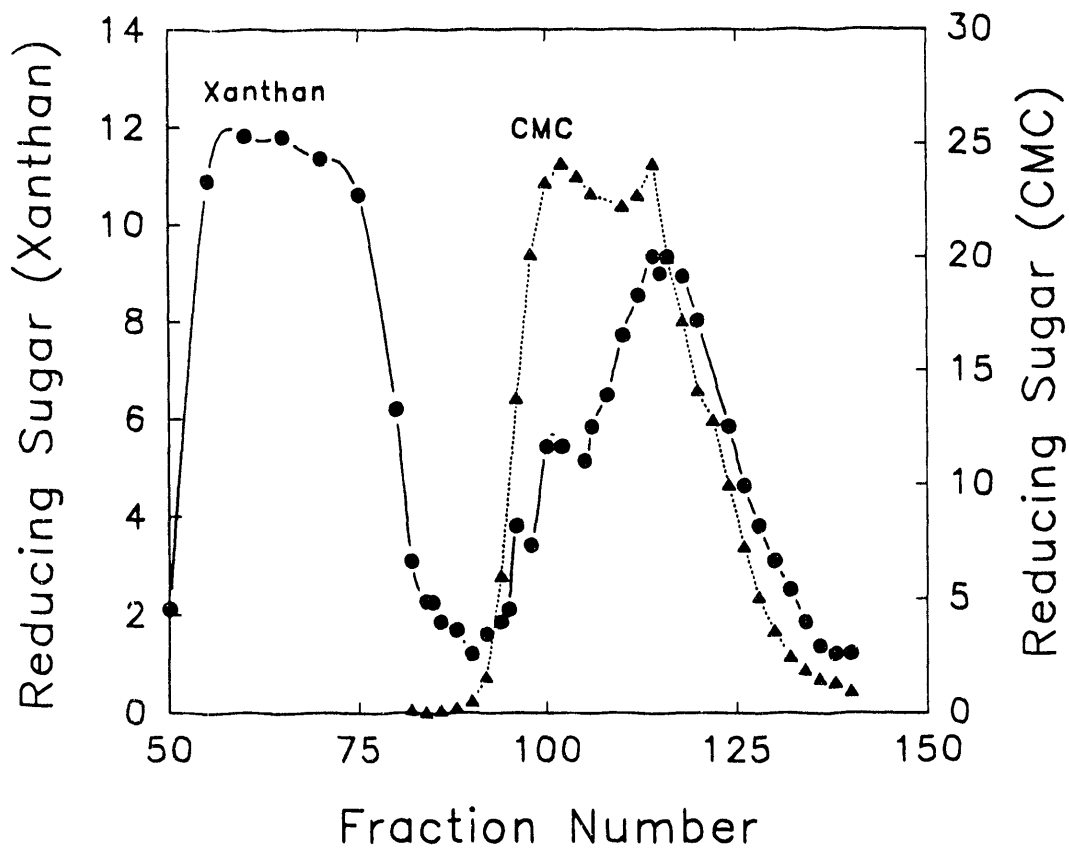


Figure 3. Separation of xanthan lyase and xanthan depolymerase by ion exchange chromatography; (●) - reducing sugar formed in 1 hr at 45°C using xanthan gum for substrate; (▲) - reducing sugar formed in 1 hour at 45°C using carboxymethyl cellulose as substrate.

NaCl. At 60°C, however, a significant stabilizing effect by NaCl can be seen. After 1 hour, 85% of the lyase activity remained relative to unheated controls when salt was added, while only 40% of the activity remained after 1 hour if no salt was added to the purified enzyme. After 3 hours, 75% of the activity remained when salt was present, compared to only 15% if salt was omitted.

5. SUMMARY

The xanthan gum-degrading enzymes produced by this bacterial consortium when xanthan gum is used as the primary carbon source have enhanced thermal stability and the ability to function in salt-containing solutions. While there are deep

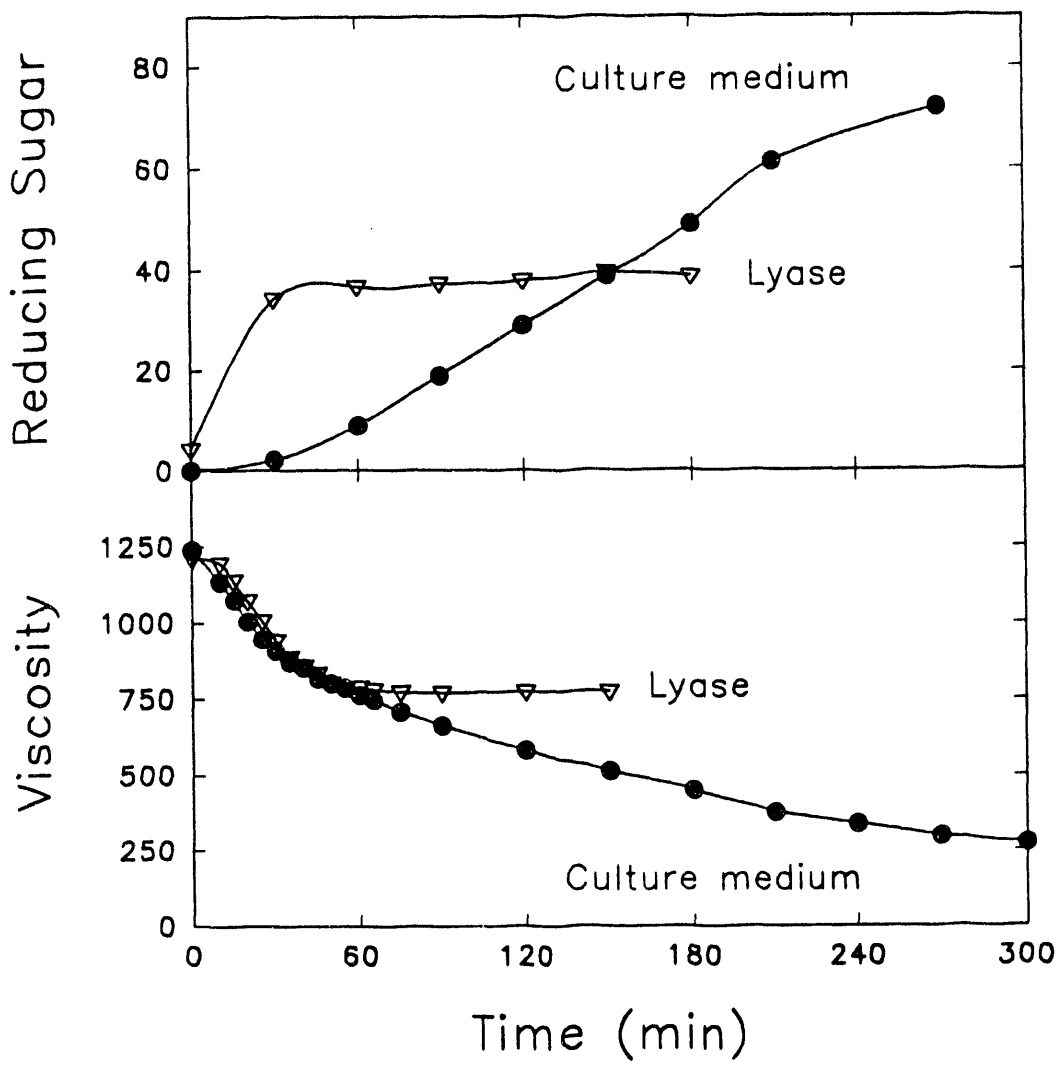


Figure 4. *Upper panel:* Time course of reducing sugar formed at 45°C using xanthan gum as substate; (•) - concentrated culture medium added; (▽) - purified lyase added. *Lower panel:* Time course of reaction solution viscosity caused by the addition of enzyme; (•) - concentrated culture medium added; (▽) - purified lyase added.

wells that are at temperatures above the range that these enzymes can function, there are many situations where the temperatures are at or below 65°C, which suggests that these enzymes could be used to lower the viscosity of xanthan gum-based drilling and fracture fluids. As the degradation of the polymer will be proportional to the amount of enzyme added and the temperature of the formation

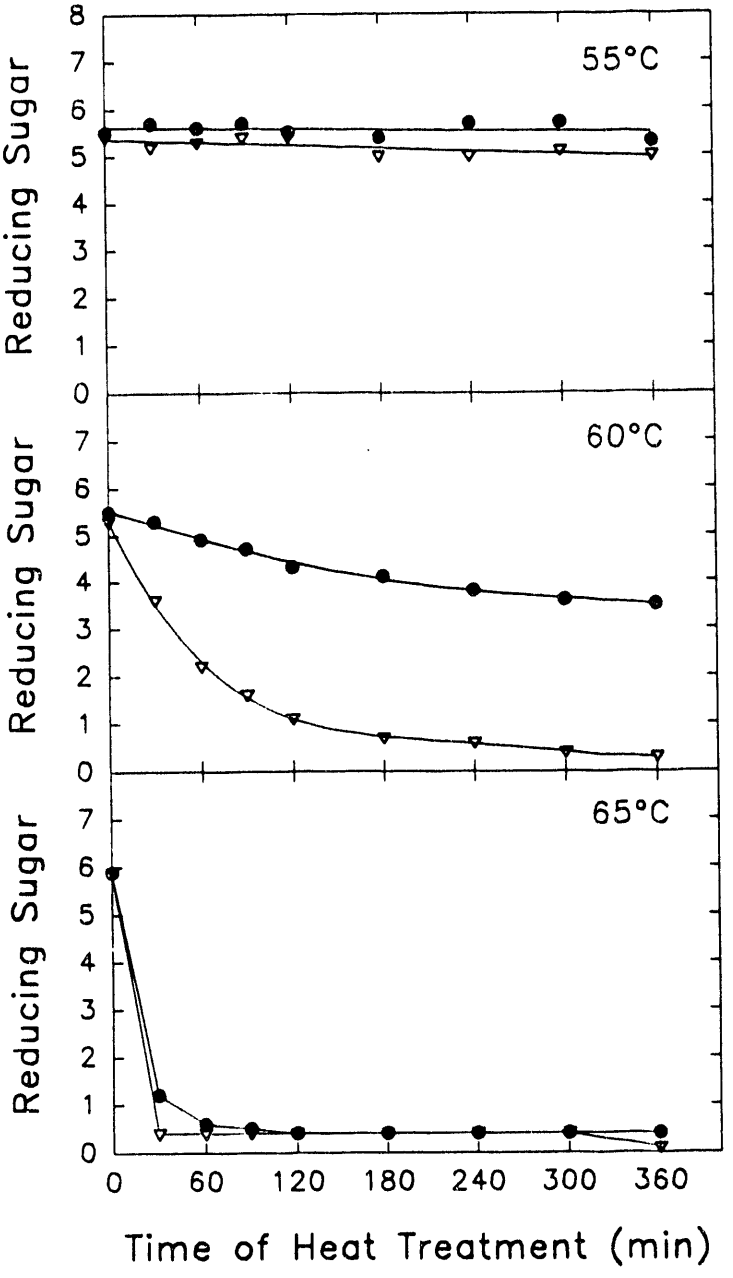


Figure 5. Heat stability of the purified lyase. Purified xanthan lyase was heated for the times indicated (0 - 360 min), with (●) and without (▽) 0.25 M NaCl, and the residual enzyme activity measured in a separate 1 hr at 45°C assay using xanthan gum. *Upper panel:* enzyme activity remaining after heat treatment at 55°C; *middle panel*: enzyme activity remaining after 60°C treatment; *lower panel:* enzyme activity remaining after heat treatment at 65°C.

that they would be used in, it should be possible to control the rate of viscosity reduction by adjusting the ratio of enzyme to xanthan gum. Fracture fluids can be made out of freshwater, brine, or even seawater, because the enzymes are functional in salt-containing solutions; some adjustment of the pH of the fluid to near 6 would improve hydrolysis of the polymer. The advantage of this technology is that it is non-hazardous and non-toxic, unlike the current chemical means to reduce the viscosity of xanthan gum-based fluids. Therefore, the risks of groundwater contamination and operator exposure to hazardous chemicals should be greatly reduced.

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Subsurface Application of *Alcaligenes eutrophus* for Plugging of Porous Media

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Abstract

Alcaligenes eutrophus, which produces a massive amount of intracellular polyester--poly-3-hydroxybutyrate(PHB)--as high as 70% of the cell weight, was selected for porous media plugging studies. To simulate the subsurface environment, both static drainage and pressurized pumping flow systems of *A. eutrophus* living cells and PHB suspensions through laboratory sand packs were investigated. In the static drainage flow system, the effluent rate was reduced about 280-fold. For the pressurized pumping flow system, the relative permeability (K_1/K_0) was reduced by one million. Both dead cells and living cells of *A. eutrophus* plugged the sand pack columns in the static drainage flow system. The dead cell suspension reduced the effluent rate by 4.3 times within 5 hrs., while the living cell suspension made a 280-fold reduction. A PHB water solution, a commercial product in powder form which disperses well but is not totally dissolved in water, showed the plugging effects solely dependent on the concentration of PHB. These facts signify that *A. eutrophus* and its microbial product, PHB, are efficient plugging agents. They have potential applications in the MEOR process, such as selective plugging, because of their relative non-agglomerating cell size, their rod shape of 0.7 m in diameter and 1.8-2.6 m in length, and their lack of any exopolymer in culture solutions, especially the beneficial biopolymer (PHB) produced internally in cells.

1. INTRODUCTION

In the microbial enhanced oil recovery (MEOR) process, bacterial plugging has two extremely different effects. In the secondary production of oil, bacteria in waterflooding injection waters may plug the injection at the formation face, causing a loss of injectivity which can seriously jeopardize the overall production of oil. However, the waterflood performance can be improved by reducing fluid channeling through high-permeability zones. Therefore, bacterial plugging in porous media has been studied for a long time. There are two types of general mechanisms of microbial plugging likely to occur in the wellbore region: particulate plugging with the bacterial cells' aggregates and the buildup of biofilms in the wellbore region by viable microorganisms able to colonize the rock surface [1]. The presence of exopolymers produced by bacteria greatly enhances the formation of biofilm in the second general plugging mechanism.

Improvement of waterflood, repairing of damaged reservoirs, and solving water coning problems in MEOR can be achieved by plugging the wellbore region. The key to fulfilling these improvements is to select the proper plugging agents. For this work, *A. eutrophus* (ATCC #17699), a strain using a variety of organic compounds as the sole carbon source and soil and water as habitats, has been investigated for the plugging agent of porous media. Commercial PHB powder, which is the intracellular product of *A. eutrophus*, showed potential plugging in sand pack columns when its water solution was used as injection fluid. The plugging efficiency of the PHB water solution is related to its concentration in solutions quite well. This means that it may become a good plugging agent for those situations in which control of the extent of plugging is needed. We found

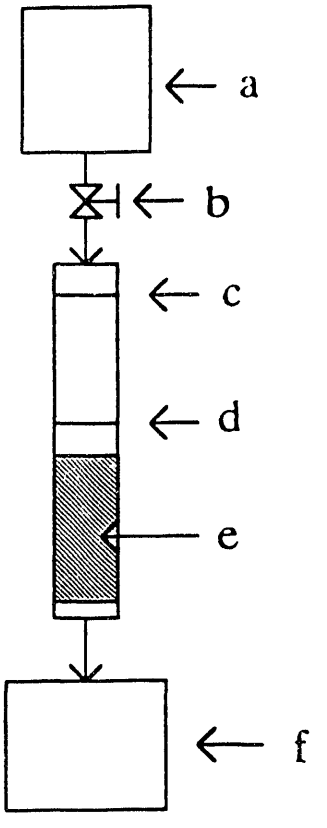
that the plugging effects of *A. eutrophus* matched the two general mechanisms above. The dead cell suspension plugging showed the particulate plugging result, and plugged the sand packs column by the cells themselves without any help. The plugging effects of the living cell suspension can be explained by the biofilm formation mechanism. In this case, with no exopolymer or any other agents, the living cell suspension showed more plugging effects than the dead cell suspension, which led to the conclusion that it is caused by the growth of *A. eutrophus* on the sand surface.

This marks the first time that *A. eutrophus* and its bioproduct, PHB, have been studied in the application of plugging for the MEOR process. Bacteria, which do not produce any extracellular products, have broad applications in selective plugging, water coning, and fouled reservoir repair. More promising applications have been expected for those bacteria who form intracellular polymers, such as *A. eutrophus*, instead of exopolymers. In particular, the bioproduct of *A. eutrophus*, PHB, has many unique characteristics that have drawn our attention.

2. EXPERIMENT

2.1. Static Drainage System

Bacterial suspensions or other test fluids drain through small sand pack columns to be designed as a preliminary study tool. With this rapid test, the



(A) bacterial suspension container

(B) flow rate control clamp

(C) upper mark

(D) lower mark

(E) sand pack

(F) effluent collector

Figure 1. Schematic diagram of the static drainage flow system.

plugging effects of test fluids are differentiated roughly but clearly. Figure 1 shows this simple system, containing a suspension container, a sand column, and an effluent collector. The test fluids are drained from the container at a certain rate to maintain a liquid level in the column between the upper and lower marks. The sand columns were made of 20 g., 32-100 mesh standard Ottawa sand held in glass tubes with an average porosity of 46.4%. The glass tube was vertically set up, with both of its two ends being open to the atmosphere, but the bottom end having a screwed cap with a piece of screen in it which held the sand.

Three types of fluids were run in this static drainage system: (1) a living cell suspension of *A. eutrophus*, (2) a dead cell suspension of *A. eutrophus*, and (3) commercial PHB water solutions. The living cell suspension was the culture of two-day growth of *A. eutrophus* in nutrient broth. The dead cell suspension was gained by sterilizing the living cell suspension in autoclave for 20 minutes. Three PHB water solutions, A, B, and C, were made from one original solution. The original solution was 0.913 g. PHB powder in 1 liter of distilled water, sonicated for 16 hours at 250 setting to disperse the PHB well in water, because PHB has very little solubility in water. After sitting overnight, 250 ml of that solution were filtered out with filter paper. The filtrate was collected as solution A, and had a concentration of 0.380 g. PHB/L. Solution B was 250 ml of

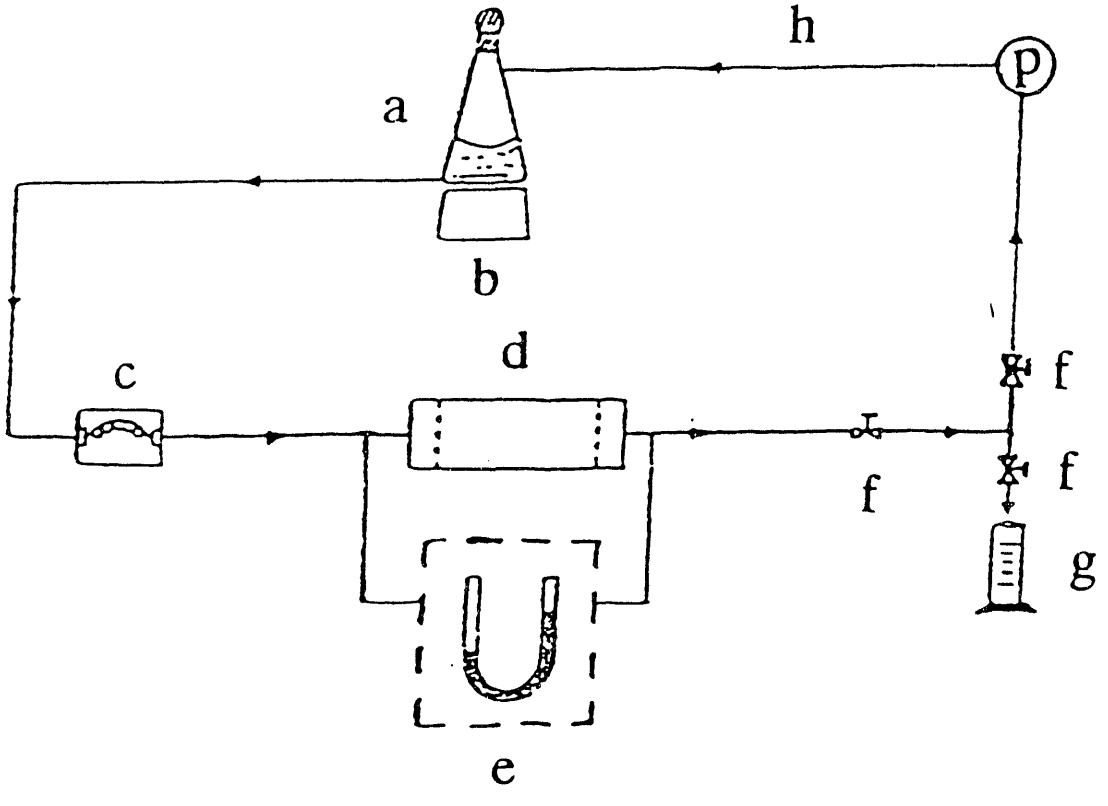


Figure 2. Schematic diagram of the pressurized flow system. (A) bacterial suspension container; (B) magnetic stirrer; (C) peristaltic pump; (D) sand pack; (E) U-shape tube pressure difference measurement with mercury; (F) control valves; (G) effluent collector when effluent rates were measured; (H) recycle line of the effluent; P recycle pump.

the original solution without filtration, containing 0.630 g. PHB in 1 liter of water. The remaining original solution became solution C, which contained 1.193 g. PHB/L.

2.2. Pressurized pumping flow system

An experimental apparatus was carefully designed in such a way that the actual hydrodynamic conditions for bacteria transport in porous media could be simulated. Figure 2 shows the equipment. The source flask was kept at a high elevation to minimize the settling of bacteria in the inlet flow line. Its bacterial source would continuously be stirred with a magnetic stirrer to make the suspension uniform. A U-shaped glass tube filled with mercury was added in the set-up to monitor the pressure drops across sand pack columns. The column was an acrylic cylinder of 10 cm in length and 2.5 cm in diameter. Standard Ottawa sand, SX 75, 30-50 mesh size, obtained from Matherson Coleman & Bell, was used as the packing material. Before packing the column, the sand was thoroughly washed with distilled water to remove fines and then dried at 80° C for at least 48 hours, until totally dried. To prepare the bacterial suspension, *A. eutrophus*

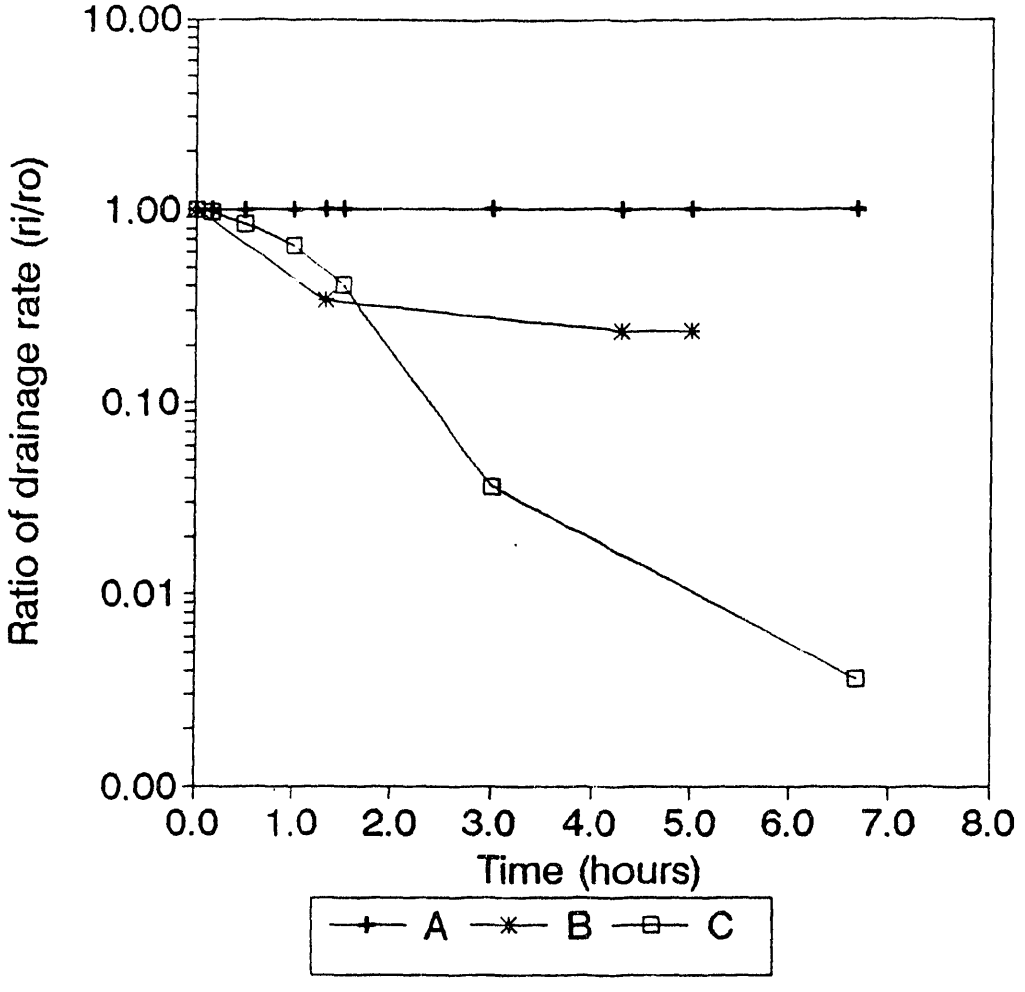


Figure 3. Plugging effect of *A. eutroupus* cell suspension in sand pack columns determined by static drainage flow system. (A) nutrient solution only; (B) dead cell suspension; (C) living cell suspension. The cell suspensions were two-day growth cultures. The dead cell suspension was prepared under autoclave operation.

was grown in a nutrient broth for 2 days. Bacterial suspensions were replaced by fresh 2-day ones after being recycled in the test system for 3 days to maintain the live cell solution state being used in the plugging tests. According to the *A. eutrophus* growth curve made in this laboratory by the viable counts method, the doubling phase of *A. eutrophus* starts after 1 day of growth in nutrient broth and lasts 2 days until the third growth day. Several cells are still alive after five days.

3. CALCULATION OF EXPERIMENTAL RESULTS

3.1. Static drainage system

The draining time for the test fluids from the upper mark down to the bottom mark was recorded originally at regular intervals. Since the distance between the two marks were fixed for all the columns, the ratios of the drainage rates can be expressed as $r_i/r_o = t_o/t_i$.

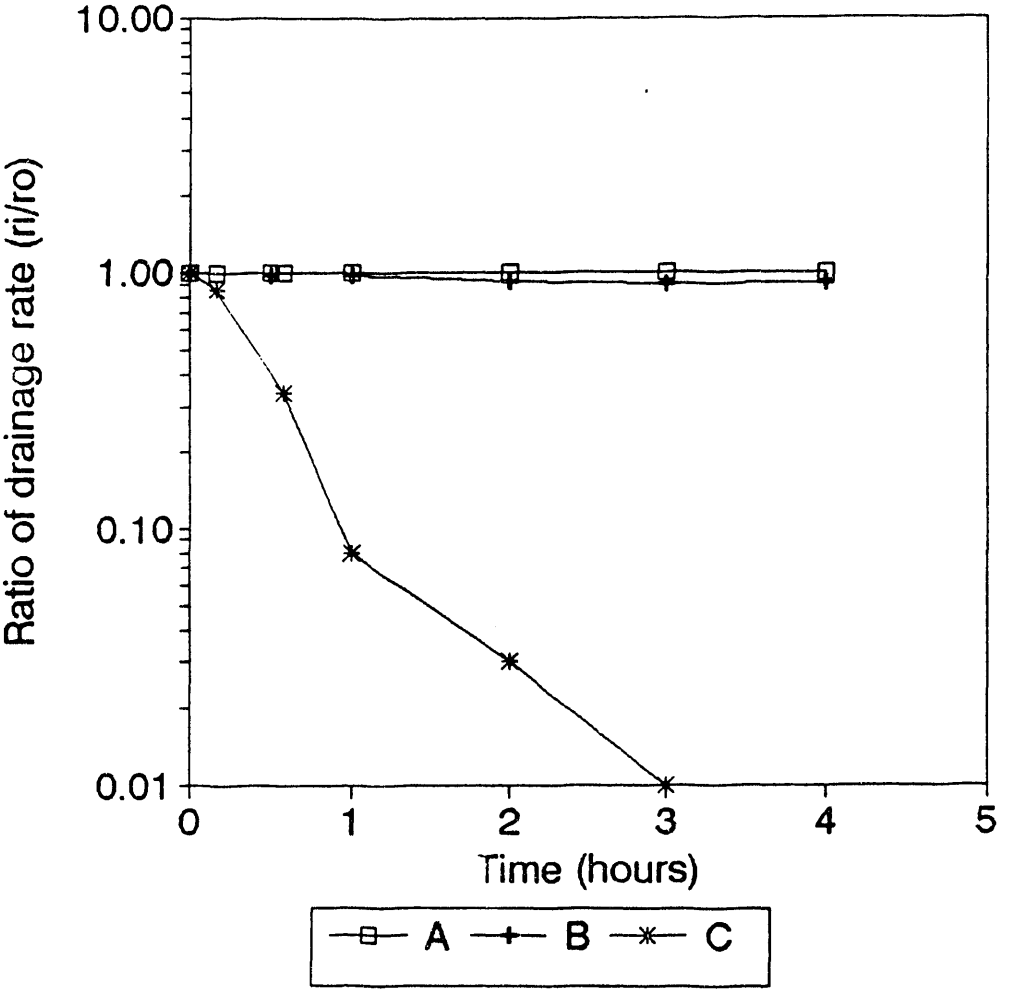


Figure 4. Plugging effect of commercial poly-3-hydroxybutyrate (PHB) powder dispersed in distilled water in sand pack columns determined by static drainage flow system. Solutions were sonicated at 250 setting for 16 hours. (A) distilled water and 0.380 g PHB/L; (B) 0.630 g PHB/L; (C) 1.193 g PHB/L.

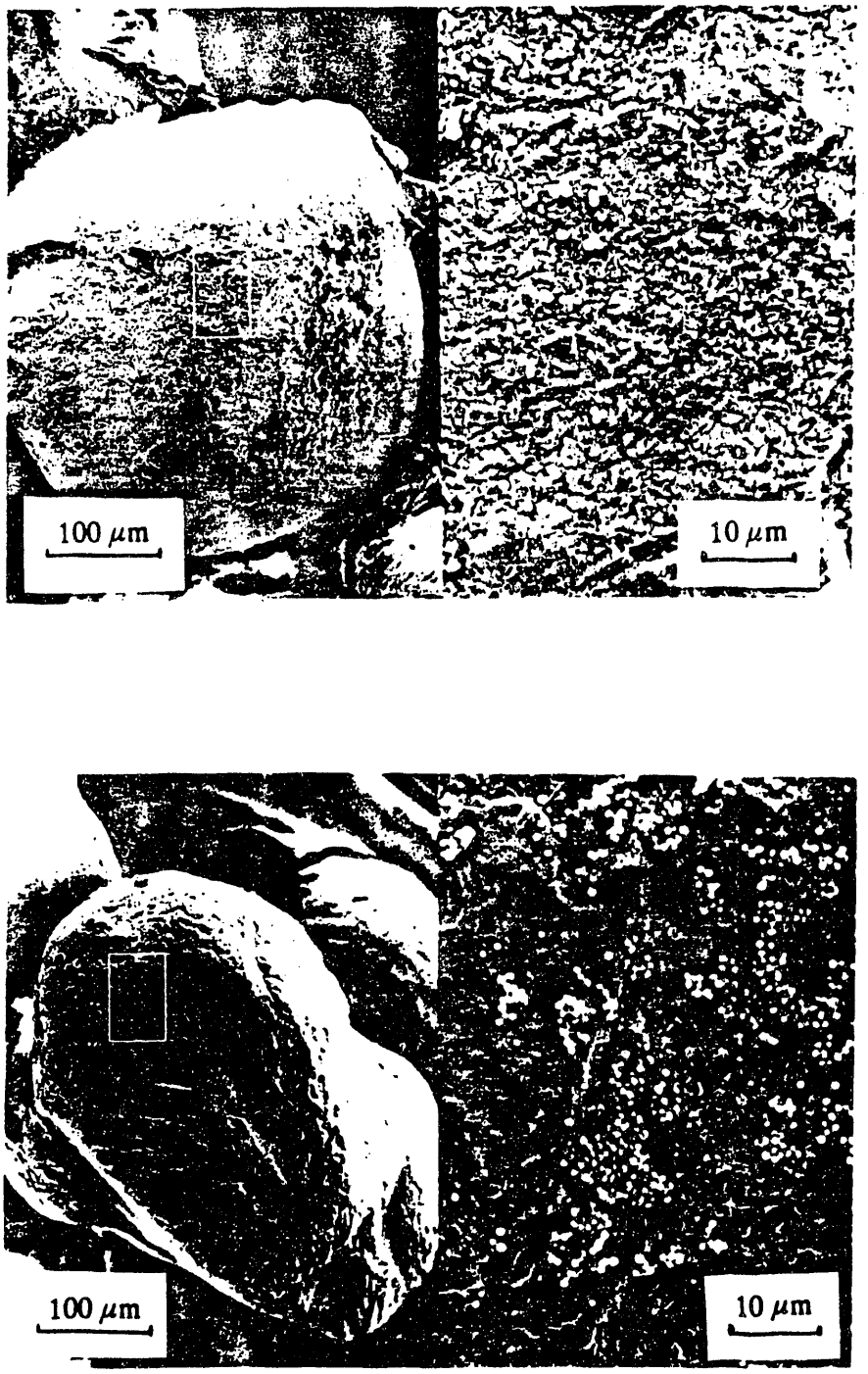
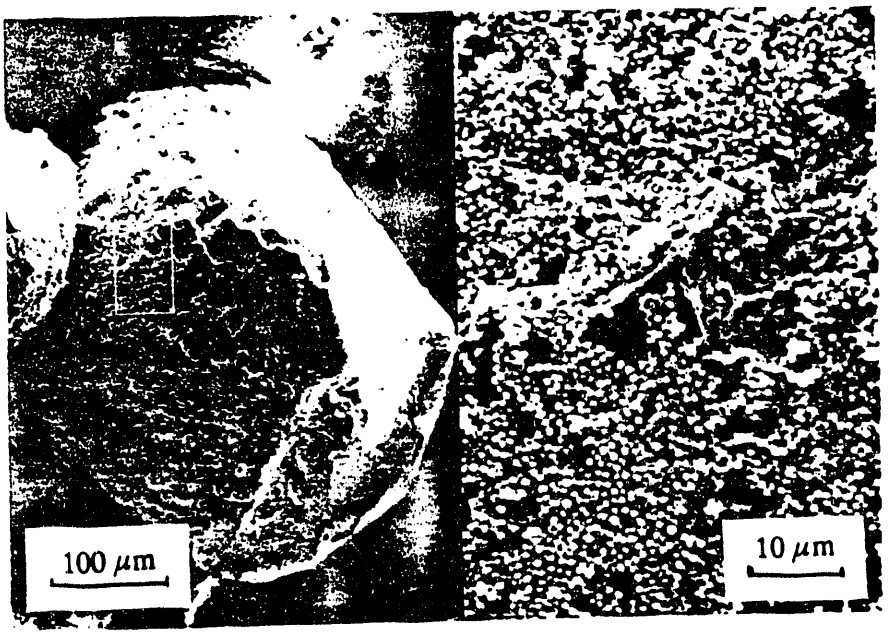


Figure 5. Scanning electron micrographs of sand surface from the packed columns after plugging test with commercial PHB powder water solutions. The clumps of PHB beads on the right side show the detail of the small rectangular on the left side. (A) solution A; (B) solution B; (C) solution C. The following were taken on the surface area where few clumps were found: (D) solution A; (E) solution B; (F) solution C.



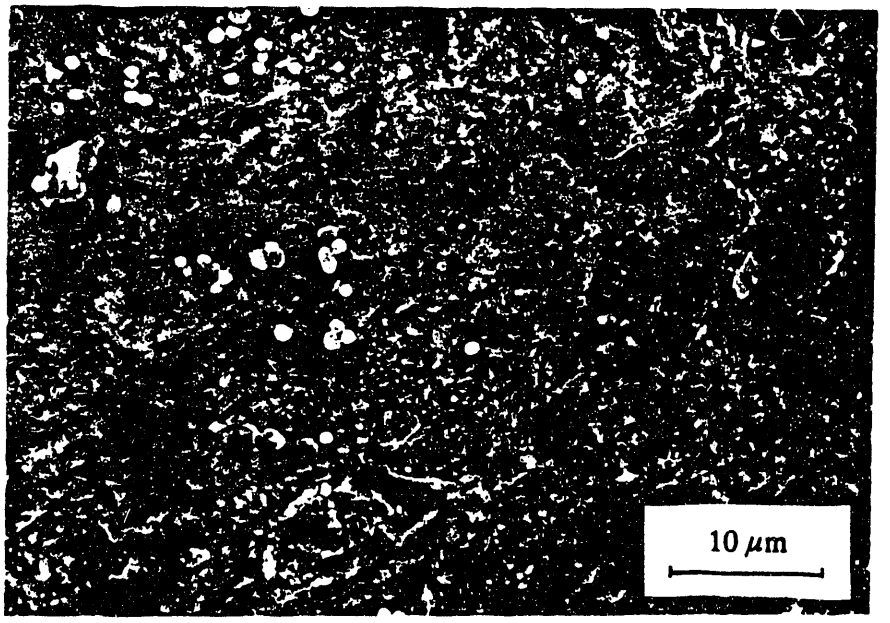


Figure 5 (C) and (D). See caption for Figure 5 for details.



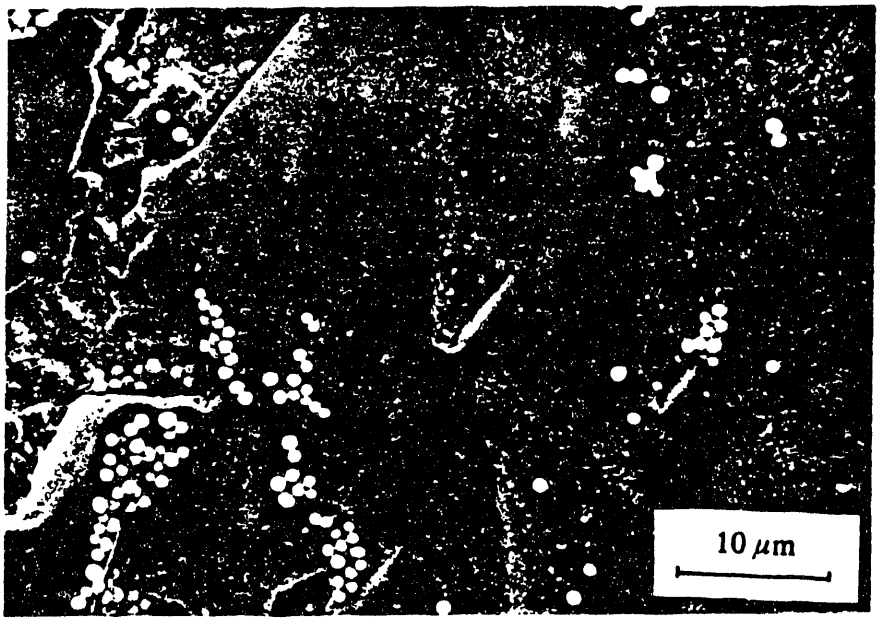


Figure 5 (E) and (F). See caption for Figure 5 for details.

3.2. Pressurized pumping flow system

In this system, the column was horizontally mounted in the set-up, and the pressure recording device was connected across the column. Before pumping the bacterial suspension in each run, sterilized distilled water was pumped through the flow system at the same rate as the bacterial suspension to clean the system. The pressure difference of complete plugging in the column was 30 psi, determined by closing the outlet control valve. The peristaltic pump provided this pressure difference constantly to force the suspension through the system. Before any plugging, the flow rate (which is determined by collecting the effluent at the outlet of the system) was measured at around 3.65 mL/min., and the pressure difference was zero. As the pressure difference was increasing, the measured flow rate was decreasing. The pressure difference across the sand pack column was continuously monitored and recorded while a bacterial suspension was injected into the sand pack column, with the rate of the effluent being measured at regular intervals. The expression of the plugging effect can be derived from permeability changes during the experiments. The permeability values at each interval of injection were calculated based on Darcy's equation,

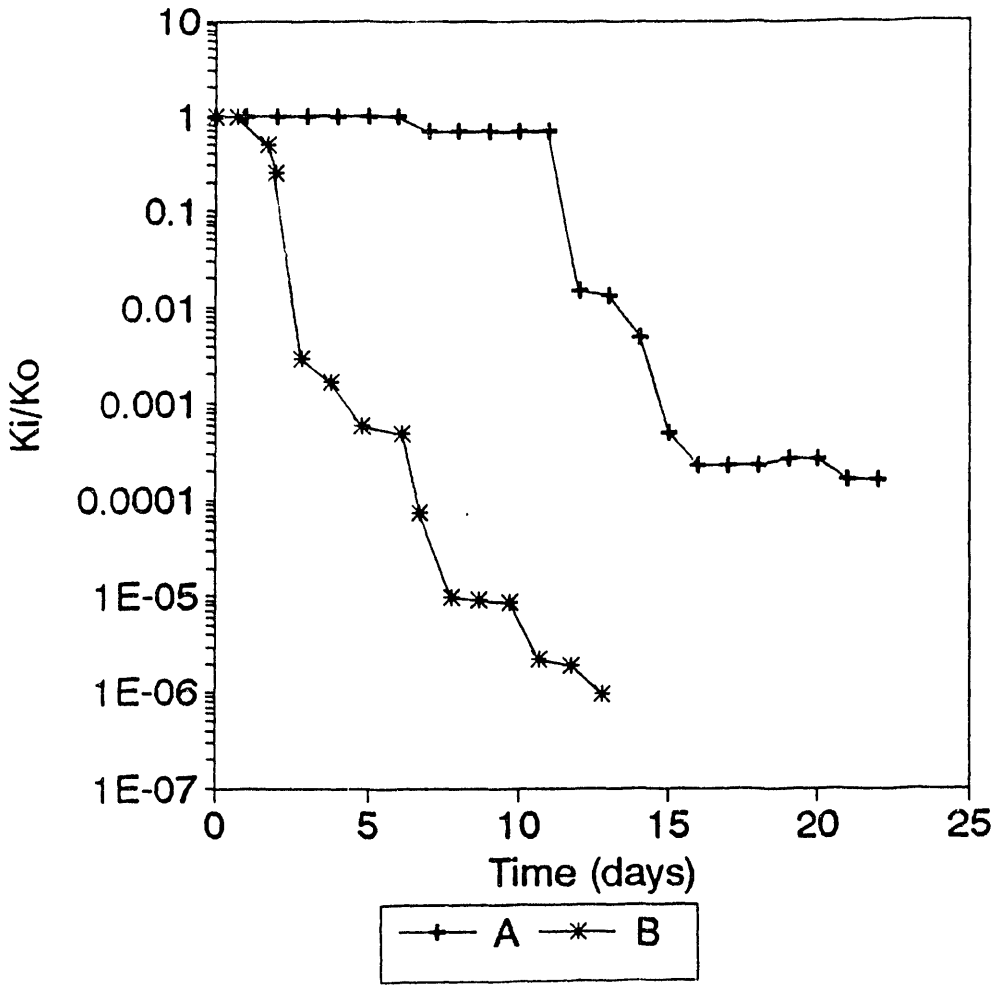


Figure 6. Plugging effect of *A. eutrophus* cells suspension in sand pack columns determined in pressurized flow system. (A) first run; (B) second run. The suspensions were two-day growth cultures and replaced after three days recycle in the system.

$$K = Q\ L\ \mu\ /(A\ \Delta p) \quad (1)$$

where K is the permeability in Darcy, Q is the flow rate in $cm^3/sec.$, Δp is the pressure difference in atmosphere, L is the column length in cm, μ is the liquid viscosity in cp, and A is the column cross sectional area in cm^2. The ratios of permeability values at different intervals to the initial permeability value, K_i/K_o, are used to indicate the plugging effect. In equation (1), L and A are apparently constants. When similar bacterial suspensions are injected, and the effect of the temperature change from day to night in the laboratory on the μ value is negligible, μ can be considered as a constant as well. In this case, equation (1) can be simplified as follows:

$$K_i/K_o = (\Delta p_o\ Q_i)\ /\ (Q_o\ \Delta p_i) \quad (2)$$

where i refers to any interval when the pressure difference and effluent rate are recorded.

4. RESULTS

The differences in the plugging effect of the test fluids were screened out by draining through small sand pack columns. Figure 3 shows the results between living cells and dead dells of *A. eutrophus* indicated by the change in the ratio of drainage rate (r_i/r_o) with the test time. The nutrient solution, which is the medium without inoculum, had no change in the drainage rate while passed down the sand columns. Within 5 hrs., the dead cell suspension of *A. eutrophus* made a 4.3-fold reduction in effluent rate. Its r_i/r_o value is 0.232 in Figure 3. The effluent rate was reduced about 90-fold by the living cell suspension in 5 hours drainage time. Unlike the dead cell suspension, this decreasing trend was kept for living ones after 5 hours, showing a 2.65 cycles drop of logarithmic scale in the r_i/r_o axis that is about 280-fold reduction at 7 hours.

The inaccuracy of those results, due to uncontrolled parameters (e.g. pressure variation with liquid level in the test column) constrained detailed conclusions which could have been drawn from this system. However, certain indications of a complex system with multiple factors were indeed gained from such data both in this research and in other previous works. McCalla [2] reported that the plugging effects of adding a sucrose solution resulted from its percolation through columns packed with organic-deficient Peorian loess. There have been other similar studies carried out using sand packed columns rather than soil to observe the permeability reduction by Gruesbeck and Collins [3]. Furthermore, the results revealed in the experiments agreed with the previous results [1], which also showed a higher plugging effect of living cells than that of dead cells, despite the fact that a pseudomonas species was used.

The plugging effect of commercial PHB powder in water was revealed by draining 3 different concentration solutions through the sand pack columns. Figure 4 shows that concentration is the sole factor that affects the plugging results. A low PHB concentration of 0.380 g/L is no different from distilled water in the plugging of the columns. The slight plugging of sand columns showed up as the PHB concentration increased to 0.630 g/L (indicated by a small drop of r_i/r_o). A more significant plugging occurred, however, for the solution with 1.193 g PHB in 1 liter of water, a sharply dropping value of r_i/r_o being calculated as a 100-fold reduction in the drainage rate within 3 hours. The follow-up scanning electron microscope pictures taken for the 3 post-test sand columns show that the PHB particles deposited on the sand surface have the exact same appearance as

before, and that distinct layers of coating can clearly be seen (Figure 5). These pictures are well related to the plugging effect resulting from a difference in concentration. The picture of solution A exposes a few clumps of PHB particles and a chip-pile-like coating. More and larger PHB clumps as well as larger pieces of chip-pile-like coating are revealed in the sand surface plugged by solution B. Consequently, the largest PHB clumps and pieces of rock-like coating are found in the picture of solution C. Those observations express that PHB powder water solution, though not a soluble solution, can be used for porous media plugging. It is possible to control the extent of plugging by adjusting the concentration of the PHB injected. Certain treatments, such as sonication, may be needed to help to disperse PHB in water, because PHB powder has a strong tendency to clump together.

In a designed pressurized pumping flow system, the *A. eutrophus* living cell suspension tremendously reduced the K_i/K_o value (indicating the plugging effect) down to 4 log cycles after 16 days and 6 log cycles after 11 days for two repeat experiments (Figure 6).

K_i/K_o values are calculated from the changes in both pressure difference across the column and flow rate (Equation 2). The pressure difference is more sensitive than the flow rate because its change was observed before that of flow rate. Even though the pressure difference reached the ultimate value of plugging (30 psi), effluent still trickled down from the column. This effluent is probably due to the nonuniform pore sizes caused by irregular shapes of the sand used. That means that most of the pores were plugged; but there were a few through which the effluent could escape. The nonuniform pore sizes of the packed sand are observed under light microscope with a magnification of 5.

5. DISCUSSION

Many reservoirs are heterogeneous (i.e., the permeabilities are highly variable), and consequently, flow channeling and fingering become a serious problem. Our work with intracellular biopolymers is most unique in that the plugging is not due to a short distance of wellbore for a surface plugging. We are anticipating a much longer plug through the fingering zone. Two general mechanisms of microbial plugging likely to occur in a porous media, particulate plugging and plugging due to biofilm formation, were suggested by Jack et al. [1]. In the former the bacterial cells alone cause a reduction in permeability or a decrease in drainage rate by plugging and filter cake formation. Both dead and living cells are capable of this type of plugging. The latter resulted from the build up of biofilms in the porous media by viable microorganisms. This only belongs to the living cell suspension plugging. Beyond their points, plugging by many kinds of extracellular products has been well documented.

In the *A. eutrophus* culture, there is no help for plugging the porous media from any slimes or polysaccharides because it is not a extracellular product producing bacterium. Other possible interfering factors existing in the culture also can be neglected for the results in the drainage flow system because the dead cell suspension was a half portion of the living cell suspension, sterilized in autoclave. It is implied that those two cell suspensions had the same chemical components in their cultures. Therefore, the drainage rate reduction by dead cell suspension is the result of cell aggregation in the porous media. The living cell suspension plugged the sand pack column with an aggregation of cells and biofilm formation on the sand surface. If this is true for the obtained results, the difference of the drainage rate reduction between dead and

living cell suspensions presents the effect of *A. eutrophus* cell growth inside the sand column. This difference is very significant, being 90-fold when compared to 4.3-fold of r_i/r_o reduction. In terms of its batch growth curve in the same medium, the *A. eutrophus* population only increases about 4 times within 5 hours, for its doubling time is 3 hours. So some other factors may play roles in creating that difference (e.g., the change of cell surface characteristics due to autoclave treatment may reduce the ability of cell aggregation).

Biopolymers have been used in the MEOR process for a long time. The use of these kinds of polymers have significant effects in the plugging of porous media. Jack et al. [1] have proved the positive effect of soluble dextran polymer production of plugging in their model core system. Hoefner et al.[4] reported the study of using xanthan-based profile control gels to selectively plug the high-permeability "thief" zones.

The study of the plugging effect by PHB was conducted in this research. PHB is an intracellular polyester which has been reported to be accumulated in a wide variety of bacterial species [5]. The level of PHB in cells can be drastically increased from a low percentage to over 80% of the dry cell weight when bacterial growth is limited by the depletion of essential nutrients, such as nitrogen, oxygen, phosphorous, sulfur, or magnesium. Schlegel et al. [6] demonstrated that *A. eutrophus* accumulated PHB up to 70% of the total dry cell weight when chemolithotrophic growth was limited by ammonium consumption in a batch culture. PHB powder is commercially available; however, this provides more choices between in-situ PHB produced by bacteria and on-site preparation in bulk.

Although PHB is a highly crystalline thermoplastic with a melting temperature around 180°C, the glass-transition temperature of PHB polymer is very low, at around 4°C. It is an excellent pore-filling agent in terms of applied pressure. The lab experiments showed that the water solubility of PHB is zero from 10°C to 100°C. In the range of pH 5.5 to 9.0, PHB is not soluble in water. Outside this range, for example at pH≈2 the solubility increases up to 2.3 g/L. Those unique characteristics lead PHB to promising applications, such as its uses as a plugging agent for many aggressive liquids.

As discussed, viable microorganism cultures have a higher plugging effect in porous media than dead bacteria, which is proven by the fact that microbial activity clogs the pores with the products of growth, cells, slimes or polysaccharides. Few publications have been reported about the plugging with intracellular polymers. The current research is trying to fill this gap. *A. eutrophus* does not produce extracellular products at all, but instead produces the biopolymer PHB. The plugging of the sand pack column by the *A. eutrophus* living cell suspension is an accumulative effect because the two curves in Figure 6 show the steps at the beginning. In this process, the plugging particles are entrained again because the drops of Δp were recorded on the 9th day in the first run and after 7 days in the second run. Thus, the phenomenon can be explained by the mechanisms of particulate plugging of cell aggregates and plugging due to biofilm formation. The buildup of biofilm (i.e., the growth of *A. eutrophus*) on the sand surface is the main reason the result was such a degree of plugging, since these pluggings occurred without the presence of any other agents, such as exopolymers. During the plugging tests, however, 2 days' growth of *A. eutrophus* nutrient broth culture was used in the exponential phase of its growth curve, and those nutrients needed for the growth were also available. This is only an initial trial for subsurface plugging. More studies should be done on the in situ bacteria growth kinetics, seeding protocol, and injection strategy.

6. CONCLUSIONS

A. eutrophus cell suspensions both dead and living can plug porous media, such as sand pack columns. Since *A. eutrophus* does not produce any kind of slime or extracellular polymers, it has the potential to be a plugging bacteria used in the MEOR process in which non-exopolymer producing bacteria are required for successful injection [1]. The difference in the plugging effect between dead and viable *A. eutrophus* cell suspensions provides the choice for a different need of plugging extents.

Commercial PHB powder water solution also can plug sand pack columns. It is another potential plugging agent in the MEOR process. The different plugging extent can be easily obtained by using an appropriate concentration of PHB water solution. Additionally, its unique characteristics may create more usages.

The plugging of porous media by *A. eutrophus* culture may last a longer time than other plugging bacteria, which does not contain any polyester. Based on this research, three types of plugging have occurred in *A. eutrophus* culture system: a) living cell suspension, b) dead cell suspension and, c) intracellular product - PHB solution. A plugging by living cell suspension of *A. eutrophus* can possibly last for a long time even though some changes in this system may happen. If cells died, a new plugging agent, PHB, would be released.

Both static drainage flow systems and pressurized pumping flow systems have plugging results which match the plugging mechanisms of particulate plugging by the microbial cells and viable bacterial plugging through biofilm formation.

7. ACKNOWLEDGEMENTS

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Halotolerant and Extremely Halophilic Oil-Oxidizing Bacteria in Oil Fields

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Abstract

Field experiments have shown that oil-oxidizing microflora are widely distributed in the oil fields of Russia. Oil-oxidizing microorganisms were isolated from stratal waters with salinities of up to 272 g/l. Only single oil-oxidizing microbial cells were found in the stratal waters of production wells. High salinity stratal waters (with salinity over 140 g/l) are characterized by "oil-positive" microflora that are concentrated in the oil. Oil-oxidizing eubacteria, active in media with salinities up to 15% NaCl, and the extremely halophilic oil-oxidizing archaeobacteria, active at salinites to 32% NaCl, were isolated from oil samples of the Bondyuzhskoye oil-field. These microorganisms were characterized by high oil-emulsifying activity. Some properties of the halophilic oil-oxidizing microorganisms are discussed.

1. INTRODUCTION

The pioneer studies of E. Bastin and T. Ginzburg-Karagicheva showed that oil reservoirs are not sterile but contain microorganisms of various groups [1]. The later works of C. Zobell, S. Kuznetsov, J.B. Davis, and others formed the basis of up-to-date views on the role of microorganisms in the transformation of the organic and mineral components of petroleum-bearing sediments [2]. According to these views, the oil-bearing rocks can be considered an original ecosystem in which various microbiological processes occur.

The wide distribution of hydrocarbon-oxidizing microorganisms present in the stratal waters of oil fields under development was shown previously. Generally, the maximum numbers of microorganisms were found in the near-bottom zone of injection wells used for pumping fresh surface water to maintain high reservoir pressure. On moving away from this zone, the number of hydrocarbon-oxidizing bacteria drops rapidly. It is assumed that the growth of these microorganisms in the oil fields is restrained by the salinity of the stratal waters and by the access of oxygen to the reservoir. Studies of eubacterial cultures isolated from the brines of oil fields located in the basin of the Upper Kama showed that the optimal salinity for their growth is 20 g/l NaCl, and in media with salinities above 100 g/l, the development of microorganisms slows down rapidly. As the salinity of the water increases, the taxonomic variety of microorganisms decreases, and in brine-type stratal waters, members of genus *Rhodococous* predominate [2].

Investigations also have shown that microorganisms isolated from the saline stratal waters are to some extent halotolerant [3].

The mechanism of haloadaption of hydrocarbon-oxidizing microorganisms isolated from oil-bearing strata has not been well investigated. It is known that halophilic aerobic archaeobacteria can grow in highly mineralized ecosystems [4]. However, until now, the extremely halophilic bacteria have not been isolated from oil-bearing strata.

The increased interest of scientists in studying hydrocarbon-oxidizing microorganisms is primarily due to their high biotechnological potential. These microorganisms are widely used for the natural and anthropogenic ecosystem

Table 1
Chemical composition of the formation waters of the Bondyuzhskoye oil field

Number of well	Salinity $g.l^{-1}$	pH	Eh mV	Content, $mg.l^{-1}$				
				HCO_3^-	SO_4^{2-}	CH_3COO^-	Ca^{2+}	Mg^{2+}
366*	0.8	7.3	+280	122	40	0.6	62	12
303	6.0	6.8	+135	220	18	0.1	400	120
304	18.1	7.2	+100	305	24	0.1	1200	240
15	82.2	6.6	+140	268	28	0.1	6000	1320
323	147.1	6.4	+120	177	26	4.4	10400	2580
283	159.0	6.3	+145	165	24	0.1	11200	2400
365	172.5	6.3	+110	177	15	1.8	12200	2760
351	181.5	6.5	+140	195	26	0.1	12800	2880
352	195.8	6.1	+170	146	31	0.1	13800	3000
353	207.9	6.1	+140	165	18	4.8	15200	3000
273	272.3	5.5	+180	104	19	0.1	19800	4200
367	272.4	5.9	+120	134	19	0.1	19600	4080

*Data on the chemical composition of the surface fresh water (pumped)

refinement of oil impurities. They also are used as producers of active oil-displacement agents (such as CO_2, fatty acids, alcohols, polysaccharides, and surfactants) in creating biotechnologies for enhancing oil recovery.

The aim of the present work was to study the hydrocarbon-oxidizing microorganisms in an exploited oil field. Especially interesting were the halotolerant and extremely halophilic hydrocarbon-oxidizing microorganisms, and the perspectives for their application in various biotechnologies.

2. OBJECTIVES AND METHODS

The field experiments were carried out in the Bondyuzhskoye oil field, located on the right bank of the Kama river in the northeastern part of the Tatarstan. The oil-bearing reservoir rocks are sandstones and silts of the Upper Devonian. The depth of the oil-bearing horizon formation is about 1500-1700 m, and the formation's temperature is between 30° and 400°C. The crude oil from the Bondyuzhskoye oil field belongs to the highly sulfurous paraffin type of oil.

The natural stratal waters are of the chloro-calcium type, with a total mineralization of up to 330 g/l. A low sulfate and hydrocarbonate content is typical of these waters. The exploitation of the oil field has continued for 30 years. Flooding of the oil reservoir with fresh surface waters and waters of various salinity levels has been used to maintain high pressure in the reservoir. The application of fresh water for waterflooding causes changes in the chemical composition of the original, high-salinity stratal waters.

Table 1 summarizes data on the chemical composition of the stratal waters from the Bondyuzhskoye oil field area used for waterflooding with fresh waters. As soon as the oil-field brines become fresher, the concentrations of Ca^{2+} and Mg^{2+} cations in the water decreases, that of hydrocarbonate ion increases, and pH values vary from slightly acidic to neutral. The redox potential values of stratal waters correspond to the slightly reduced environmental conditions. On

the whole, flooding the reservoir with fresh waters results in the appearance in the rocks of a wide spectrum of ecological niches, with water of different salinity levels and chemical composition.

Methods of aseptic sampling from the injection and production wells were described previously [3]. "Aqua-Merck" kits, purchased from Merck, were used in standard hydrochemical procedures for chemical analysis of the stratal waters. The redox potential and pH value were measured using the millivoltmeter pH-150. The microbial content of the oil reservoir was determined by serially diluting samples of the stratal waters and oil, then by plating the diluted samples on the appropriate solid medium. Raymond's mineral medium containing oil or hexadecane as an organic substrate was used to quantitate the aerobic hydrocarbon-oxidizing eubacteria. Isolation of pure cultures was carried out on the meat peptone agar, peptone agar (mineral medium + 15 mg/l peptone), and the solidified Raymond's medium (2% agar) with 10% NaCl [3].

The oil sample was spread evenly on the medium's surface. Individual colonies of microorganisms were transferred into liquid Raymond's medium containing 1, 10, 15, or 20% NaCl. Yeast extract (0.05%), acetate, and glucose (0.5% each) as well as some alkanes (octane, decane, tetradecane, hexadecane [2%]) and oil from the Bondyuzhskoye oil field were used as organic substrates.

Halophilic archaeobacteria have been isolated by oil-drop spreading on the surface of the solidified medium usually used to cultivate extremely halophilic archaeobacteria. Individual colonies of microorganisms were transferred into liquid medium of the same composition. The purity of the cultures was checked by their retransfer onto the solidified medium.

Hydrocarbon utilization was studied in Raymond's mineral medium with various concentrations of NaCl. $MgSO_4.7H_2O$ (2%) and yeast extract (0.25%) were added to the medium for halophilic archaeobacteria. The oil and hexadecane were autoclaved in advance, separately, and added to the medium to a final concentration of 2% v/v immediately before the start of the experiment. Cultivation was carried out at 37°C for 3 weeks in small vials (20 ml) closed with the rubber stoppers, and sealed with aluminium crimp seals. The microbial growth was monitored visually and microscopically. Hydrocarbon-free medium was used as the control. Changes in the oil's composition were monitored by gas-liquid chromatography. Exopolysaccharides were determined by precipitation with organic solvents from the medium. Surface tension at the interface was determined according to Babalyan et al.[5].

3. RESULTS

Our studies show that microorganisms able to use the hydrocarbons in oil penetrate the reservoir together with the surface water. There were thousands and tens of thousands of oil- and hexadecane-oxidizing microbial cells in one milliliter of the injection surface water. The numbers of hydrocarbon-oxidizing bacteria in the stratal waters was one to two orders lower, and there were only single cells or several tens of cells in one milliliter.

Analysis of the fluid samples from the production wells showed that the content of aerobic microorganisms in the stratal waters was as low as that of hydrocarbon-oxidizing bacteria, and there was no correlation with the salinity of the water. By contrast, the concentrations of bacterial cells in oil were much higher, reaching thousands, sometimes even tens of thousands, of bacterial cells in one milliliter (Table 2). We noted that more colonies developed after oil-drop distribution on pure peptone agar (PA) than on meat peptone agar (MPA).

Table 2
The number of aerobic bacteria in stratal fluids of the Bondyuzhskoye oil field

Well Number	Salinity g/l	Number of bacteria, cells/ml			
		Stratal waters		Oil	
		MPA**	PA*	MPA**	PA*
303	6.0	200	400	1000	100
323	147.0	0	300	10000	10000
283	159.0	30	20	100	-
365	172.5	100	100	1000	100000
351	181.5	0	0	10000	10000
353	207.9	60	1000	1000	-
273	272.3	2000	2000	1000	100000
367	272.4	1000	300	1000	10000

*Pure peptone agar
**Meat peptone agar

The colonies of microorganisms were very small, uniform, and mainly white although some light pink and brown colonies also appeared.

The ability of microorganisms to concentrate in oil is very important for the quantitative estimation of microflora in the oil fields. Probably the accumulation of microbial cells in oil is due to the lipophilic properties of the cell wall of many hydrocarbon-oxidizing microorganisms, such as rhodococci, nocardia, corynebacteria, and mycobacteria. These lipophilic properties depend on the presence of high molecular-weight mycolic acids in the cell wall.

Over twenty pure cultures of microorganisms able to oxidize oil or individual hydrocarbons were isolated from the stratal waters of the oil field. Five eubacterial cultures with oil-oxidizing ability within a wide range of salinity (Table 3) were isolated from oil of the well 367 (salinity of 272.4 g/l). The properties of isolate 367-1 resemble those of the genus *Micrococcus*, whereas isolate 367-2 resembles the genus *Rhodococcus*. However, low GC values equal to 32.5 and 30.1 mol%, respectively, do not allow us to include the isolates in these genera. According to the Bergey's Manual, the GC value of DNA for the genus *Micrococcus* ranges between 65 and 75 mol%, and that for the genus *Rhodococcus* is between 59 and 73 mol%.

The isolated microorganisms metabolize acetate, propionate, butyrate, yeast extract, glucose, individual hydrocarbons, and crude oil. All the microorganisms tested can use NH_4^+ and NO_3^- as nitrogen sources, and their growth was observed within a wide range of pH (5-9) and temperature (6-37°C). Taking into account the peculiarities of the ecological niche of these microorganisms (brines' salinity 272.4 g/l), we studied their tolerance to increased NaCl concentrations. The inhibitory effect of the NaCl concentration depends on the type of organic substrate used. In mineral medium containing acetate, microbial growth ceased at a concentration of 10-12%, whereas in the glucose-containing medium, growth ceased at 15% NaCl. The addition of yeast extract increased salt tolerance up to 20% NaCl. The colonies developed on potato agar in the presence of 25% NaCl.

The growth rates of *Rhodococcus* sp. strain 367-5, *Arthrobacter* sp. 367-4 and 367-2 on media of different salinities are shown in Figure 1. These strains differ in the extent of their NaCl requirements (halophilism) [6].

Table 3
Some properties of the oil-oxidizing bacteria of the Bondyuzhskoye oil field (Substrate: Fatty acids, glucose, n-paraffins, crude oil)

Microorganism	Morphology and size, μm	Hours of generation	Optimum pH range	Salinity range NaCl %
Str. 367-1	Spherical rods 0.5-3.5	30	4.7-8.1 6.0	0.50-25
Str. 367-2	Rods 0.6-0.8x	23	5.2-9.1 5.8	0.15-25
Micrococcus sp. 367-3	Spherical rods 0.3-3.5	18	4.7-9.1 6.6	0.15-20
Arthrobacter sp. 367-4	Rods 0.6-0,8x 10-12	12	6.2-9.8 8.0	0.20-20
Rhodococccus sp. 367-5	Rods 0.6-0,8x 10-12	18	6.2-9.2 8.0	0.20-20

Rhodococcus sp. and *Arthrobacter* sp. are slightly halophilic and their growth rate decreases sharply at low NaCl concentrations of about 4-6%. Strain 367-2 is moderately halophilic and is characterized by the optimum growth at a concentration of 15%.

All the cultures adapted at a high rate to the low salinity medium (1-2% NaCl) after transfer from medium with high salinity (10-20%). At the same time, oil-oxidizing microorganisms brought into the reservoir with fresh water did not grow in media with a NaCl content over 8%. It seems that the tolerance of the reservoir's microflora to high salt concentration depends on adaptation and selection mechanisms involved in the formation of this microbial community. Some cultures isolated from the oil stratum synthesize extracellular polysaccharides during growth in n-alkane-containing medium (Table 4).

During cultivation of the oil-oxidizing microbial association in the presence of crude oil, biosurfactants are released into the medium which lower the interphase surface tension at the water-oil boundary from 26.95 at 3 days of cultivation $mN.m^{-1}$ to 23.23 $mN.m^{-1}$ after 11 days, and to 10.91 $mN.m^{-1}$ after 47 days.

These results point to possible mechanisms of microbiological technologies for enhancing recovery and provide perspectives on the investigation of the isolated microorganisms.

The bacterium, strain H-352, was isolated from production well No. 352 (mineralization of the brine is 196 g/l). The optimal growth of the strain was observed at NaCl concentrations between 15 and 32%. When the NaCl concentration was reduced to 10%, the culture's growth practically ceased (Figure 2).

Table 4
Formation of polysaccharides during growth of hydrocarbon-oxidizing microorganisms

Microorganism	Substrate	Polysaccharide, mg/l					
		Days of cultivation					
		3	7	13	20	24	29
Str. 367-2	$C_{16}H_{34}$	-	75	680	243	-	80
Rhodococcus erythropolis, 376-6	$C_{15}H_{32}$	32	100	-	500	190	50
	$C_{16}H_{34}$	35	85	970	380	258	143
Yarrowia lipoytica, 367-3	$C_{16}H_{34}$	-	-	-	250	205	117

- not determined

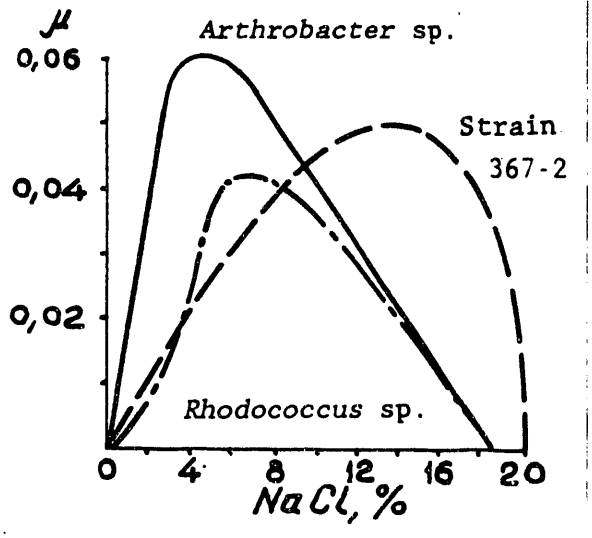


Figure 1. Changes in specific growth rate on acetate-containing medium for three cultures of oil-oxidizing eubacteria at different salinities.

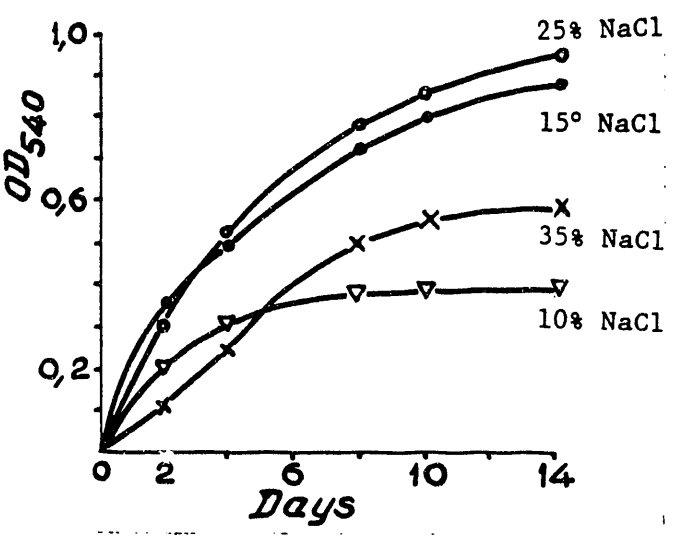


Figure 2. Growth of *Halobacterium* sp. H-352 on acetate-containing medium at different salinities.

Table 5
Characteristics of *Halobacterium distributum* H-352

Colonies		Purple-red, 3-4 mm
Shape and size of cells		Pleomorphic rods, lysed in distilled water; 0.2-0.5 x 1-8μm
Intervals of	Salinity	15-32%, optimum 20-25%
	pH	5-8, optimum 7.2
	Temperature	26-55°C, optimum 37-40°C
Antibiotic sensitivity		Pencillin resistant Bacitracin sensitive
Carotinoid pigments		Bacterioruberins with adsorption maxima at 370, 388, 495, and 527 nm
Lipid composition		Glycerol diphytanyl esters
GC composition of DNA		62.4 mol%

The cells of this strain are pleomorphic rods, shortening to 1.0-1.5 μm after cultivation for three to four days. In hypotonic solutions containing 5-10% NaCl and in distilled water the cells round up, exhibiting partial lysis. The overall characteristics of this isolate (Table 5) (halophilism, lipid composition, pigment composition, and sensitivity to antibiotics) show that it belongs to the order Halobacteriales. Its morphological, physiological, and genotypical features allow us to include the strain in the genus *Halobacterium* and to identify it as *Halobacterium distributum*.

Detection of viable eu- and archaeo-bacterial cells in the stratal brines with natural salinities up to 272 g/l raises the question whether the isolated bacteria can use the oil-derived hydrocarbons as a source of carbon in the reservoir fluids.

Chromatographic analysis revealed that cultivation of the microbial association of five isolated eubacterial strains in medium containing 15% NaCl in the presence of oil results in the complete utilization of normal and branched alkanes and naphthenes within a month (Figure 3a,b). The liquid culture of *Halobacterium distributum* in the presence of 25-30% NaCl and crude oil also actively biodegraded the oil components in three weeks (Figure 3c).

4. DISCUSSION

The findings described above have contributed significantly to our understanding of the fact that oxidation of oil-derived hydrocarbons by

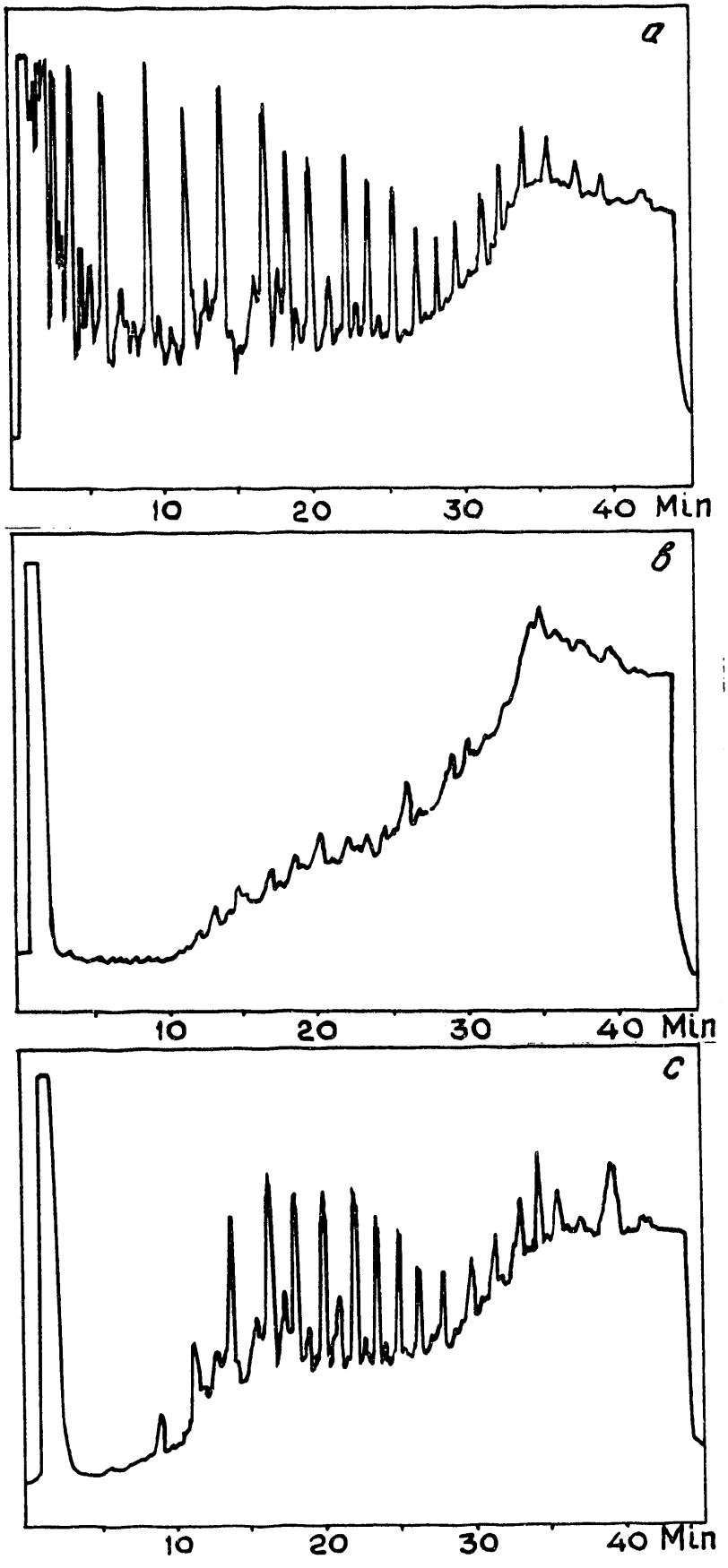


Figure 3. Biodegradation of crude oil by halophilic oil-oxidizing bacteria.

a. crude oil control sample.

b. crude oil sample after cultivation with a consortium of eubacteria.

c. crude oil sample after cultivation with *Halobacterium* sp. H-352.

3% SE 30 on Chromation N-AW (0.12-0.16) 2m x 3mm ID, Glass. Col. Temp. Prog. Flow Rate- 40 ml/min, FID (64×10^{-11} AFS) Sample: 1 μl in hexane.

microorganisms is quite possible in an environment of extreme salinity. Isolation from oil-field brines of microorganisms able to actively oxidize oil within a wide range of salinities (10 to 32%), allows for a discussion dealing with the development of these microorganisms in such an ecosystem. We note that although eubacterial cultures grow under a wide range of salinity (0.5-25% NaCl), they have the ability to oxidize the hydrocarbons only at salinities lower than 15% NaCl. These differences could be explained by adaptation to the reservoir conditions of eubacteria brought in from the soil surface during contour flooding by fresh water injection into the reservoir.

Inoculation of the reservoir with the extremely halophilic archaeobacteria is unlikely. The salinity of water injected into the reservoir does not exceed 0.5 g/l. Such salinity causes an immediate cell lysis of the isolated extremely halophilic strain. We suggest that isolation of this strain indicates the existence of an indigenous microflora, including the extremely halophilic archaeobacteria. However, independent of the possible ways in which the halophilic archaeobacteria are inoculated into the reservoir, we suppose that some peculiarities of the isolated archaeobacteria are indicative of their ability to grow in their own econiche in the oil field studied.

Data characterizing the biological peculiarities of the halotolerant and extremely halophilic hydrocarbon-oxidizing microorganisms were used to create the modern technology for ecosystems purification of oil impurities. The basis of this technology is the natural association of hydrocarbon-oxidizing microorganisms isolated from the oil-bearing strata. In this technology, definite additions also are used, sharply activating the process of biodegradation. The preparation, both as a dried microbial preparation or a concentrated wet biomass, has been designated Devouroil.

Devouroil has some distinct advantages over the known techniques of biological treatment. It consists of both hydrophilic (developing in water) and lipophilic (developing in hydrocarbon) microorganisms. Due to such a biological composition, it acts in the hydrocarbon biodegradation process both at the water-oil boundary, and directly in the hydrophobic substrate (hydrocarbon) thickness. Consequently, the time necessary for the removal of impurities is decreased and the effectiveness of purification increased.

The preparation is adapted to salinities in the media up to 150 g/l with an optimum at 5-8% NaCl. Investigations carried out at the Research Center "Oil and Gas" of the Russian branch of the World Laboratory showed a high rate of oxidation of the oil-n-alkanes by the preparation in media with salinities up to 10% NaCl (Table 6).

The preparation can purify water containing about 5% of oil and soil with a concentration of impurities of over 20 kg/m^3. After treatment, only easily degraded bacterial protein and ecologically neutral oil-degradation products are found. Investigations by the Russian Ministry of Health have shown that the microorganisms in the preparation are nontoxic and nonpathogenic.

Natural studies of the Devouroil in an oil field of the Tatarstan have shown the high effectivity of the dried preparation at a concentration 2 kg/ha. A further increase in the rate of purification process is possible if the applied dose of the preparation is increased up to 5 kg/ha. The treatment should be combined with the addition of a mixture of mineral salts of definite composition prepared from usual mineral fertilizers. When the preparation is used in saline ecosystems, addition of a biostimulator-osmoprotector is necessary.

Table 6
Dependence on salinity of the biodegradation of saturated hydrocarbons in crude oil by Devouroil

Days of Incubation	Salinity, NaCl %				
	0.1	1	3	5	10
3	5	50	55	50	15
5	7	80	80	80	45
10	7	90	87	88	60
15	8	95	94	96	75
20	10	95	100	100	80

The preparation can be used in other technologies, such as oil well deparaffination, bore schlamm purification of hydrocarbons, and refinement of the ballast waters in oil tankers.

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The Use of Slime-forming Bacteria to Enhance the Strength of the Soil Matrix

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Abstract

Our experiments showed that the strength of soil can be enhanced by either applying slime-forming bacteria directly to the soil matrix to produce a biopolymer inside it, or by applying biopolymer from slime-forming bacteria or commercial products such as poly-3-hydroxybutyrate (PHB), xanthan gum, and sodium alginates to the soil matrix. The cross-linked support networks result from the interaction of biopolymer with the soil matrix.

Zonal bioremediation or environmental encapsulation are potential applications of this research. Reduction of soil erosion and enhancement of the soil strength in the foundations of hydraulic systems may also be good prospects for geotechnical and agricultural exploration.

1. INTRODUCTION

The slime-forming bacteria *Alcaligenes faecalis* or *A. viscolactis* belong to an order of higher bacteria (the *Myxobacterales*) that form colonies capable of creeping slowly over a layer of slime that they secrete. The chemical composition of the slimes consists mostly of polysaccharides and minor amounts of lipids and proteins. Viscosity up to 50,000 cps can be achieved in the proper medium [1-4].

The organic soil fraction, i.e., the soil organic matter (SOM), existing in dynamic equilibrium, is continuously subjected to polymerization, condensation, and degradation processes, and is more susceptible under ecological changes than is the inorganic soil fraction. Consequently, the strength of the soil matrix will be reduced by the tremendous losses of SOM under exploitative cultivation practices or under erosion.

Soil colloids can be roughly classified as inorganic colloids (such as alumino-silicates, hydrated sesquioxides, and allophanes) and organic colloids (such as humic acids, polysaccharides, and other organic components of humus). Many microbially derived biopolymers, such as poly-3-hydroxybutyrate (PHB), the slimes from slime-forming bacteria, or the gellan gum of polysaccharides, behave as humus which acts as a hydrophilic colloid with numerous functional groups as donor atoms. Humus is closely associated with positively charged colloids such as sesquioxides, allophanes, and other clay constituents. The interfacial chemical interactions within the domains and microaggregates of the soil matrix are either adhesion or binding. As a result, the binders and the newly introduced biopolymers are expected to increase the strength of the soil. It is possible to chemically alter the structure of biopolymers by cross-links through coordination of transition metals (possibly employing wastewater, such as plating wastes, consisting of aqueous solutions of Co^{2+}, Ni^{2+}, and Zn^{2+} for economy). The interaction between the soil matrix and biopolymers can be enhanced by curing. For example, heating the soil matrix, which contains a biopolymer, can open the functional groups in the biopolymer and cross-link the soil matrix and biopolymer by free radicals and unsaturated backbones. If the strength of the soil matrix can be enhanced by applying a biopolymer to it, then this inexpensive technology

can be applied to locations which are vulnerable to contamination (such as the periphery of landfills and underground storage tanks) to control the migration of leachates from hazardous wastes.

With this objective in mind, experiments were undertaken to test the feasibility of enhancing the strength of the soil matrix either by growing bacteria inside the soil matrix or applying a biopolymer to the soil.

2. EXPERIMENTAL

2.1. Growth of slime-forming bacteria

Two kinds of slime-forming bacteria were used in this investigation. Both of them were obtained from the American Type Culture Collection (ATCC). One was *A. faecalis*, (ATCC # 49677); the other was *A. viscolactis*, (which is renamed now as *Corynebacterium* sp., ATCC # 21698). These two bacteria can produce very hydrophilic and viscous slimes in a suitable environment. Both were grown in an appropriate medium: one medium is simple, consisting of nutrient broth only. The other medium has many ingredients; we felt that it was difficult to simulate in a natural environment at this time. Evidently, the yield of biopolymer is larger in multiple ingredient media. Also, for comparison, 0.1% PHB, 3% xanthan gums, and 2% sodium alginate solutions which are commercially available were studied. The detailed procedure was as follows.

For growing of *A. faecalis*, we prepared two mediums, one was 1 liter of 0.8% nutrient broth, and the other was 1 liter of Burk's nitrogen-free basal medium [5] (its component is listed in Table 1), mixed with 0.6% peptone as the nitrogen source and 3.4% glucose as the carbon source. The *A. faecalis* seed culture was inoculated into the medium and the bottle was incubated at room temperature (23-27°C) with mechanical shaking for 4 days.

For growing *A. viscolactis*, a 1 liter of 0.8% nutrient broth, and a 1 liter of whey medium were prepared. The whey medium containing 7.0% commercial whey powder was adjusted to pH 6.7 with a saturated solution of sodium hydroxide, then centrifuged at 6,000 rpm for 30 minutes. In a 1 liter flask, 250 ml of the clear supernant were inoculated with 2 ml of an actively growing 24-hour nutrient broth culture. The flask was agitated on a rotary shaker for 25 minutes.

After 4 days of incubation, the bacteria growth was examined under a microscope (200X). The *A. viscolactis* in the whey medium showed poor growth.

Our first experiment was to apply the bacteria culture to a sterilized soil matrix and monitor the production of biopolymer inside the soil matrix to see if there was any enhancement in the strength of the soil. Practically, it is difficult to keep the soil matrix in a shape rigid enough to test the soil strength after sterilization. Therefore, in the second attempt, we grew the bacteria in the medium first, isolated the biopolymer, and then applied the biopolymer to the soil matrix instead of growing the bacteria inside the soil matrix. In this manner, we obtained a better soil matrix for the test.

2.2. Preparation of biopolymer

To make the gum solution from the two slime-forming bacteria, the bacterial medium was centrifuged at 2000 rpm for 40 minutes. Then, the viscous precipitates were removed from the bottom of centrifugation tube, redissolved in distilled water, and stored. To make the gum solution from PHB, xanthan gums, and sodium alginates, the biopolymer powder was simply dissolved in distilled water by mechanical shaking.

Table 1
Burk's nitrogen-free basal medium*

KH_2PO_4	0.2 gram	Mo, Fe	1 ml**
K_2HPO_4	0.8 gram	Sucrose	10-30 grams
$MgSO_4$	0.1 gram	$CaSO_4 \cdot 2H_2O$	0.1 gram
Distilled Water	1000 ml		

*The two phosphates were dissolved first, then the other salts were added to the distilled water. The quantity of sucrose used depended on the duration of the experiment. The pH of the medium was around 7.0-7.2. Sterilization was carried at 15 lb for 30 minutes with the bottle in an upright position. Incubation was done in flat position.
** Stock solution containing 1 mg Fe and 0.1 mg Mo per ml.

2.3. Test of strength of soil matrix

Soil is composed of three different types of soil matrices: sand, clay, and silt. In this study, various types of soil matrix -- sand (washed and dried), silica (SiO_2, about 240 mesh), and clay (gray modeling clay) -- were used to determine how biopolymers are effective in increasing the strength of different types of soil matrix. Depending on the type of matrix, two methods were used to measure its strength. One was the unconfined compression test for cohesive materials such as clay and silica, and the other was the torvane test for very soft to stiff soil matrix such as sand[6].

For the unconfined compression test, cylindrical shaped specimens were used. To make the specimens, 12 ml of each bacteria culture or gum solution were added to 200 g of each soil matrix, and then each mixture was shaken vigorously to spread the bacteria culture or gum solutions uniformly in the matrix. Next, the soil matrix was placed in a cylindrical specimen frame and compacted. Then, the soil matrix was taken out and cut into cylindrical shapes whose height-to-diameter ratio was larger than 2. The specimens were put into the oven at 80-85°C for 2-3 hours to remove the moisture and to simulate curing. The finished specimens were placed on the bottom of the loading devices (Karol Warner Inc., model #545, loading capacity from 0 to 1000 lb), and the loading ram was lowered so that it barely touched them. At this point, the displacement and force dial gauges were adjusted. Then, the lower plate was raised by the loading frame crank, which was turned by hand at a constant speed, until the specimen failed (at peak strength). During the test, dial readings of displacement and force were recorded simultaneously at representative intervals. The applied compressive stress was plotted against the axial strain to compare the strength of the soil matrix.

For the torvane test, 20 ml of each bacterial culture was added to each soil matrix in a 50 ml beaker and set aside for 2-3 days to let the bacteria penetrate the soil matrix completely and produce the biopolymer inside the soil matrix. Then, the strength of the soil matrix was measured by the hand torvane test device (Soiltest, model #CL600A, stress range from 0 to 1 kg/cm^2). The proper vane was chosen first and the flatness of the surface of the sample was ensured. Torvane was pressed into the soil matrix to the depth of the blades and a constant vertical pressure was maintained while turning the knob. A rate of rotation was recommended such that failure developed in five to ten seconds. After failure developed, the remaining spring tension was released slowly and the index mark on the knob indicated the maximum shear value. Then, the water

content of each soil matrix was determined from representative portions of the soil matrix. The torvane stress was plotted against the moisture content to compare the strength of the soil matrix.

3. RESULTS

Three types of soil matrix were studied -- sand (washed and dried), silica (SiO_2, about 240 mesh), and clay (gray modeling clay).

3.1. Sand

A torvane test was conducted to compare the shear strength of sand in this set of experiments. A high shear strength means that the sand can withstand more shear stress under allowable strain or deformation. Many factors may affect shear strength, such as the void ratio or relative density, the shape and size of the particles, distribution of grain size, roughness of the particle surface, water content, and over condensation or prestress [7]. We used same type of sand (Mallinkrodt 7062, washed and dried) to avoid errors due to various types of sands, and the shear stress was measured under different moisture contents to assess how moisture content would affect the strength. The results of improvement in the strength of the sand samples by different sample treatment are summarized in Figure 1.

PHB is known to be capable of plugging the porous media and forming a barrier around it. The repeating unit of PHB monomer is a four 3-hydroxybutyric acid sequence. Therefore, we measured the strength of soil matrix with a sample of sand mixed with PHB solution and found that it had a higher torvane stress (0.19 kg/cm^2) than one without PHB (0.15 kg/cm^2) with the same moisture content. This result showed that PHB can enhance the strength of the sand.

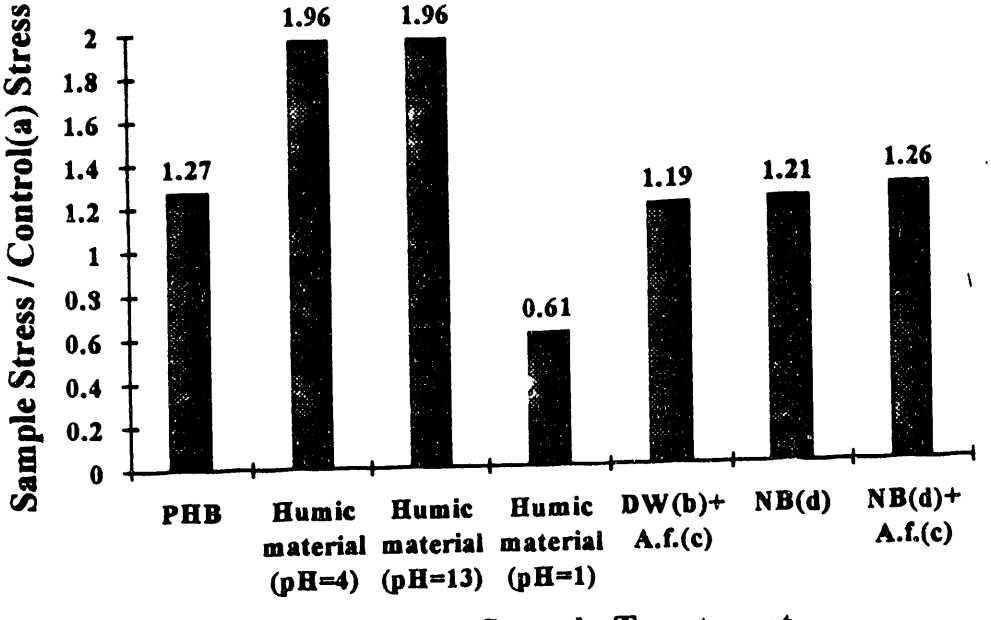


Figure 1. Ratio of sample stress to control stress of sand sample: (a) sample with distilled water only; (b) distilled water; (c) *Algaligenes faecalis*; (d) nutrient broth.

Humic material, such as that from lignite, is quite abundant in natural soils or in landfill sites. Therefore, we studied the effect of humic material on the strength of the soil matrix. The sand sample mixed with humic material from lignite gave high torvane stress at pH 4, which is around the PZC (point of zero cross) of humic material, and at pH 13 because of the attraction force between the organics. The opposite trend for low pH (pH 1) is due to the repulsion force within the organics.

If nutrients are well provided, bacteria can grow in the soil matrix and form biopolymers to bind with it. The results showed that a sand sample mixed with nutrient broth and *A. faecalis* can reach a higher torvane stress compared to others without nutrients or without bacteria. Therefore, the growth of slime-forming bacteria inside the sand, with adequate nutrients, can enhance its strength more effectively because more biopolymers are produced.

3.2. Silica

The silica used was about 240 mesh, which is very fine, so the saturated silica is somewhat like cemented soils that retain their intrinsic strength after the removal of confining pressure. The unconfined compression test is more suitable for measuring the strength of silica than the torvane test. Figure 2 shows that the sample which contained distilled water mixed with *A. faecalis* gave the highest torvane stress. However, these results are tentative because the torvane test may not be a good measurement for the strength of silica.

On the other hand, we used the unconfined compression test to measure the strength of silica and found out that a specimen mixed with PHB solution had a threefold higher compressive stress than a specimen mixed with water only (control). Thus, the strength of silica is enhanced by the application of PHB (Figure 3).

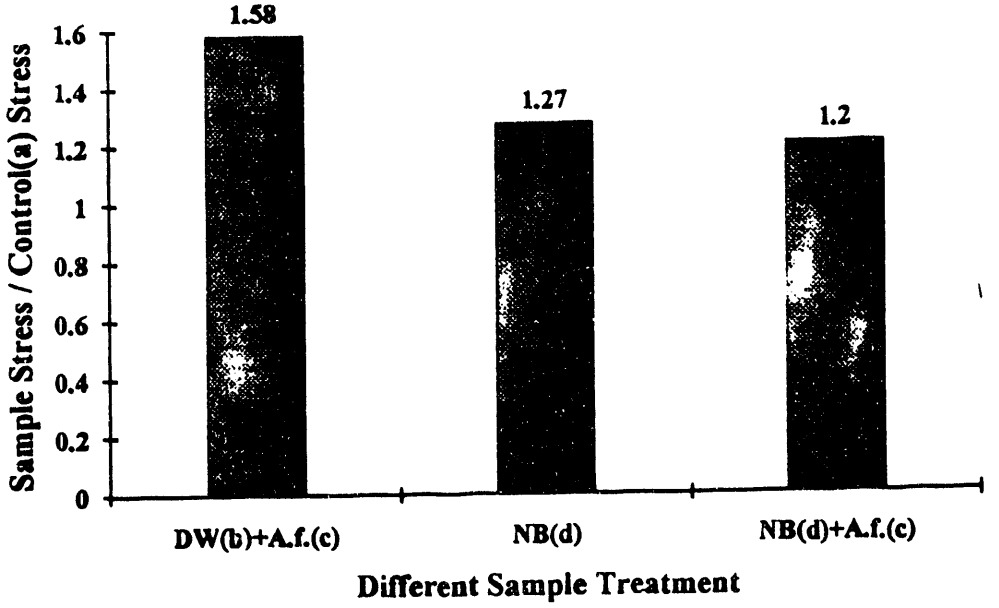


Figure 2. Ratio of sample stress to control stress of silica sample: (a) sample with distilled water only; (b) distilled water; (c) *Algaligenes faecalis*; (d) nutrient broth.

3.3. Clay

The results from the unconfined compression test are summarized in Figure 4. From the results we can see how the biopolymer solutions perform in clay samples. Different concentrations of different biopolymers were prepared. The biopolymer from *A. faecalis* growing in the recommended medium gave the largest enhancement in the strength of clay, probably due to the good yield and appropriate functionality of the biopolymer. Xanthan gum also worked well, based on its special structures and properties. The polymer backbone of xanthan is made up of β-1,4-linked D-glucose residues and is, therefore, identical to the cellulose molecule. It provides high solution viscosity at low concentration. As for PHB, biopolymers from *A. faecalis* growing in nutrient broth, biopolymers from *A. viscolactis* growing in nutrient broth, and sodium alginates, gave different, smaller enhancements. Overall, all these biopolymers enhanced the strength of clay.

4. DISCUSSION

Since different concentrations of several biopolymers were used in this study, it is difficult to conclude which biopolymer is the best one for enhancing the strength of the soil matrix because the polymers have different properties and structures. However, regardless of concentration, the strength of soil matrix is enhanced. In this study, we concentrated on the potential and feasibility of applying this technology, but there are still several factors which may affect the ability of biopolymers to enhance the strength of the soil matrix. For example, different concentrations and viscosities may cause different enhancements, as may different nutrient supplies, temperatures, and pH. If we can establish the ultimate concentration of a biopolymer solution, in addition to a suitable nutrient supply, the correct temperature and pH for maximizing the increase of strength while reducing costs, this would be a worthwhile application.

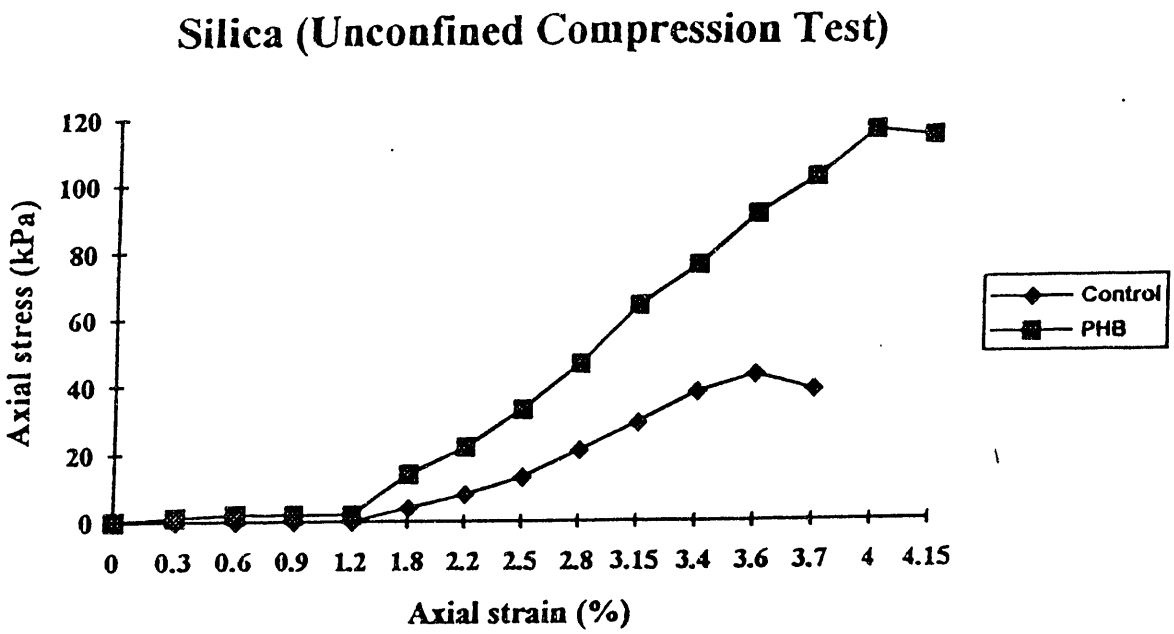


Figure 3. Effect of PHB solution on soil matrix strength of silica sample.

Soil erosion is a world-wide problem, and is now more severe than ever. It may destabilize foundations and cause massive loss of soil, with the corresponding geotechnic, civil engineering, and agricultural problems. Improving the strength of soil would be the solution to preventing the erosion problem. The study showed that the strength of the soil can be enhanced by applying biopolymers to it because of the cross-linking interaction of biopolymers and the soil matrix. We anticipate that this technology can be applied to different types of soil.

Liquefaction of sand may also present a big problem. For example, the failure of the Ft. Peck Dam in Montana is said to have been due to this phenomenon [6]. When loose saturated sands are subjected to strains or shocks, there is a tendency for the volume of the sand to decrease. This tendency may cause an increase in pore pressure which results in a decrease in effective stress within the soil mass. Once the pore pressure becomes equal to the effective stress, the sand will lose all its strength, and is said to be in a state of liquefaction. Through the study, we showed that the strength of sand can also be enhanced via this application. Therefore, if this technology were developed, the bases of large hydraulic complexes such as dams would be able to bear a heavier loading and avoid collapse under conditions of large statically induced strains like earthquakes.

In-situ bioremediation of cleaning hazardous sites has become increasingly important. As the study showed, microbially derived polymers can be efficiently used as binding agents to help the soil matrix become stronger and less permeable, and thereby, stop the migration of hazardous leachates [8]. Also,

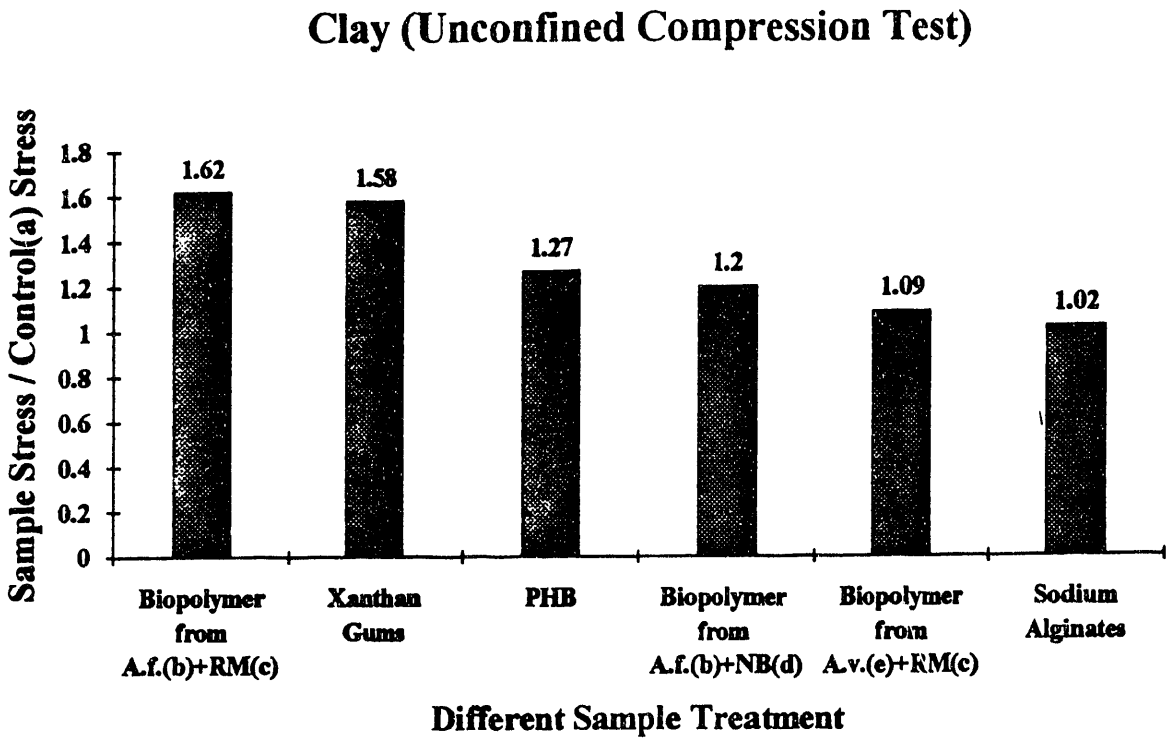


Figure 4. Ratio of sample stress to control stress of clay sample: (a) sample with distilled water only; (b) *Algaligenes faecalis*; (c) recommended medium; (d) nutrient broth; (e) *Alcaligenes viscolactis*.

biopolymers can prevent fluid migration by promoting the generation of biopolymer-filled soil layers to generate a capsule around the spill similar to a slurry wall. Furthermore, this technology can be extended to minimize risks associated with land subsidence due to fossil-energy exploitation. It is expected that biopolymers will encapsulate the oil-contaminated soil in a dynamic natural environment and seal off pollutant plumes so that natural bioremediation can occur preferentially in the remaining regions. In conjuction with other techniques such as air stripping and vacuum venting, the creation of a shield may also eventually prevent further migration.

5. ACKNOWLEDGMENTS

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Parameters Affecting Microbial Oil Mobilization in Porous Media

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Abstract

Prior work at NIPER has defined some of the key mechanisms responsible for improved oil mobilization by microbial formulations; the development of simulation models capable of predicting reservoir and production behavior of MEOR technology also has been started. The simulator development and laboratory testing aspects were integrated, so that a feedback loop existed. Mechanisms considered to be important for oil recovery include changes in properties such as interfacial tension, wettability, and adsorption/retention that govern oil mobilization and affect fractional flow and relative permeabilities. Other oil recovery mechanisms traditionally associated with fluid flow changes include polymer and biomass production by microorganisms.

Laboratory experiments were conducted to obtain input data for the simulator regarding adsorption, clogging/declogging, and wettability alteration. Computer tomography (CT) studies demonstrated that fluid and gas distributions altered by microorganisms can reduce residual oil saturation in porous media.

This paper describes ongoing laboratory investigations conducted to evaluate microbial mechanisms for improved oil recovery in porous media, and to further improve the simulator.

1. INTRODUCTION

Application of microbial EOR technology to increase oil production requires careful laboratory design of the microbial formulation to optimize its performance for the particular application. Research at NIPER has improved understanding of the mechanisms of oil mobilization by microbial formulations and transport of microbes and their metabolites in porous media. Results have shown that a key component in the oil mobilization mechanism is the capability of microorganisms to produce localized high concentrations of products at an oil-water interface.

The unusual complexity of oil recovery by microbial formulations requires close coordination between laboratory mechanistic studies and oil displacement experiments under carefully controlled conditions to develop and validate a numerical simulator. A simulator for MEOR methods can best be developed through an integrated program of acquisition of laboratory and field data with the feedback loop being the numerical simulation model. This paper describes the results of various laboratory experiments that were conducted to validate the numerical simulator and improve our understanding of microbial mechanisms of oil mobilization.

Laboratory experiments were conducted with microbial isolates that produce mainly gases and surfactants when fermenting sugars such as those present in commercial molasses. Experiments were conducted to evaluate microbial wettability alteration of rock. Studies also were started to determine if microbial wettability alteration contributed significantly to oil mobilization in porous media. CT imaging experiments were conducted to evaluate changes in fluid and gas distributions caused by microbial activity in Berea sandstone core

and to determine the effects of these distributions on improved oil recovery by microbial formulations. Microbial oil recovery mechanisms, based on these experiments, will be incorporated into the numerical simulator.

2. EXPERIMENTAL APPARATUS AND PROCEDURES

2.1. Coreflood apparatus and procedures

Unfired Berea sandstone core and field core from North Burbank Unit, OK (a fluvial-dominated deltaic reservoir, Class I by DOE reservoir identification system) were used in these studies. All experiments were conducted at ambient temperature. Core preparation and the experimental equipment were described previously [1].

2.2. Crude oil

Crude oil samples were obtained from the Bartlesville sand in Delaware-Childers and Chelsea-Alluwe fields in northeastern Oklahoma. Delaware-Childers oil has a gravity of 31° API [0.87 g/cm^3] and a viscosity of 7.5 cP at 77 °F; Chelsea-Alluwe oil has a gravity of 34° API [0.85 g/cm^3] and a viscosity of 8.1 cP at 77 °F.

2.3. Microorganisms

Results from previous studies have shown that a combination of an adapted strain of *Bacillus licheniformis*, NIPER 1A (ATCC No. 39307) and a *Clostridium* species, designated as NIPER 6, was the most effective formulation for oil recovery in porous media. These strains have been described elsewhere [1]. NIPER 7 is another species of *Clostridium* that produces a greater quantity of carbon dioxide than NIPER 6 when fermenting sucrose in molasses.

2.4. Chemicals and media

The molasses used in these experiments was obtained from Pacific Molasses Company in Oklahoma City, OK. Its composition has previously been described [1]. The concentration of molasses used in these experiments was 4% vol/vol in tap water with 0.1% wt/vol ammonium phosphate added.

The brine used for all experiments was 0.5% sodium chloride in deionized water.

2.5. Wettability experiments

Blocks of Berea sandstone were obtained from Cleveland Quarries (Amherst, Ohio) and cut into cylindrical core plugs of 0.75 in. (1.9 cm) in diameter and 1.2 in. (3 cm) in length. The plugs were then refluxed with 10% siloxane to alter them to an oil-wet condition [2].

Oil-wet field core was obtained from North Burbank Unit (NBU) and cut into cylindrical core plugs of 1 in. (2.54 cm) in diameter and approximately 1 in. (2.54 cm) in length. The plugs were then cleaned by toluene and methanol soxhlet extraction to remove connate water and oil.

The United States Bureau of Mines (USBM) centrifuge wettability method was used for all wettability measurements [3]. In this method, a positive wettability value indicates a water-wet core, and a negative value indicates an oil-wet core. The range runs from negative one to positive one. In some experiments, the microbial cells were filtered out of the solution using a 0.2μ Millipore filter, leaving only the microbial products or metabolites for testing. All wettability experiments were conducted at ambient temperature.

2.6. Adsorption studies

Static adsorption experiments were conducted with microbial cells to determine the amount of adsorption that occurred when the cells were placed in contact with the rock surface. The volume of microbial cells used (mixed with sterile brine) was 2:1 by weight to Berea or other crushed rock. Flasks containing crushed rock and microbial cells were moderately stirred for 24 hr. Control flasks containing only crushed rock and brine, or containing only microbial cells without rock, were stirred for the same amount of time with each experiment. After stirring and filtering the effluent from the flasks, the concentrations of microbial cells were measured in cells/gm. All adsorption experiments were conducted at ambient temperature.

2.7. CT imaging

The dynamics of fluid flow and microbial gas production were investigated using rock-fluid imaging techniques. Displacement experiments were monitored using X-ray computed tomography (CT) to determine gas production and its effects on fluid flow, and the alteration of fluid distributions by microbial formulations. A Siemens Somatom II computed tomography scanner was used to obtain images of cores. Detailed information about the use of these techniques were previously presented [4]. No tagging techniques were used unless oil was present. When crude oil was used, it was tagged with 20 or 30% iododecane. All CT experiments were conducted at ambient temperature.

3. RESULTS

3.1. Description of numerical simulator

A three-dimensional, three-phase numerical simulator was developed to predict the propagation and distribution of microorganisms and nutrients in porous media. This transport simulator accounts for diffusion, dispersion, clogging, chemotaxis, growth and decay of microorganisms, and consumption of nutrients by microbial systems. The simulator can account for three-phase flow and oil

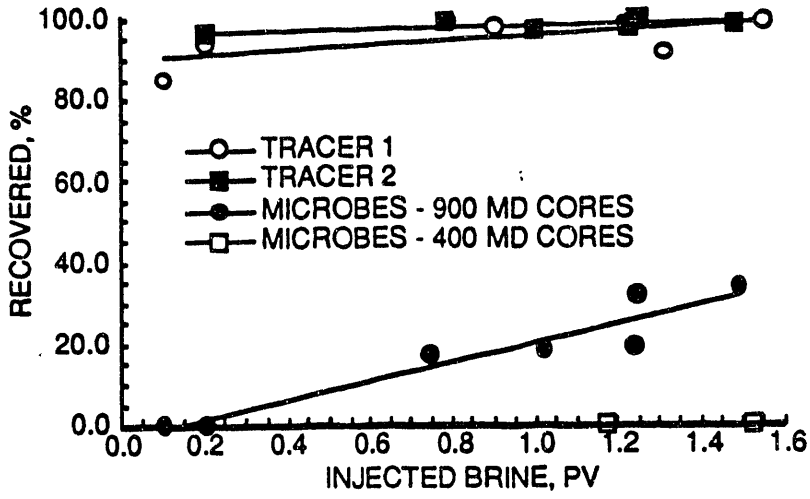


Figure 1. Recovery of microorganisms vs. amount injected in Berea coreflood experiments.

recovery by microorganisms. A more complete description of the simulator was presented in SPE paper 22845 [5]. Using this simulator, the transport of microorganisms can be investigated, and the effect of a microbial system on oil recovery can be studied.

3.2. Microbial retention tests

Coreflood experiments were conducted to obtain information on microbial adsorption and clogging/declogging in 25 cm-long Berea sandstone core samples for incorporation in the numerical model. The procedure was previously described [6]. For retention tests using high permeability cores (900 mD), a linear correlation was obtained between the amount of microbes injected and the amount of microbes recovered. Retention tests using lower permeability cores (400 mD) did not show any correlation (Figure 1). Fluorescein tracer test results were the same whether in high or low permeability cores; virtually 100% of the tracer was recovered. The pressure drop across the core during the microbial cell injection was measured and showed no significant increase in pressure. This may indicate that the greater amount of cell retention observed is due to adsorption or cell destruction, rather than a high amount of plugging. A Berea sandstone core with a permeability of 2.4 darcies was used to determine if the higher permeability had a different effect on microbial cell retention. The volume of microbes injected was 1.2 PV. Interestingly, we found that a similar amount of microbial retention was obtained using this higher permeability core when compared to the 900 mD cores (Figure 2). A retention test was conducted in a 2.8 darcy core with a 1% sodium bicarbonate preflush to determine whether the sodium bicarbonate would act as a sacrificial agent to prevent adsorption of microbial cells. The volume of microbes injected was 1.2 PV. The results showed the opposite effect, with more microbes being retained using the preflush than in the control core (Figure 3). The bicarbonate preflush appears to be having some effect on the microbial retention, possibly by changing the pH, or by altering the cell surface.

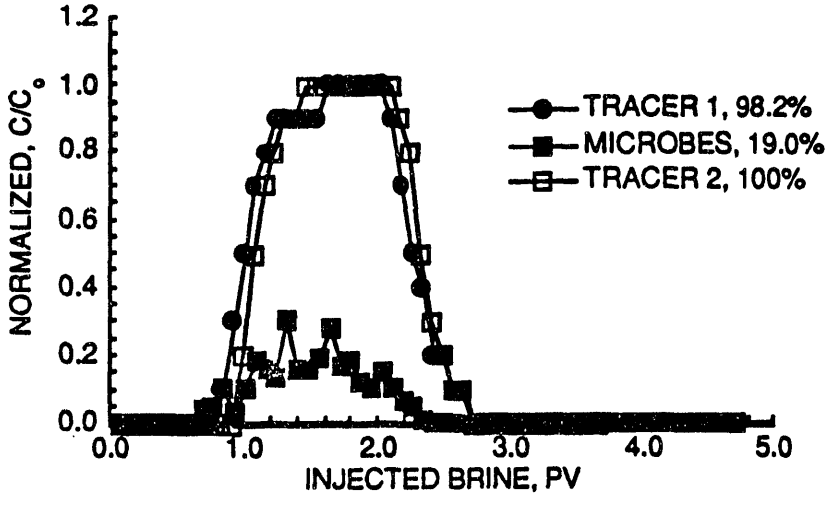


Figure 2. Microbial retention test in 2.42 darcy Berea core showing % recovered.

Table 1
Static adsorption studies with microbial cells and crushed rock

Rock type	Injected conc. cells/gm	Final conc. cells/gm
Berea	6.0×10^8	6.0×10^5
Berea	2.0×10^8	3.0×10^3
Berea	1.0×10^8	3.0×10^5
Berea[1]	2.0×10^9	4.0×10^2
NBU	6.0×10^6	4.0×10^3
NBU	3.0×10^3	6.0×10^0

[1] Preflush with 1% sodium bicarbonate

3.3. Adsorption

Static adsorption studies were conducted using Berea sandstone and crushed North Burbank, OK, core. All adsorption test results correlated with results obtained from the retention experiments. These results indicated that these microorganisms have a high degree of adsorption, and have a consistently strong propensity to interact with rock. Table 1 gives the results of these experiments. When sodium bicarbonate was used with these microbial adsorption studies, an even greater amount of cells was adsorbed, which corresponded to the results observed when sodium bicarbonate was used in the retention test.

3.4. Wettability

Previous results obtained from microbial wettability experiments using water-wet Berea sandstone have shown that microbial formulations can significantly alter wettability (Table 2). The wettability index of Berea sandstone in control

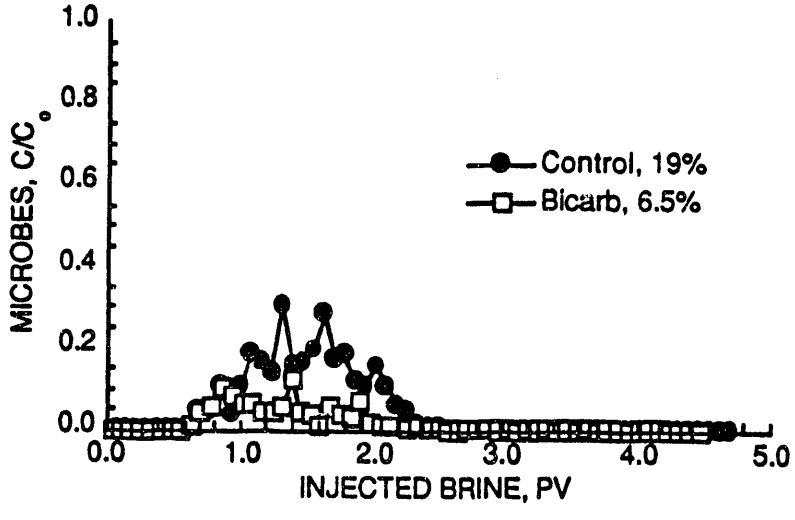


Figure 3. Microbial retention test with 1% sodium bicarbonate preflush showing % recovered.

Table 2
Wettability values

Berea Sandstone	System Used	Wettability Index
Cp1	Brine	+ 0.315
Cp3	Products	+ 0.239
Cp5	NIPER 1A	+ 0.950
Siloxane-treated Berea		
Si1	Brine	- 0.806
Si3	Molasses	- 0.689
Si5	NIPER 1A	- 0.259
NBU core		
Bu1	Brine	- 0.414
Bu3	Products	- 0.421
Bu5	NIPER 1A	- 0.257

systems using only brine and oil has consistently been in the + 0.200 to + 0.400 range (intermediate to slightly water-wet). When only the products or microbial metabolites were used, the wettability index was comparable to the control range. When NIPER 1A was used (including the cells), the wettability index was always significantly higher (+ 0.95), as shown by the example core in the table.

Several series of experiments were performed to determine the effect of microbial formulations on wettability of oil-wet rock. One series of experiments was conducted with siloxane-treated Berea core plugs using brine on two plugs, 2% molasses only on two plugs, a combination of NIPER 1A and NIPER 6 cells on two plugs, and NIPER 1A cells on three plugs. Representative results of these experiments are shown in Table 2. Siloxane core experiments using only brine

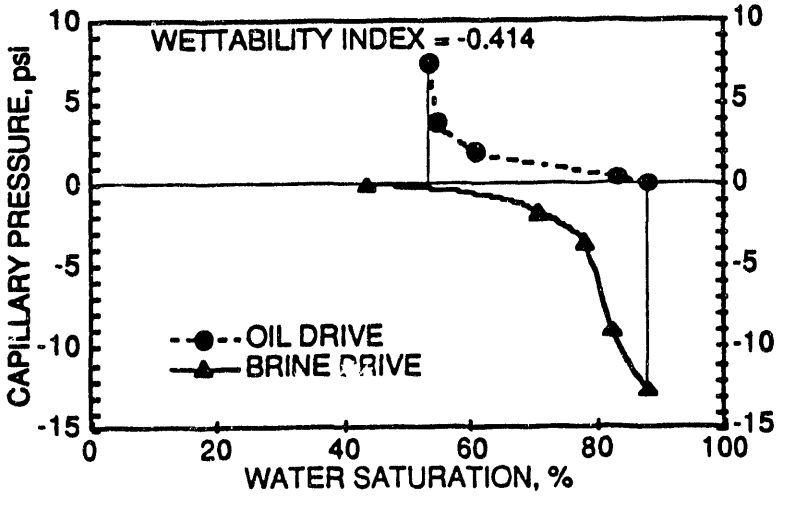


Figure 4. NBU core with brine.

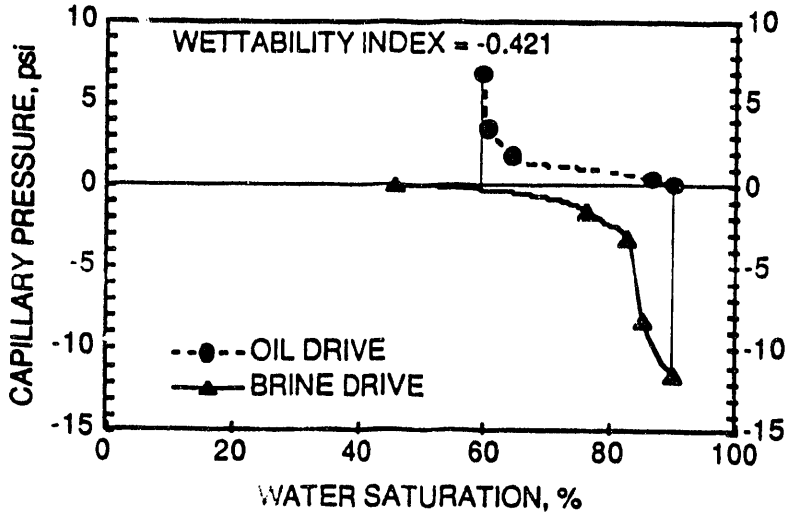


Figure 5. NBA core with microbial products.

consistently had a wettability index around -0.8, an oil-wet value, while those using molasses had wettability indices that were close to those of the control brine cores. When NIPER 1A was used, the wettability index was significantly changed to a more intermediate wettability state (an average of about -0.25).

Another series of experiments was then conducted with oil-wet core from NBU. Three of these plugs were tested with brine; two plugs were tested with a microbial metabolites (products) solution of NIPER 1A, where the cells had been filtered out, and two plugs were tested with NIPER 1A (cells included) (Table 2). The results of these experiments are shown in Figures 4 through 6. The control

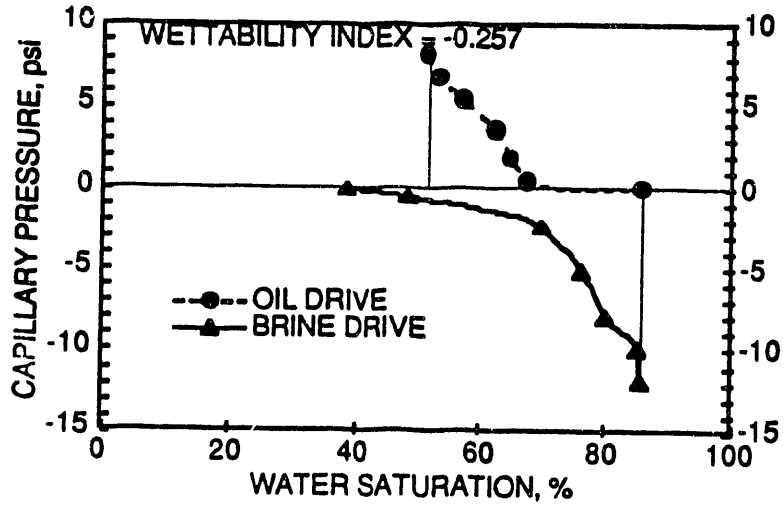


Figure 6. NBU core with NIPER 1A.

plugs using only brine had wettability indices from -0.36 to -0.54, which is in the range of the published wettability index for NBU (-0.45) [7]. Those plugs using the products solution of NIPER 1A also were in this range. When NIPER 1A cells were used, the wettability was changed to a more intermediate state.

When wettability values and their corresponding residual oil saturations were compared, a trend was observed indicating that shifting the wettability from highly oil-wet to a more intermediate value also reduced the residual oil saturation. The residual oil saturation values decreased from over 25% to less than 20% (Figure 7). This represents a 20% increase in recovery efficiency.

In these experiments, microbial formulations appeared to alter the wettability oil-wet core plugs to a more intermediate wet condition. The results obtained with plugs that had been treated to make them oil-wet were comparable to results using oil-wet field plugs. In all cases, the presence of the microbial cells significantly affected the wettability alteration. When the cells were filtered out, the wettability indices were similar to the control and nutrient-only results. Overall, by comparing these different types of plugs using the same microbial formulations and the USBM centrifuge method, we showed that NIPER 1A can significantly alter the wettability of the rock surface and reduce the residual oil saturation. Using the results of these and other concurrent experiments, mechanisms for microbial oil recovery can be further defined and incorporated into the numerical simulator.

3.5. CT imaging experiments

CT imaging experiments using microbes in Berea sandstone to observe gas and fluid distributions were published previously [6]. Another CT experiment was conducted using Chelsea-Alluwe crude oil tagged with 20% iododecane. A porosity scan was obtained by subtracting the scan of the dry core from the scan of the brine-saturated core. When measured with the CT, the average porosity for this core was 21.2%; this corresponded closely with the volumetrically measured value of 21.7%. The core was waterflooded to residual oil saturation and then injected with 0.1 PV of NIPER 1A and 7, and 0.3 PV of 4% molasses. The core was then incubated for 48 hr and the pressure was monitored. Table 3 shows the

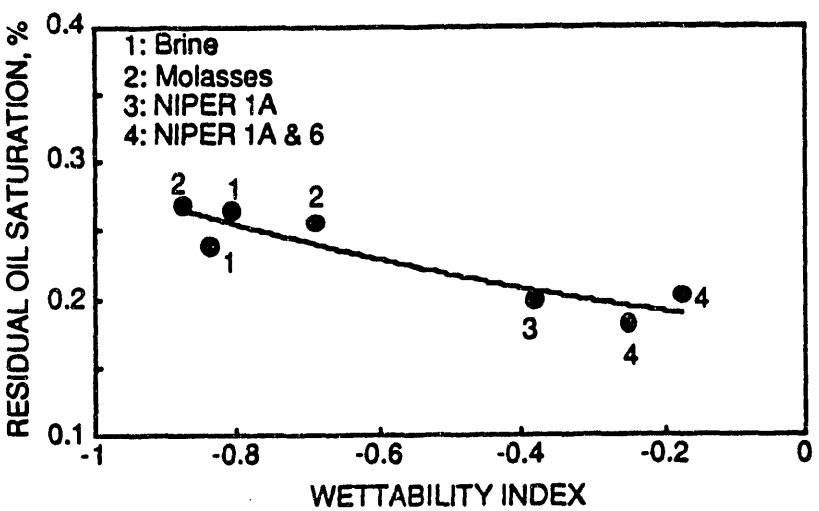


Figure 7. Wettability vs. residual oil saturation in siloxane treated Berea cores.

Table 3
CT saturation data

	Core E1	Core CT3
S_{oi}	59.2%	68.0%
S_{orwf}	34.0%	35.1%
S_{ormf}	30.2%	31.7%
E_r	11.1%	9.7%

S_{oi} = initial oil saturation, percent pore volume, %PV
S_{orwf} = oil saturation after waterflooding, %PV

S_{ormf} = oil saturation after microbial treatment and subsequent waterflood, %PV

E_r = recovery efficiency, $\frac{S_{orwf} - S_{ormf}}{S_{orwf}} \times 100\%$

saturations of this coreflood (CT3) along with the saturations of a previous coreflood (E1). These saturations and previous imaging analyses have indicated that the microorganisms used are capable of changing the fluid distributions and mobilizing crude oil in the core.

4. CONCLUSIONS

1. For the systems studied, significant microbial cell retention occurs during transport in porous media, and is more complex than simple clogging/declogging or adsorption. This phenomenon may be due to a combination of factors.
2. Cells must be present to cause an alteration in wettability. The products of this particular microbial system or nutrient alone do not significantly change wettability. Residual oil saturations are reduced by this change in wettability.
3. CT imaging can be used to evaluate changes in fluid and gas distributions caused by microbial activity in porous media.

5. ACKNOWLEDGEMENTS

This work was supported by the U S. Department of Energy under Cooperative Agreement DE-FC22-83FE60149. Fred W. Burtch and Rhonda Patterson of the DOE Bartlesville Project Office are acknowledged for their help in conducting this work. The authors thank Deanna Evans for her assistance with the retention experiments, and T.E. Burchfield, M.K. Tham, and Bill Linville for their review of this paper.

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Behavior of Microbial Culture Product (PARA-BAC^R) Isolates in Anaerobic Environments

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Abstract

The ability of selected microorganims utilized in the commercial microbial product PARA-BAC^R to survive and multiply in anaerobic environments with petroleum and paraffins is described. In environments rigidly excluding oxygen and with redox potentials of -200 mV or less, members of the product consortium exhibited doubling rates of 4 to greater than 24 hours for several transfers in a chemically defined media with either crude oil, hexadecane, or octadecane as a sole carbon source. Consortium members could be shown to produce biosurfactant type activities. The ability of the complete consortia to reduce viscosity and interfacial tension in a variety of different crude oils under anaerobic conditions is described. These properties are correlated with results obtained from field case histories.

1. INTRODUCTION

A microbial culture product, PARA-BAC^R, has been used successfully for paraffin control and production enhancement in the petroleum industry for over six years. It is composed of a group of naturally occurring, non-pathogenic microorganisms which have been specifically selected and adapted to control paraffin deposition in crude oil. The exact types of microorganisms used and their culture methods are held as proprietary information, because they represent a unique group of microorganisms not heretofore used in conventional MEOR type work. There are several different products which control paraffins of varying molecular weights or other problems associated with oil field production systems (Table 1). PARA-BAC^R has shown widespread effectiveness and commercial success on a variety of crude oils and reservoir types. The usual method of treatment is a producing well-bore type application down the well anulus rather than traditional MEOR type applications into waterfloods, although some flood work has been performed. The following work begins a series of studies reporting on the behavior of both product and individual isolates with various types of crude oils and documenting the types of changes which the products and their constituent isolates may be expected to produce under varying conditions.

A critical point, which has been brought up in discussions of microbial use in oil wells, is their behavior in anaerobic environments. Opinions differ as to the degree of anaerobicity found in oil well fluids and statements vary depending upon what point the particular researcher wishes to make at the time. While oxygen does display a differential solubility in crude oil over water, it also is a fact that significant populations of obligately anaerobic bacteria, such as sulfate-reducing bacteria and methanogenic bacteria, can be isolated from oil wells. Probably the best statement of fact is that both anaerobic and aerobic environments can exist in the subsurface environment depending upon the reservoir location studied. The widespread presence of reservoir souring due to microbially mediated sulfate reduction (an obligately anaerobic process) suggests that anaerobic environments are commonly found in the subsurface. Therefore, it

Table 1
Para-Bac products with date of commercial introduction

Product	Date
Para-Bac	1986
Para-BacX	1987
Para-Bac+	1987
Litho-Bac	1987
Para-Bac XX	1988
Para-Bac XXX	1988
Para-Bac X+	1988
Para-Bac XX+	1989
Corroso-Bac	1989
Para-Bac/S	1990
Para-Bac/S+	1990
Sulfo-Bac	1990
Sulfo-Bac C	1991

is of interest to study the behavior of microbial culture products and their individual isolates to see the effect of anaerobic environments on their ability to produce changes in crude oils relevant to paraffin deposition and/or enhanced oil recovery.

2. MATERIAL AND METHODS

2.1. Bacterial strains

The various products used were typical production lots of Para-Bac, Para-Bac X, and Para-Bac/S. Individual isolates were taken from the culture collection of Micro-Bac International and were used in the individual products. Isolate identifications and culture methods are proprietary information of Micro-Bac International. None of the isolates used are dissimilatory sulfate reducers or recognized pathogens. All are environmental isolates and are not produced using recombinant DNA technology.

2.2. Experimental design

Products were cultured in a standard anaerobic gas, 85% N_2, 10% H_2, 5% CO_2 atmosphere in a Coy anaerobic chamber. This atmosphere was scavenged for oxygen with a forced atmosphere palladium catalyst system. Redox potential was monitored continuously using a Corning PS 19 ORP probe. A resazurin solution was used as a back-up indicator. Oils, paraffins, and synthetic growth media were degassed by vacuum treatment to 28 inches of mercury and regassing with 100% nitrogen. This procedure was repeated twice and then a final cycle was performed with the anaerobic gas mixture. Growth vessels were prepared using a 50% petroleum-50% synthetic salts medium mixture (100 ml total volume). Bacterial strains were inoculated from standardized culture broths, which had been subcultured several times on a synthetic salts/petroleum medium. The inoculated vessels contained from 0.1 to 3.5 x 10^8 cells per milliliter. The growth of isolates was determined at 12-hour intervals by microscopic count of cells using phase contrast microscopy. At the end of seven days, the growth vessels were

removed from the anaerobic chambers and fluid properties (viscosity and interfacial tension) were immediately determined.

2.3. Petroleum materials

The crude oil samples used were reference samples of light South Texas and Oklahoma crudes of 28-30 API gravity. Chemically pure n-hexadecane and n-octodecane (99+ pure) also were used. The oil was mixed with bacterial solutions in a 1:1 ratio.

2.4. Determination of fluid properties

A rotary viscometer (Labline Instruments) was used to determine crude oil viscosities at 25°C. Interfacial tensions were determined using a du Nuoy type ring tensiometer (Fisher Scientific).

3. RESULTS

Several strains used in the various products showed increases in the microscopic count of bacterial cells when grown on crude oil as a sole carbon source or when grown on hexadecane or octadecane (Figures 1-3). Significant levels of variation were seen between strains; for example, Para-Bac/S isolate 1553 (Figure 1), showed good growth in the presence of the two pure hydrocarbons and the Oklahoma crude, but poor growth on the South Texas oil. Likewise, Para-Bac/S isolate 15137 showed good growth only in the presence of pure octadecane, and no growth in the presence of the other hydrocarbons. Para-Bac/S isolate 15119 showed growth only on South Texas oil and possibly on Oklahoma oil (Figure 3). Similar variations were seen with other product isolates (data not shown.)

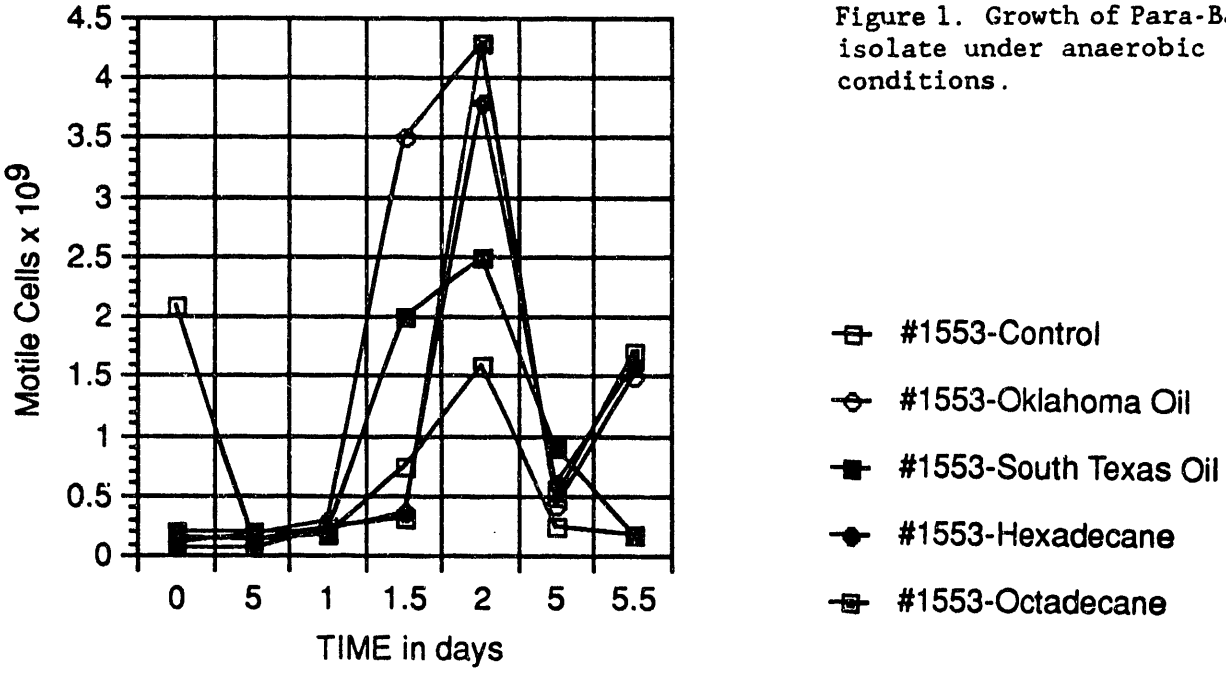


Figure 1. Growth of Para-Bac isolate under anaerobic conditions.

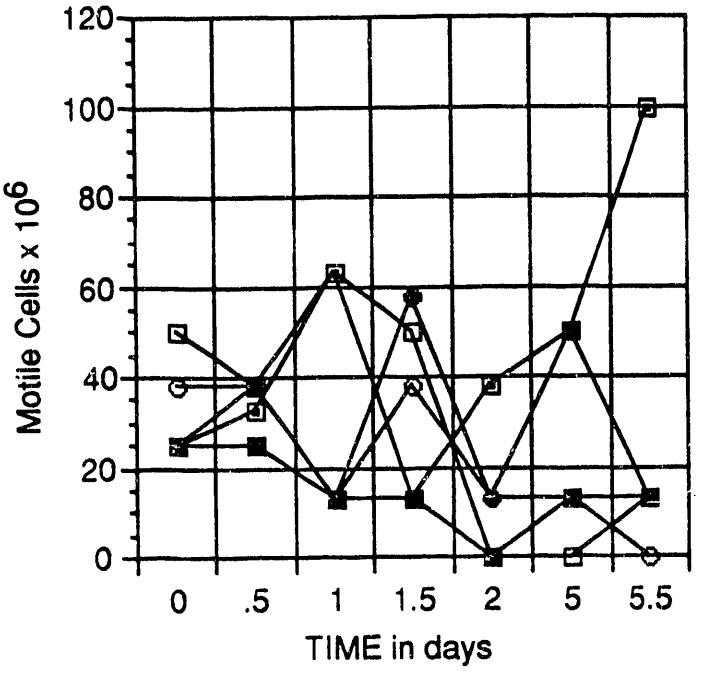


Figure 2. Growth of Para-Bac isolate under anaerobic conditions.

#15137-Control
#15137-Oklahoma Oil
#15137-South Texas Oil
#15137-Hexadecane
#15137-Octadecane

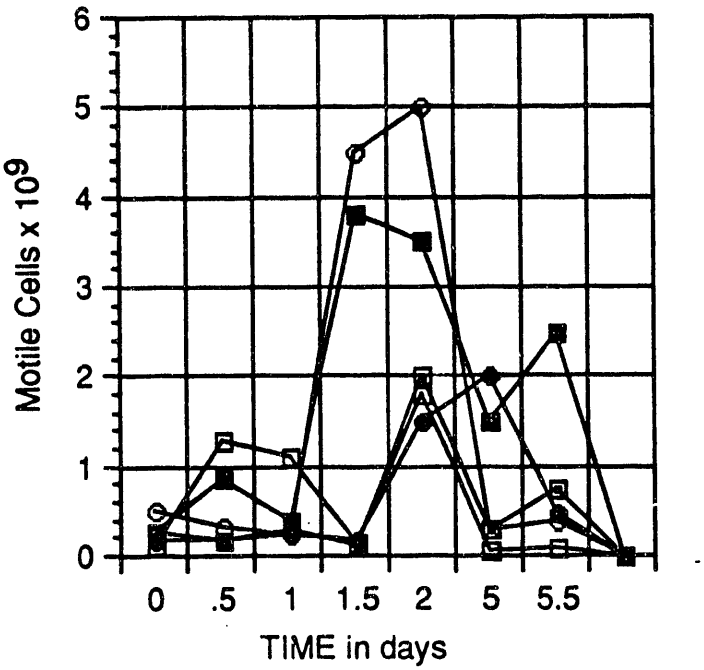


Figure 3. Growth of Para-Bac isolate under anaerobic conditions.

#15119-Control
#15119-Oklahoma Oil
#15119-South Texas Oil
#15119-Hexadecane
#15119-Octadecane

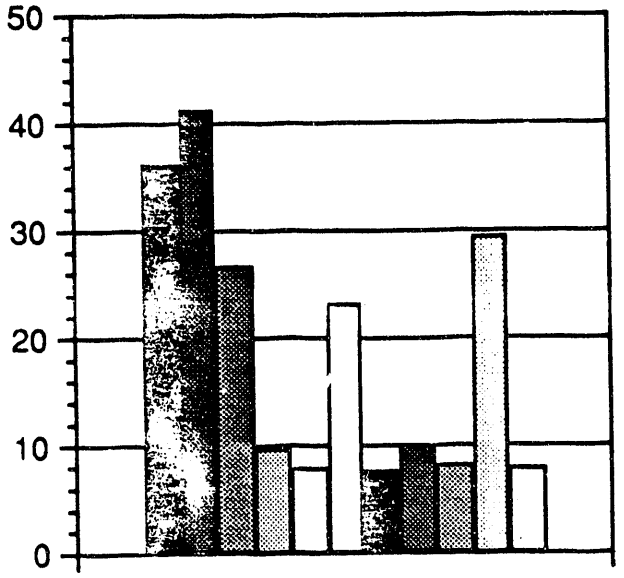


Figure 4. Apparent viscosity changes. Para-Bac Products versus Oklahoma Crude. See Figure 5, below, for key.

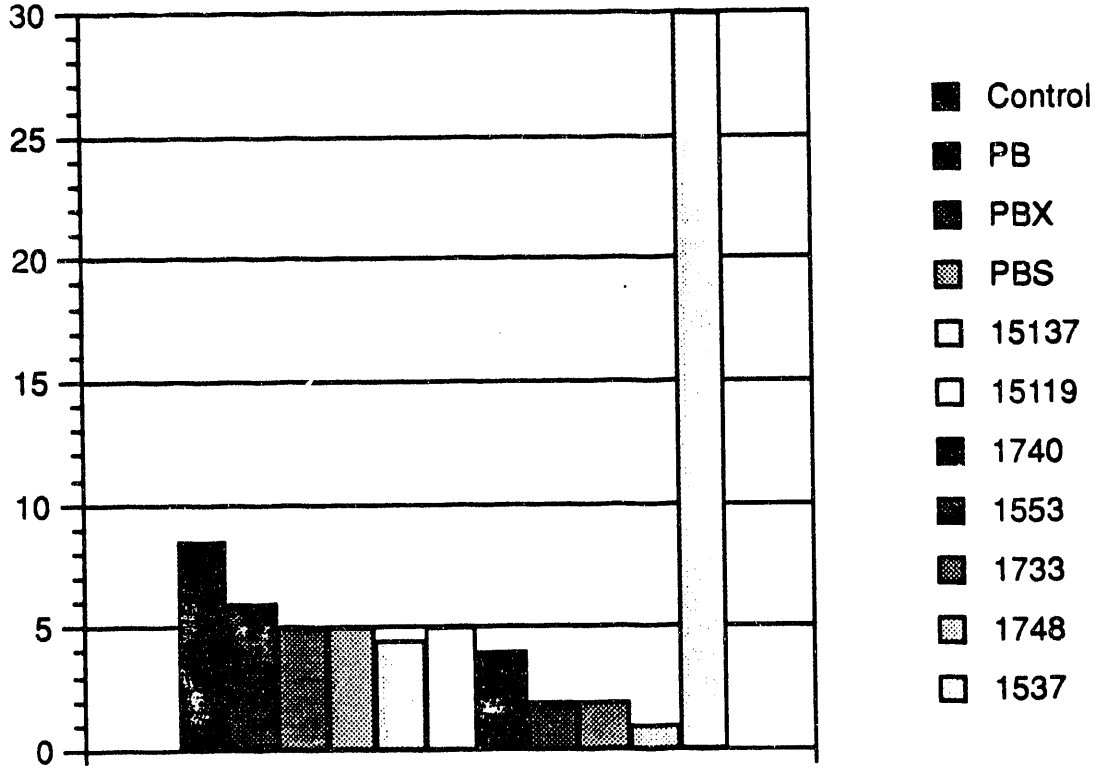


Figure 5. Interfacial tension changes. Para-Bac Products versus Oklahoma Crude.

When changes in viscosity were measured using the different isolates incubated in the presence of Oklahoma crude, significant variations also were seen (Figure 4). Interestingly, changes in viscosity did not correlate with growth capability in the oil. For example, strain 15137 showed poor growth in Oklahoma oil, but produced significant reductions in viscosity. Isolate 1553 showed good growth in the Oklahoma crude and also produced good reductions in viscosity.

A similar phenomenon was seen with changes in interfacial tension; both 15137 and 1553 produced significant reductions in interfacial tensions (Figure 5). Strain 1537, which showed a greater than 60% reduction in viscosity, actually produced an increase in interfacial tension. This strain showed poor growth in Oklahoma crude.

4. DISCUSSION

The following conclusions can be made regarding the behavior of isolates from various Para-Bac products.

1. Strains show varying capacities to increase their numbers in the presence of crude oil and purified alkanes as a sole organic carbon source using a basal salt solution as an aqueous medium.
2. Strains generally reduced viscosities; however, no correlation could be made between growth rate and the ability to reduce viscosity.
3. Strains generally reduced interfacial tensions, but showed no direct correlation between this, their growth rates, and their ability to reduce viscosities.

The claim that strains are able to multiply in the presence of crude oil or alkanes as a sole carbon source is admittedly a controversial one. Concerns raised about the presence of residual oxygen contained in the oil layer are based upon the higher solubility of oxygen in petroleum compared to water. Because the oil samples were degassed before the experiment and were held under anaerobic conditions for an extended period, it seems unlikely that this is the complete explanation for the phenomenon observed. Also, the strains generally do not grow at a higher rate under aerobic conditions. The energetics of the degradation is not unfavorable if one assumes that degradation continues past the initial oxidation step and is actually similar to a variety of other hydrocarbon degradations, such as the beta oxidation of fatty acids. Only the initial oxidation step would be thermodynamically unfavorable in the absence of oxygen. Ample precedence exists in microbial metabolism for an initial input of energy to begin a degradation process which has a net yield of energy for the organism. A further amplification of this process will be presented in subsequent publications.

Few studies have focussed upon the diversity of microbial effects which are possible on the physical properties of crude oil. The present work establishes that simple growth of a microbial strain is not sufficient to produce changes in some properties of crude oil, which have been associated with microbial enhanced oil recovery and that these properties actually vary independently from one another with various strains.

This observation notes the importance of tailoring individual treatments to individual crude oil types and specific well types and reservoir geologies. The standard Para-Bac treatment design incorporates a custom dosage blend of various microbial products to optimize paraffin control and/or production increases. This treatment design has been successfully used for over six years in field

treatments. For example, the specific oil used in these studies, the Oklahoma crude, derives from a field successfully treated for paraffin control with concomitant production increases over the last two years.

Aqueous Microbial Biosurfactant Solutions Exhibiting Ultra-low Tension at Oil-water Interfaces

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Abstract

During aerobic cultivation, cultures of *Penicillium spiculisporum* ATCC 16071, produce spiculisporic acid (S-acid), yielding at least 0.05-0.1 mol per mol glucose consumption. The solubility of S-acid in water is extremely restricted; however, neutralization of the acid with linear alkylamine gave remarkably surface-active aqueous solutions. Depending upon the length of the linear hydrocarbon chain of n-alkylamine, n-R_AA, added to neutralize the S-acid molecule, interfacial tension of about 10^{-3} mN/m can be achieved in contact with the oil phase. A wide variety of n-alkylamine salts of S-acid, dicarboxyl acid, were prepared by stoichiometrical neutralization of one carboxyl group with n-R_AA, and then by neutralization of the remaining carboxyl groups with n-R_BA. The n-alkylamine salts of S-acid, SnR_AAnR_BA, were examined for their ability to promote very low interfacial tensions between aqueous solutions of the prepared salts of S-acid and n-alkanes as the oil phase. The lowest interfacial tension of about 10^{-3} mN/m was achieved with n-octylamine salts at a concentration of 10^{-3} mol per liter. Such reduction of the interfacial tension between the oil and water phase may substantially meet the requirements for the low-tension waterflooding process for oil recovery.

1. INTRODUCTION

A basic element in the process of low-tension waterflooding, a technique to enhance oil recovery, is to have control over the capillary number and viscosity ratio. Capillary number (hereafter referred to as N_C) is the ratio of viscous to interfacial forces as described by equation (1), and is closely related to the balance of forces on oil trapped in porous media, such as sandstone formations.

$$N_C = \frac{\mu_W V_W}{\phi \gamma_{OW}} \tag{1}$$

where μ_W is the viscosity of the water phase, V_W is the Darcy velocity of the water phase, ϕ is porosity, and γ_{OW} is the interfacial tension between the oil and water phases.

W.R. Foster [1] emphasized the importance of N_C to determine the residual oil and the shape of the permeability saturation functions, as illustrated in Figure 1, where residual oil is plotted as a function of capillary number for porous media of fired Berea sandstone. The order of magnitude of N_C during ordinary waterflooding is estimated to be 10^{-6}. Figure 1 shows that residual oil decreases as N_C increases, and that Berea sandstone with an N_C of about 10^{-2} has zero residual oil. In other words, an increase in N_C of about four orders of magnitude is needed to ensure zero residual oil. This can be accomplished practically by reducing interfacial tension, γ_{OW}, in equation (1). Because interfacial tension between oil and water is about 10^1 mN/m, γ_{OW} must be reduced

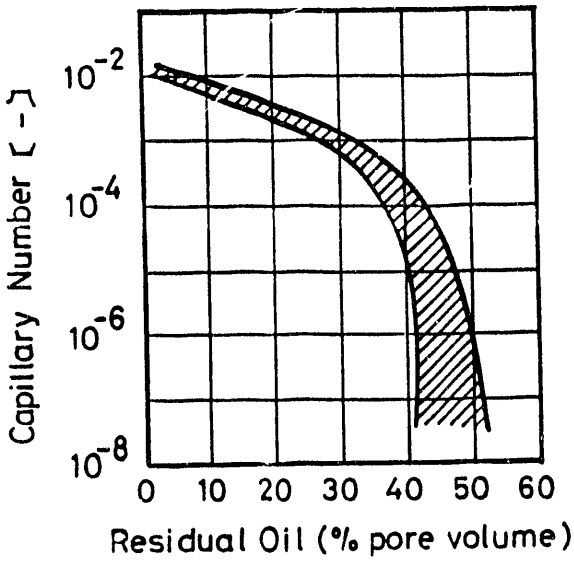


Figure 1. Dependence of residual oil on capillary number.

to an order of magnitude of 10^{-3}mN/m to almost completely recover the oil which is trapped in the porous media of Berea sandstone. Development of aqueous surfactant solutions exhibiting interfacial tensions less than 10^{-2} mN/m are an essential requirement for the low-tension waterflooding process.

Ultra-low values of the tensions at an oil-water interface can be obtained using a water-soluble, surface-active compound originating from a microbial product. During aerobic cultivation, a strain of *Penicillium spiculisporum* produces spiculisporic acid (4,5-dicarboxy-4-pentadecanoide, hereafter referred to as S-acid) yielding at least 0.05-0.1 mol S-acid per 1 mol glucose consumed [2]. The solubility of S-acid in water is extremely restricted; however, neutralization of the acid with linear alkylamine, n-RA, gave remarkably surface-active aqueous solutions.

In studies on the biochemical activities of fungi, Tabuchi et al. [3,4] found that a strain isolated from soil and identified as *P. spiculisporum* accumulated a large amount of needle-like crystals in the culture broth when grown on glucose. The accumulated compound was characterized as 4-hydroxy-4,5-dicarboxypentadecanoic (open-ring acid of spiculisporic acid; O-acid). After heating and drying, O-acid was converted to S-acid. Tabuchi et al. [4] also found that intermittent feeding of glucose improved O-acid production, and more than 110g of O-acid per liter accumulated in the culture broth after ten days in their shake flask experiments. This high production of the acid undoubtedly increases the potential for its use as a microbial resource for the production of surfactant. Presumably, the high value may be ascribable to the fact that the product is substantially insoluble in water, thus, inhibition of its production is probably negligible.

Spiculisporic acid (S-acid) originally isolated from cultures of *Penicillium* species was examined for its antibiotic properties. Though there are no published reports, the antibiotic properties were evaluated to be good. Interest

in this compound has revived due to its surface-active properties. Ishigami and Yamazaki [5] discussed the surface active properties of sodium salts of S-acid. Because S-acid is a dicarboxylic acid containing one lactone ring, the mono-, di-, and tri-sodium salts of the acid can be prepared by varying the pH of the aqueous medium by neutralization and by saponification of the acid with aqueous sodium hydroxide. The mono-sodium salt of S-acid caused the highest reduction in surface tension among these salts and was comparable to ordinary anionic surfactants, such as sodium dodecylsulfate, sodium dodecylbenzenesulfonate, and sodium laurate.

Ishigami and Yamazaki [5] prepared each sodium salt of both S-acid and O-acid, and investigated the surface active properties of each aqueous solution in terms of surface tension at a critical micelle concentration (CMC). The surface tension of the aqueous sodium salts of spiculisporic acid varies according to the pH of the aqueous medium in the range between 33 mN/m (corresponding to the mono-sodium salt, S-1Na) and 56 mN/m (corresponding to the tri-sodium salt, S-3Na). Such a pronounced pH-dependency of the surface activity may restrict the application of these sodium salts as surfactants, and careful control of the pH of the aqueous medium to between 4.0 and 5.0 is necessary to keep the surface tension below 33 mN/m.

In addition, the hydrophilic-lipophilic (or hydrophobic) balance, the so-called HLB, even of the mono-sodium salt of the S-acid molecule inclines slightly to the hydrophilic side, and thus, the surface activity of S-1Na is not sufficiently high. It is desirable to introduce hydrophobic group(s) into the molecule of S-acid to achieve the most appropriate balance between hydrophilic and hydrophobic moieties.

Because of the polyfunctionality of the molecular structure of S-acid, there is the potential to broaden the spectrum of surface active properties by developing various derivatives from S-acid. Preparation of n-alkylamine (n-RA) salts of S-acid is, presumably, one practical way to achieve this goal because the hydrophobicity of the resultant molecule increases as the alkyl chain length of the n-alkylamine increases.

In the present paper, we discuss a series of experiments concerning the preparation of a wide variety of n-alkylamine salts of spiculisporic acid, and an evaluation of their surface activity. Depending upon the length of the linear hydrocarbon chain of n-alkylamine used, an ultra-low surface tension, in the order of 10^{-3} mN/m, can be achieved in a contact with oil phase. We discuss the development of aqueous solutions exhibiting ultra-low interfacial tension against oil phases, such as hydrocarbons.

2. EXPERIMENTS

2.1. Microorganism

The most valuable culture for the production of spiculisporic acid by *Penicillium spiculisporum* is probably *P. spiculisporum* ATCC 16071, which is the subject of U.S. Patent No. 3,625,826 (Dec. 7, 1971). This culture, which appears to only synthesize 4-hydroxy-4,5-dicarboxypentadecanoic acid, was used in this experimental study. Figures 2 and 3 show the biosynthetic pathway proposed by T. Ban [6] for the production of 4-hydroxypentadecanoic acid during the growth of *P. spiculisporum* ATCC 16071.

2.2. Preparation of inoculum

The medium consisted of 10% glucose, 0.1% NH_4Cl, 0.1% KH_2PO_4, and 0.02% $MgSO_4 7H_2O$. The microorganism was inoculated into 100 ml of the medium in a

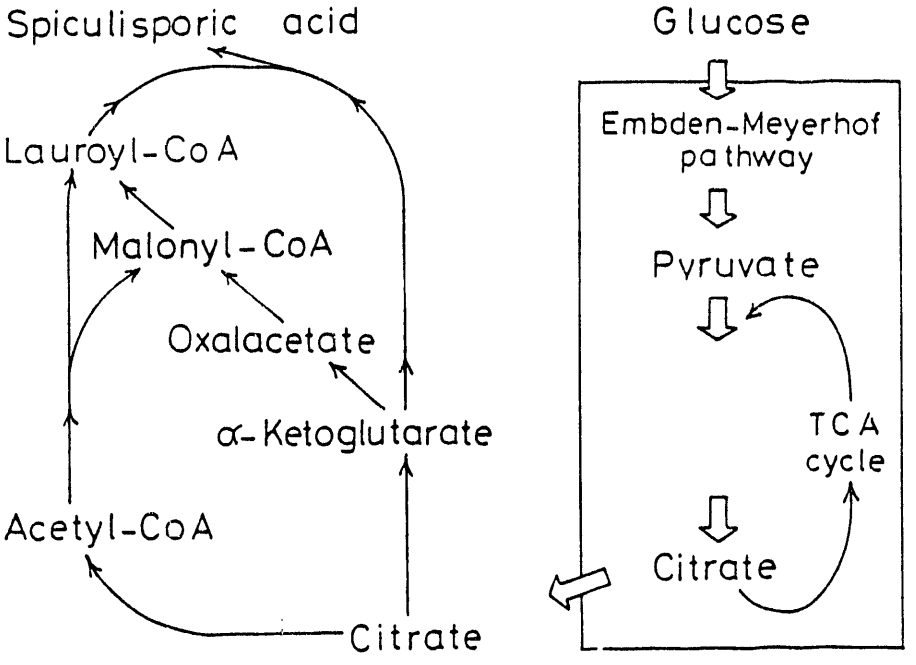


Figure 2. Proposed pathway for the production of spiculisporic acid.

500 ml flask, and incubated at 30°C for two days on reciprocal shaker at 120 oscillations per minute with an amplitude of 7 cm.

2.3. Cultivation in fermentation apparatus

The cultivation and production of spiculisporic acid by *P. spiculisporum* in an experimental-scale fermenter (Figure 4) was made under the following conditions.

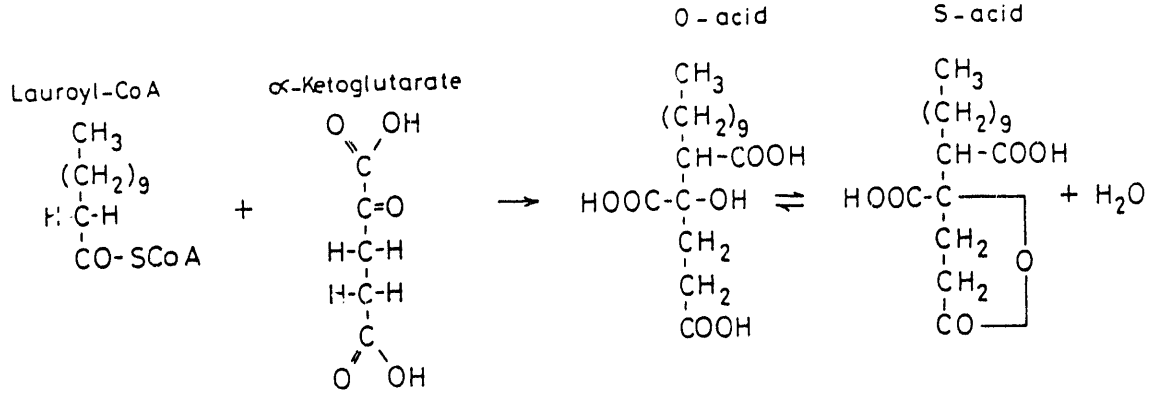


Figure 3. Biosynthesis of O-acid and S-acid.

Production medium: 1,500 ml of medium, the composition of which is given in Section 2.2, was prepared, autoclaved, and cooled at 30°C.
Inoculation: 50 ml of inoculum was used.
Temperature: 30 ± 0.1°C.
Agitation speed: 350 rpm.
Aeration: 30 ml per minute of oxygen.

2.4. Recovery of spiculisporic acid from the fermentation broth

At the end of the cultivation of *P. spiculisporum* in the fermenter, the entire fermentation broth was used for the recovery of S-acid; Figure 5 shows the procedure as a flow chart. First, the broth was heated to 90°C for 30 minutes to dissolve the S-acid. While the broth was still hot, the mycelia were removed by filtration. S-acid is lost if the system cools. The filtrate was heated to 80°-100°C for 30 minutes to convert all O-acid to S-acid, then cooled for three hours. All the S-acid present should be precipitated and recovered by this procedure.

2.5. Preparation of n-alkylamine salts of S-Acid

2.5.1. Materials:

Tetrahydrofuran (THF), Diethyl ether (Ether), Ethylmethyl ketone (EMK), and n-alkylamines (nRA).

All the materials used in this study were analytical grade and purchased from either Wako Pure Chemicals Co. Ltd., Japan, or Tokyo Chemical Industry Co., Ltd., Japan.

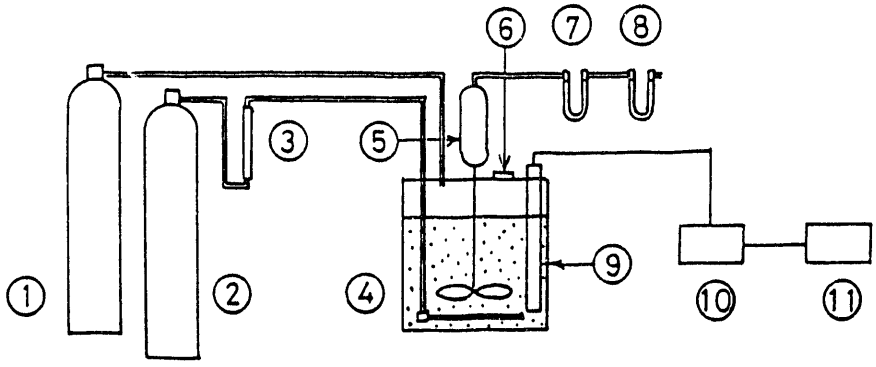


Figure 4. Experimental fermentation apparatus.

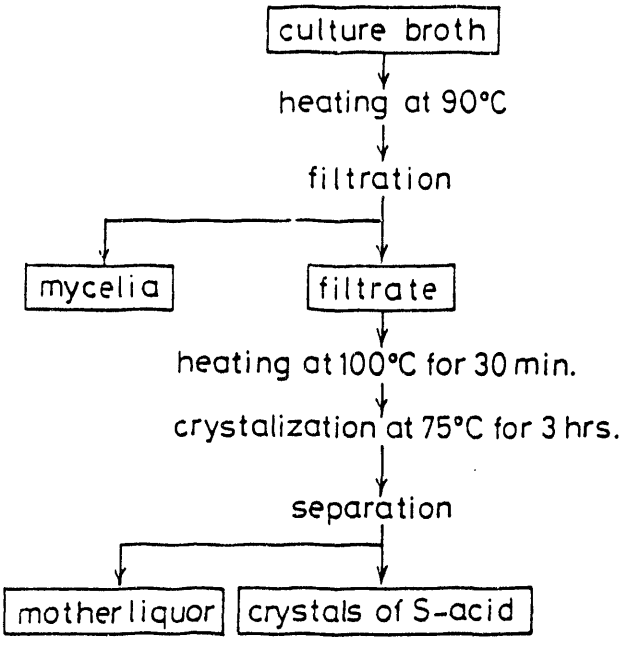
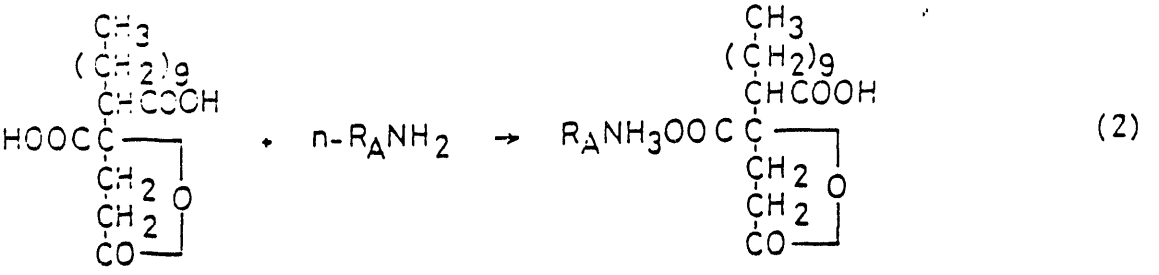


Figure 5. Recovery of spiculisporic acid from the fermentation broth.



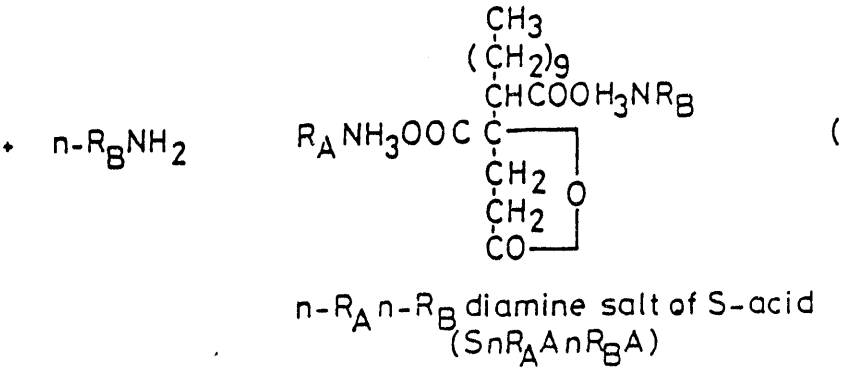


Figure 6. Stepwise neutralization of S-acid with n-alkylamine.

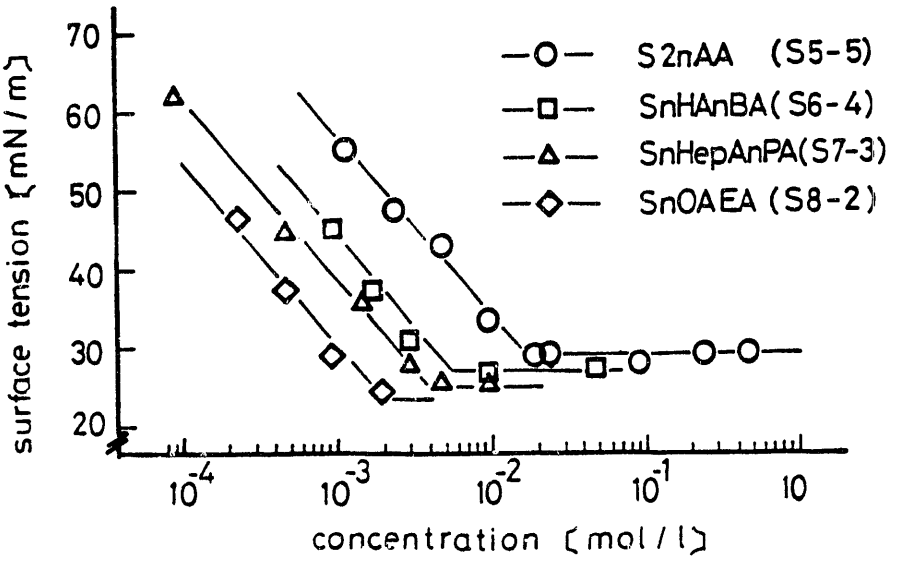


Figure 7. Surface tension of various n-alkylamine salts as a function of their concentration.

2.5.2. **Preparation:**

Because S-acid is a dicarboxylic acid having two carboxyl groups in its molecule, it can be neutralized by alkylamine in two steps (Figure 6).

First, the carboxyl group at the position of C-4 is neutralized by a stoichiometric amount of n-alkylamine (nR_ANH_2, or, in shorter form, nR_AA) as

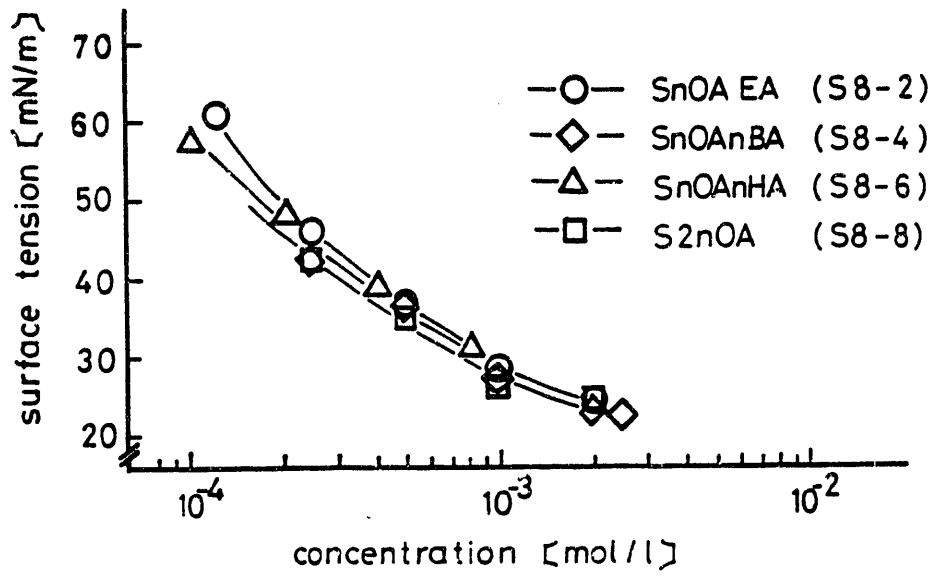


Figure 8. Surface tension of various n-octylamine salts as a function of their concentration.

shown in equation (2) in Figure 6; then, the remaining carboxyl group at C-5 position was stoichiometrically neutralized by nR_BNH_2 according to equation (3).

These two steps were carried out by dissolving S-acid in a mixed medium of 95% tetrahydrofuran and 5% water, and by adding the stoichiometric amount of n-alkylamine drop by drop.

All the neutralized products of the n-alkylamine salts of S-acid were refined by washing with a solvent, diethyl ether-ethylmethyl ketone, to remove residual reaction materials.

The hydrophilic-lipophilic balance (HLB) of the resultant salt can be easily changed by changing the hydrocarbon chain length of the n-alkylamine used to neutralize S-acid. Therefore, we prepared a series of n-alkylamine salts of S-acid having a wide variety of HLB, and evaluated their surface activity in terms of surface free energy, or surface tension of the aqueous solution of the salts and interfacial tension between aqueous and oil phase.

2.6. Measurement of surface tension

The surface tension of aqueous solutions prepared from refined alkylamine salts of S-acid were measured using Wilhelmy's vertical plate tensiometer at 30 ± 1°C.

2.7. Determination of interfacial tension

Tensions at the interface between aqueous solution of the alkylamine salt of S-acid, and an oil phase, such as n-alkanes, were determined by means of the spinning drop method described by Cayias et al. [7].

3. EXPERIMENTAL RESULTS

3.1. Surface tension at aqueous n-alkylamine salt solutions

Table 1 shows various n-alkylamine salts of spiculisporic acid prepared according to equations (2) and (3) in Figure 6. A salt, abbreviated to SnOAnBA (or, more simply, as S8-4) is a compound whose structure is shown in Figure 6, where R_A is n-octyl and R_B is n-butyl, respectively.

Table 1 shows their solubility in water, critical micelle concentration (CMC), and surface tension at the concentration corresponding to the CMC of each n-alkylamine salt of S-acid.

The critical micelle concentration of a surface active compound can be determined by measuring the surface tension of a series of aqueous solutions of different concentrations and plotting surface tension as a function of concentration. Although surface tension depends upon the concentration of the surface active compound when the concentration is lower than the CMC, and tension decreases as concentration increases, there is no significant reduction of surface tension when the concentration is greater than CMC. Figure 7 shows plots of surface tension as a function of concentration for various n-alkylamine salts of S-acid. From the Figure, the critical micelle concentration as well as the surface tension at the concentration of CMC of each salt can be determined; Table 1 lists the results.

Figure 8 shows plots of surface tension as a function of concentration for n-alkylamine salts having n-octyl as a longer alkyl chain with a different shorter alkyl chain, including the alkylamine salts of SnOA-EA (S8-2), SnOAnBA (S8-4), SnOAnHA (S8-6), and S2nOA (S8-8). The curves of surface tension vs. concentration for all these alkylamine salts are almost identical, suggesting that surface tension is predominantly governed by the length of the longer alkyl

Table 1
Various n-alkylamine salts of S-acid and their physical properties

Alkylamine salt		Solubility in water mol/l	Critical micelle concentration mol/l	Surface tension at CMC mN/m
S2nBA	(S4-4)	1×10^{-1}	3×10^{-3}	37
S2nAA	(S5-5)	1×10^{-1}	2×10^{-2}	33
SnHA-EA	(S6-2)	1×10^{-2}	1×10^{-2}	28
SnHAnBA	(S6-4)	1×10^{-2}	1×10^{-2}	28
S2nHA	(S6-6)	1×10^{-2}	1×10^{-2}	28
SnHepAnPA	(S7-3)	1×10^{-3}	4×10^{-3}	25
SnHepAnAA	(S7-5)	1×10^{-3}	4×10^{-3}	25
S2nHepA	(S7-7)	1×10^{-3}	4×10^{-3}	25
SnOA-EA	(S8-2)	1×10^{-3}		25
SnOAnBA	(S8-4)	1×10^{-3}		25
SnOAnHA	(S8-6)	1×10^{-3}		25
S2nOA	(S8-8)			25

chain, in these cases, the n-octyl chain, and that the length of the shorter chain has no significant effect upon surface tension.

The important findings shown in Table 1, Figures 7 and 8, can be summarized as follows:

1. The surface tension of the alkylamine salt of S-acid is governed by the chain length of the alkylamine used to neutralize the S-acid. As shown in Table 1, the longer the length of n-alkyl chain added, the greater the surface activity of the resultant n-alkylamine salt.

2. When alkylamine salt, expressed as SnR_AAnR_BA, is prepared from two different n-alkylamines, R_AA and R_BA, for stepwise neutralization of S-acid (Figure 6), the surface activity of the resultant salt of SnR_AAnR_BA is predominantly governed by the alkyl chain length of either R_AA or R_BA, whichever is longer.

3. As the alkyl chain length of longer alkylamine increases, the values of the critical micelle concentration, CMC, decreases (Figure 8).

3.2. Interfacial tension against oil phase

Special attention should be given to a fact that aqueous solutions of S7- and S8-series of salts of S-acid produce extremely low surface tensions around 25 mN/m (Table 1). Lowering of surface tension achieved by commercial surface-active agents manufactured chemically generally does not exceed 30 mN/m. Therefore, in expectation of achieving extremely low tensions at the interface between oil and water phases, determinations of interfacial tension were made with a series of aqueous n-alkylamine salts against n-hexane as an oil phase (Figure 9).

The interfacial activity of four alkylamine salts, S2nAA (S5-5), SnHAnBA (S6-4), SnHepAnPA (S7-3), and SnOA-EA (S8-2), was examined by the spinning drop method. The total chain length of hydrocarbon added to S-acid was set at ten while the longer chain length added ranged between five to eight, namely, n-amyl, n-hexyl, n-heptyl, and n-octyl, respectively.

Figure 9 shows that interfacial tension against the oil phase is strongly governed by the hydrocarbon chain length of the longer alkylamine; n-octylamine

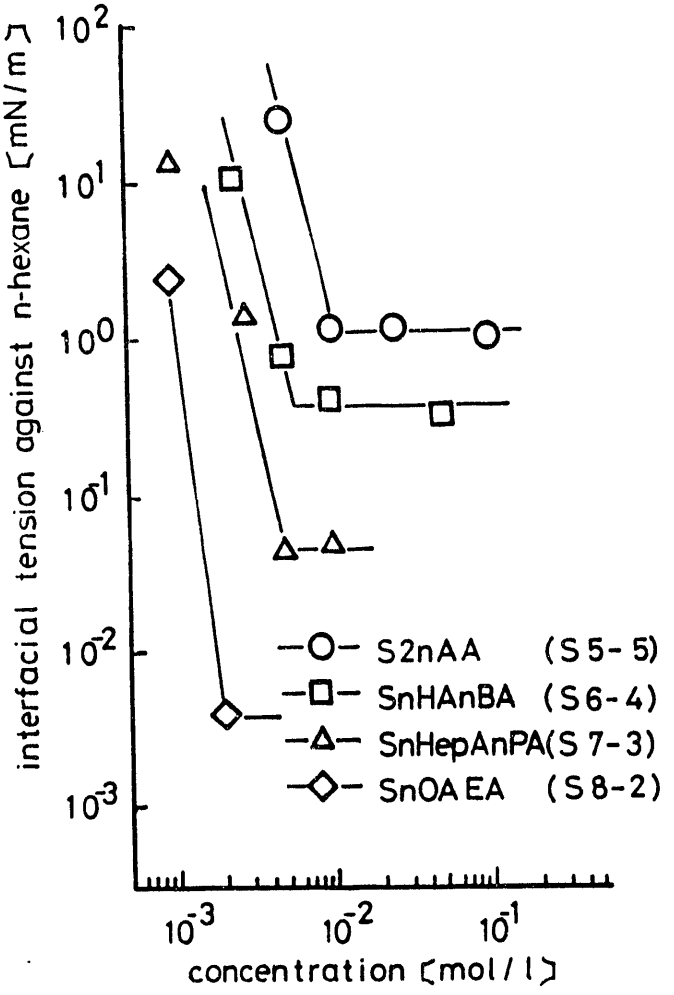


Figure 9. Interfacial tension against n-hexane of various n-alkylamine salts as a function of their concentration.

can produce aqueous solutions which exhibit extremely low interfacial tensions of about 10^{-3} mN/m.

We note that the n-octylamine salt of S-acid seems to have a well-balanced hydrophilic and lipophilic molecular structure. Such reduction of the interfacial tension between oil and water to 10^{-3} mN/m may substantially meet the requirements of low-tension waterflooding for oil recovery, as illustrated in Figure 1.

4. CONCLUSIONS

During aerobic cultivation on glucose, a strain of *Penicillium spiculisporum* produces S-acid (spiculisporic acid or 4,5-dicarboxy-4-pentadecanoide). Because of the molecular polyfunctionality of S-acid, the potential for broadening the

spectrum of surface active properties may be achieved by developing various derivatives of S-acid. Preparation of n-alkylamine salts of S-acid is one practical way to achieve this goal.

In this study, a variety of n-alkylamine salts of S-acid were prepared by stepwise neutralization, explained in equation (2) and equation (3) in Figure 6. The properties of their aqueous solutions were evaluated in terms of surface and interfacial tensions.

Depending upon the length of linear alkyl chain of n-alkylamine used to neutralize the S-acid, an extremely low interfacial tension could be achieved. Prime attention should be given to the n-octylamine salt of S-acid which can develop an aqueous solution exhibiting an ultra-low interfacial tension of 10^{-3} mN/m because the salt has the most well-balanced structure in terms of hydrophilic and lipophilic molecules.

Reduction of the interfacial tension between oil and water phases to about 10^{-3} mN/m may substantially meet the requirements of low-tension waterflooding for oil recovery.

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The Compatibility of Biosurfactants on Degassed Oil and the Displacement Efficiency of Biosurfactant/Sulfonate - Alkaline - Polymer System

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Abstract

Microbial biosurfactants are surface-active agents synthesized by microbial cultures. Biosurfactants consist of hydrophilic and hydrophobic molecules, just as other chemical surfactants. Thus, biosurfactants can reduce surface tension (air-water), and interfacial tension (IFT) in liquid-liquid and liquid-solid systems (e.g. wetting phenomena). Tests of five biosurfactants showed that the surface tension of rhamnolipids and sophorolipids/trehalolipids is 27.0 and 26.9 mN/M, respectively, and the critical micelle concentration is 0.0016 and 0.0015 wt%, respectively.

Experiments show that interfacial tension between solutions of trehalolipids and rhamnolipids and Daqing degassed oil at the Daqing oil field (temperature 45°C, average salinity of formation water 4456 ppm) is 0.4 and 0.6 mN/m, respectively. Better systems, with mixtures of trehalolipids and rhamnolipids, or trehalolipids and synthetic sulfonates 3A were obtained, and interfacial tension between the system and the Daqing degassed oil reached 0.1 mN/m.

The interfacial tension between alkaline solutions (NaOH) with trehalolipids and Daqing dressed oil is 0.03 mN/m when the alkalinity is 0.8 wt%. Mixtures of trehalolipids and petroleum sulfonates show efficient synergism; the IFT between the mixtures and Daqing degassed oil of low acidity reached very low values of 0.006 mN/m when the concentration of total surfactants was 0.4 wt% and alkalinity was 0.5 wt%. When alkaline concentration was 1.0 wt%, the interfacial tension fell to 0.002 mN/m. The salinity requirement diagram (SRD) shows that ultralow IFTs can be reached with the total surfactant concentration between 0.2-0.6 wt% and salinity below 15000 ppm. The results in the flooded core show that tertiary recovery can be increased by 11.3% and water cut can be reduced from 98% to 74%.

1. INTRODUCTION

Some microbes, such as *Torulopsis sp.* and *Pseudomonas sp.* under appropriate conditions of culture, which include sources of carbon and nitrogen, organic nutrients, pH and ambient temperature, produce a number of useful metabolites for enhanced oil recovery. These include surface active agents. A "microbial biosurfactant" usually acts in the same way as synthetic surfactants with the following characteristics. It reduces surface tension, especially interfacial tension between oil and water. Surfactants can form micellar particles, emulsify hydrocarbons, and change the hydrophobic characteristics of the surface of rocks. In general, a biosurfactant is easily dissolved in connate or injection water and acts favorably on the interface between oil and water. Microbial biosurfactants can wash oil films off reservoir rocks and have the ability to disperse crude oil while lessening its retention. According to expert predictions, the cost of

biosurfactants is 30% lower than that of synthetic surfactants. From an ecological viewpoint, a biosurfactant is particularly advantageous because unlike many synthetic surfactants, it is non-toxic. Because of these merits and, because biosurfactants can be produced by microbial metabolism and fermentation, biogenic engineering circles have paid increasing attention to the technology [1-4].

In the 1970s, biosurfactants began to be used to enhance oil recovery. In the 1980s, some methods for the preparation of biosurfactants became available. For example, investigators in the Soviet Union suggested use of lipoid biosurfactants to develop oil reservoirs [5]. The interfacial tension achieved between the aqueous biosurfactant and crude oil was less than 0.1 mN/m. In 1987, the Shanghai Institute of Organic Chemistry, the Chinese Academy of Sciences, provided us with seven biosurfactants, including trehalolipids, sophorolipids - ethyl sophorolipids, rhamnolipids and emulsion to investigate the compatibility of biosurfactants with Daqing crude oil and to implement oil displacement tests on the Daqing reservoir core.

2. EXPERIMENTS

The following equipment and chemical agents were used for our studies. Physical Equipment: Model CBVP-A3 Surface Tensiometer, made in Japan; Model 500 Spinning Drop Tensiometer, from University of Texas, USA; Model DA-101.B Automatic Density Meter, made in Japan; Model 51 Pocket pH Meter, from YOKOGAWA HOKUSHIN; Model CA-A Contact Angle Meter, made in Japan; Coreflooding apparatus manufactured by Geomecanique, France; Rheometer, low shear-30, from Contrave, Switzerland.

Chemical Agents: Biosurfactants such as trehalolipids, sophorolipids, sophorolipids-C_2H_5, rhamnolipids and emulsions from Shanghai Institute of Organic Chemistry, the Chinese Academy of Sciences. Petroleum sulfonate 3A, from the Yumen oil field. The cationic indicator was dimidium bromide, and the anionic indicator disphium blue. The titrant used was Hyamine 1622. NaOH was produced in China. Brine used in this study was average formation water [6]. Degassed crude oil was from the cross station, the First Oil Production Co., Daqing oil field. Polyacrylamide V228 was obtained from BASF Chemical Co., West Germany.

2.1. The measurement of surface and interfacial tension

In this study, the model CBVP-A3 Surface Tensiometer was used to measure the surface tension of lipoid solutions. The tests were run at temperatures of 20, 25, 30, 35, 40 and 45 $\pm$ 0.1°C. The error of measurement of IFT was $\pm$ 0.1 mN/m. Usually, Critical Micelle Concentration (CMC) is determined in the surface tension-surfactant concentration coordinate system at the point at which the surface tension drops rapidly. The spinning drop technique was used to determine low interfacial tension and the equilibrium time was between 2 and 4 hours. The test was made at 45 $\pm$ 0.1°C. The value of interfacial tension is calculated by the following formula:

$$\sigma = 5.21 \times 10^{-7} \cdot \Delta\rho \cdot d^3/T^2$$

where $\Delta\rho$ is the density difference in g/cm^3, d is the drop diameter in division, and T is the period in ms.

2.2. The rheologic investigation

The low shear - 30 rheometer was used to determine the rheology of the chemical slug which was used for coreflooding. The function of shear rate as viscosity was determined to investigate shear degradation in order to assess a reasonable level of mobility control in coreflooding at 45°C.

2.3. The salinity requirement diagram

In this study, we investigated the relationship of interfacial tension between the chemical slug and Daqing crude oil with changes in biosurfactant concentration as well as salinity to determine an optimum formulation and to explain the displacement phenomenon on the Daqing reservoir core.

2.4. Coreflooding experiments

The Alkaline-Micellar flooding apparatus from Geomecanique, France, was used to measure oil displacement at a constant temperature (45°C), constant injection velocity, and high pressure (12 MPa).

The high pressure-coreholder, effluent collector, and middle containers were all placed in the constant temperature box. The hollow between the holder and core was filled with a low melting-point alloy. Inner core pressure was kept at a given level by adjusting the back pressure valve. The pressure drop across the core was measured and recorded continuously by a pressure transducer. The chemical slug was injected at a constant rate with the model FDS-200 pump, manufactured by Core Lab, USA.

3. RESULTS AND DISCUSSION

3.1. CMC for a biosurfactant system and the effect of temperature on surface tension

At 25°C, the surface tension of pure water is 72 mN/m which can be lowered if a biosurfactant is added, although it usually remains above 20 mN/m. Correspondingly, the minimum is 27 mN/m, CMC 0.0016 wt% for the rhamnolipids, 36 mN/m, 0.0042 wt% for the sophorolipids, and 26.9 mN/m, 0.0015 wt% for a mixture of sophorolipids/rhamnolipids (Figure 1). In this study, surface tension and CMC decrease slightly with an increase of temperature for a biosurfactant system. Usually, the surface tension is constant although it becomes higher when the concentration of biosurfactant is over CMC (Figure 2).

3.2. The interfacial tension properties between a biosurfactant system and Daqing degassed oil

In average formation water, the interfacial tension between sophorolipids or trehalolipids and Daqing degassed oil is 0.6 mN/m and 0.4 mN/m, respectively (Figure 3). For the trehalolipids, the interfacial tension can fall to 0.3 mN/m as the concentration of biosurfactant is increased to 0.5 wt% but cannot become lower even if the concentration is further increased.

3.3. The interfacial tension properties between Daqing degassed oil and a mixture of biosurfactants or a biosurfactant and petroleum sulfonate

To study the effects of a mixture of two biosurfactants on the interfacial tension between the mixture and Daqing degassed oil, the biosurfactant sophorolipids-C_2H_5 was mixed with emulsion or trehalolipids, and sophorolipids were combined with trehalolipids. The results show that the mixture of trehalolipids and sophorolipids had favorable effects on surface tension.

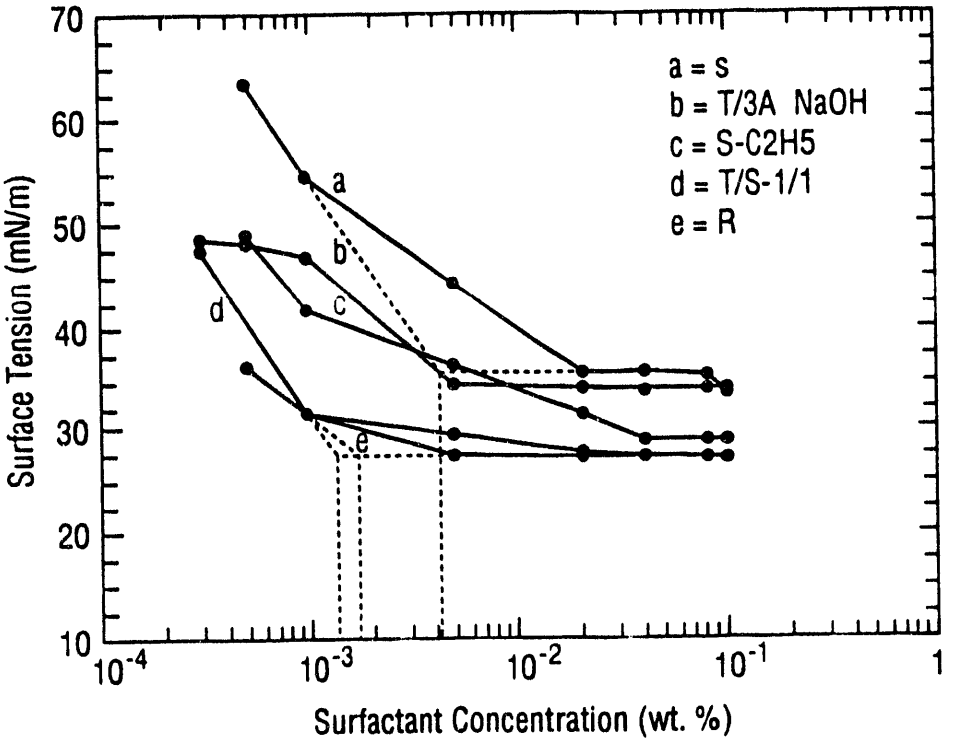


Figure 1. CMC for a single biosurfactant or its combination at 45°C.

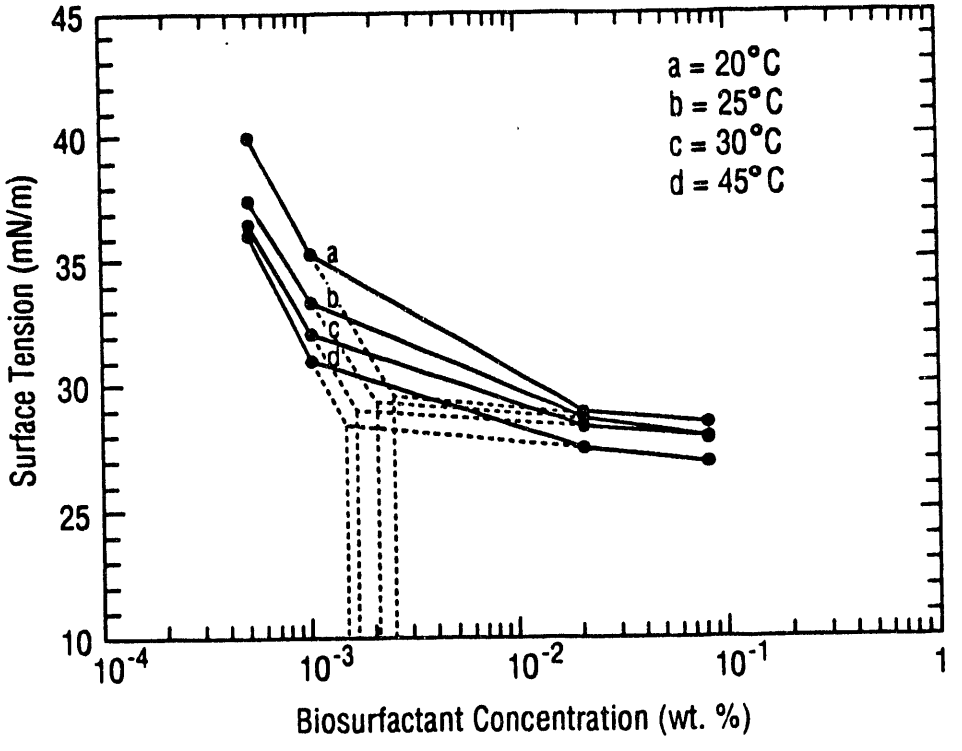


Figure 2. Effect of temperature on CMC for biosurfactant R.

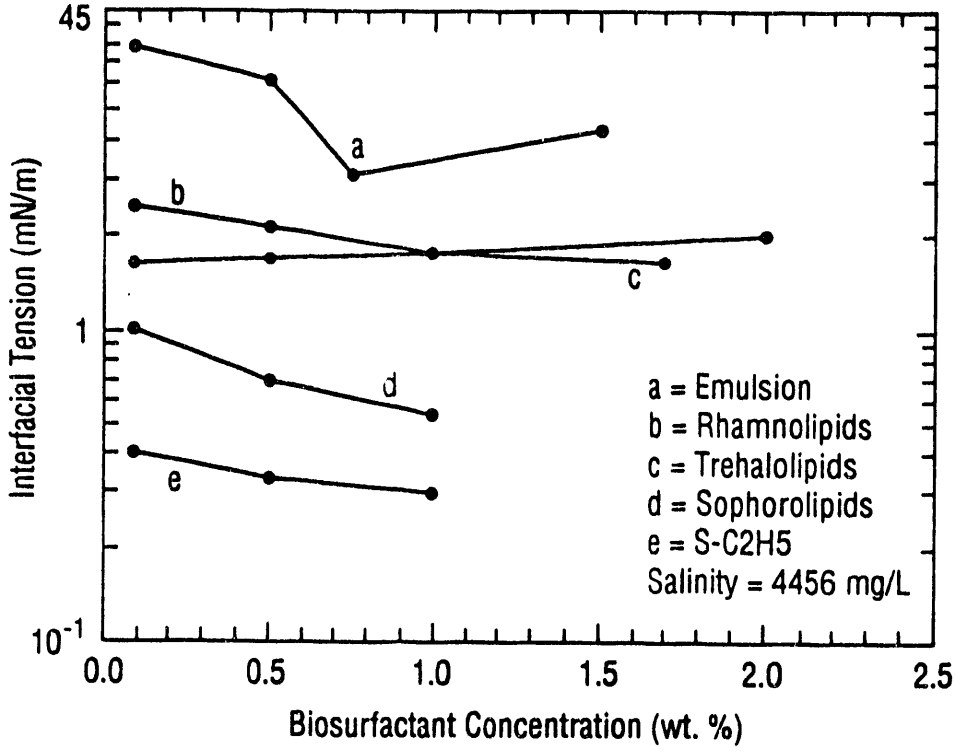


Figure 3. IFT between a biosurfactant brine and Daqing degassed oil at 45°C.

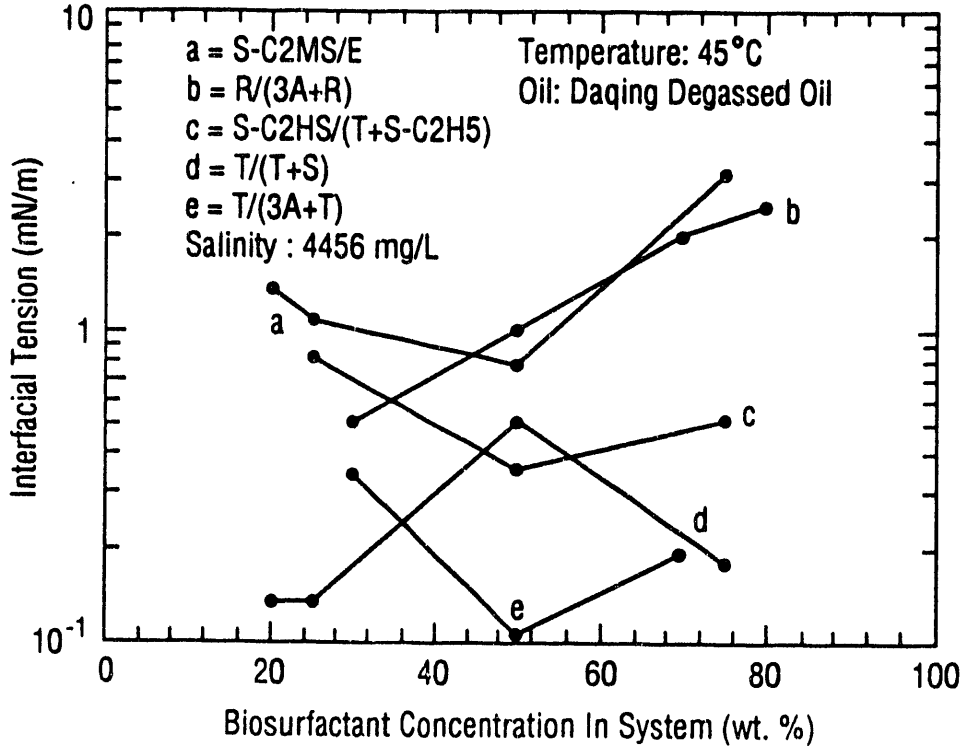


Figure 4. IFT between a biosurfactant system and Daqing degassed oil at 45°C.

Interfacial tension between the mixture and the oil can fall to 0.2 mN/m when trehalolipids are 75 wt% in mixed biosurfactants. If trehalolipids are mixed with petroleum sulfonate 3A as 50 wt% in mixed surfactants, the interfacial tension was lowered to 0.1 mN/m (Figure 4); it did not decrease further even though the ratio of trehalolipids/sophorolipids-C_2H_5 or trehalolipids/petroleum sulfonate 3A was changed.

3.4. The compatibility of the combination of biosurfactant-alkaline-polymer with the Daqing degassed oil with low acidity

In general, the interfacial activity of a biosurfactant will be markedly improved if an alkaline is added into the system (Figure 5). For the mixture of sophorolipids and trehalolipids, for example, the IFT between the mixture and oil with low acidity could be decreased to 0.03 mN/m if 0.75 wt% NaOH is added when total concentration of biosurfactants is 0.4 wt%. For the combination of trehalolipids and NaOH, it can reach to 0.035 mN/m at 1.0 wt% NaOH and 0.5 wt% trehalolipids.

For combinations of trehalolipids and petroleum sulfonate 3A, the interfacial tension between the mixture with 0.5 wt% NaOH and Daqing degassed oil is 0.006 mN/m; at the Daqing reservoir, surfactant concentration in mixture is 0.4 wt%, the average reservoir temperature is 45°C, and the average salinity of the formation water is 4456 mg/L. At 1.0 wt% NaOH the interfacial tension was lowered to 0.0015 mN/m. This fact not only suggests that the interfacial

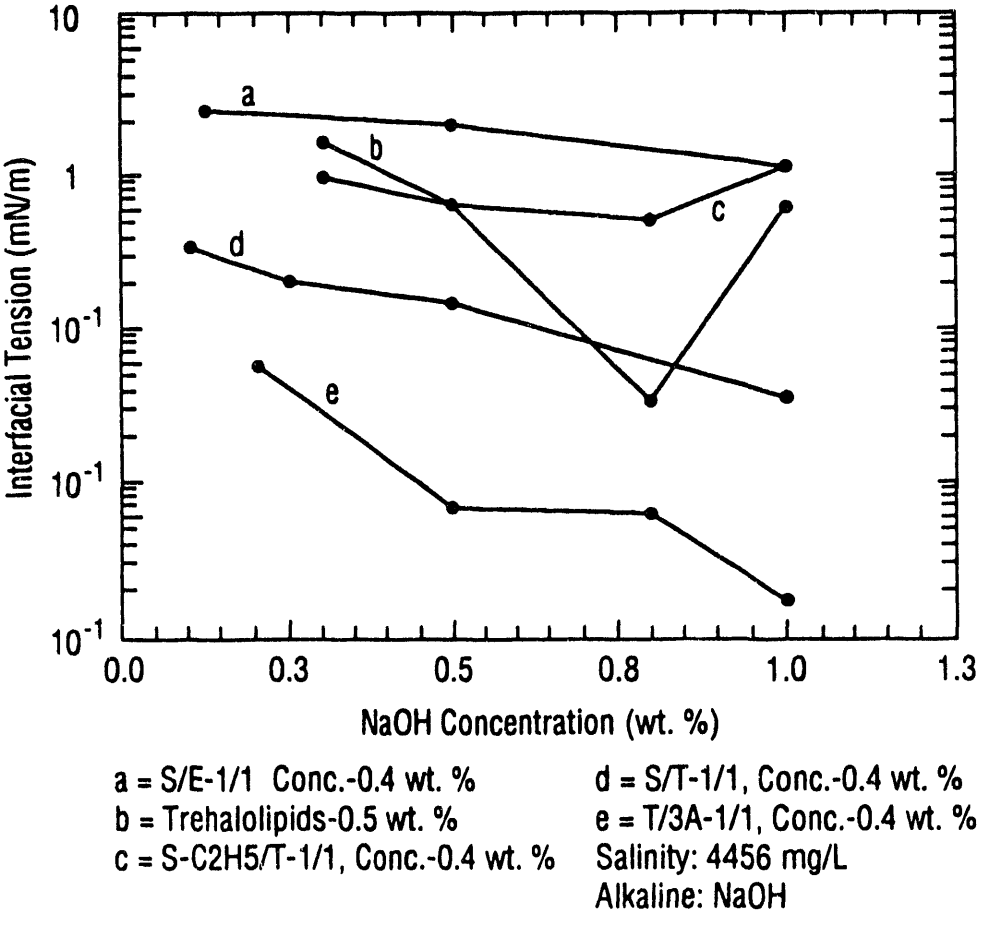


Figure 5. IFT between alkaline and Daqing degassed crude oil at 45°C.

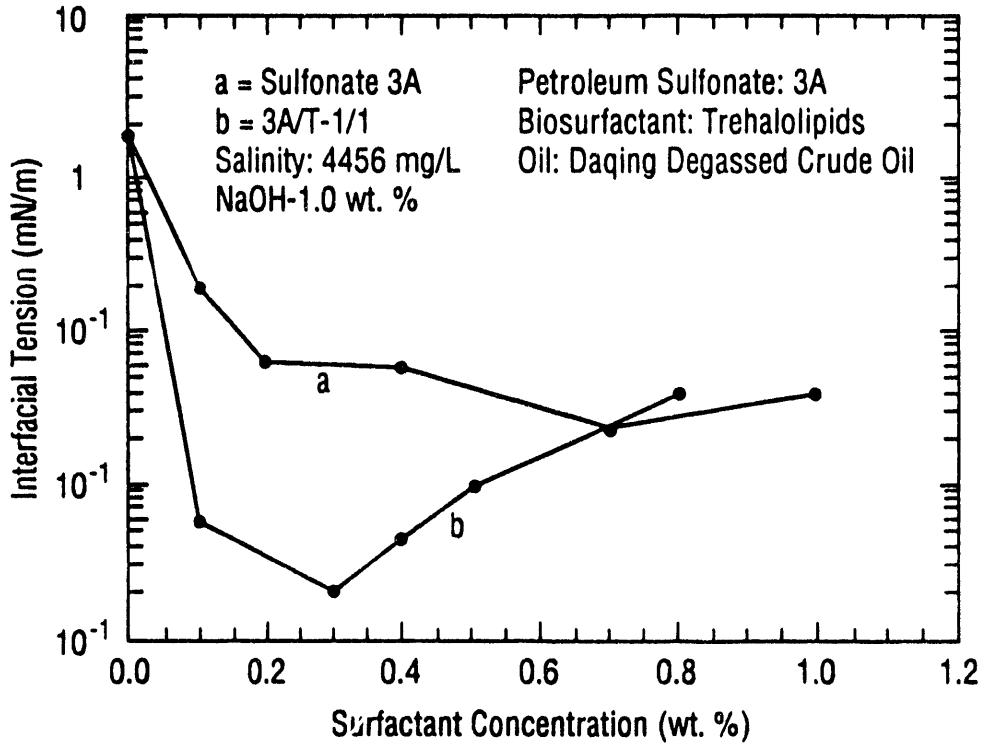


Figure 6. Effect of trehalolipids on IFT at 45°C.

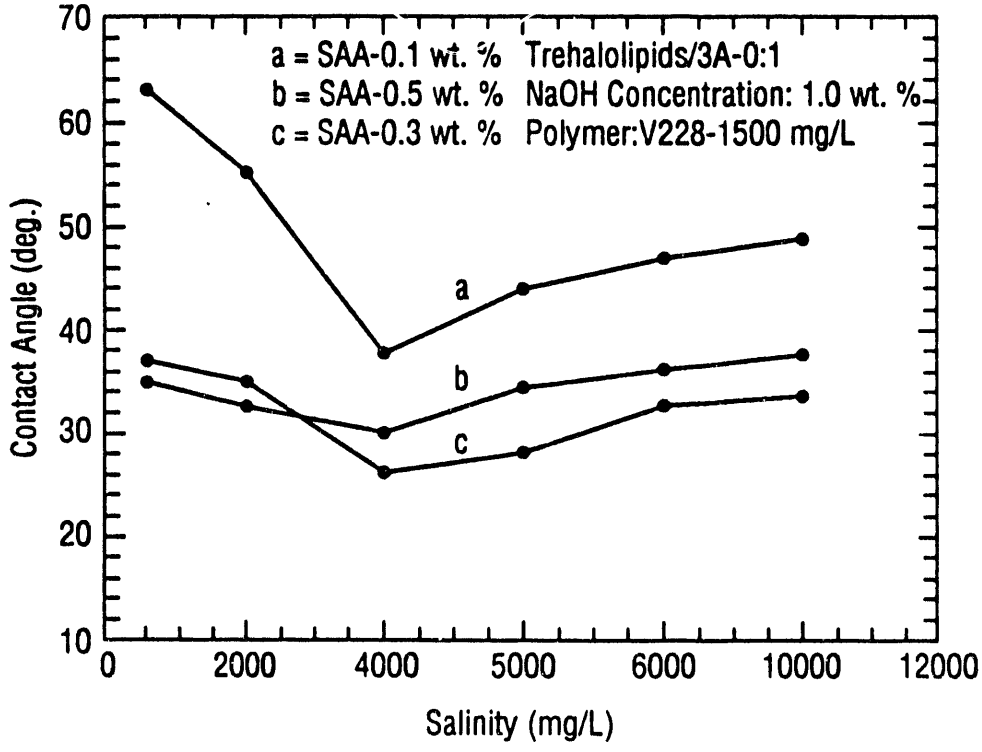


Figure 7. Effect of SAA concentration on contact angle for ASP at 45°C.

activity of a degassed oil with low acidity can be improved if an alkaline is added but also shows that trehalolipids and sulfonate 3A act synergistically, so that interfacial tension could be decreased into the ultralow range.

To go a step further, to explain the action of a biosurfactant in a mixed system, the effect on interfacial tension of a combination of trehalolipids/3A-alkaline were compared with that of the combination of 3A with alkaline (Figure 6). The interfacial tension decreased with an increase of petroleum sulfonate 3A and reached a minimum, 0.022 mN/m, at 0.70 wt% 3A while the minimum was further lowered to 0.002 mN/m as trehalolipids were added into the combination at 0.26 wt% of the total concentration of surfactant. This result implies that interfacial tension could be further lowered as a consequence of favorable synergism of trehalolipids and petroleum sulfonate.

3.5. The effect of Alkaline Surfactant Polymer (ASP) system with biosurfactant on wettability

For this experiment a glass matrix was rinsed in turn by a detergent, propanone, and distilled water before it is staved. Then it was well covered with a silicon oil with methylic hydrogen to change its oil wettability. Next, the matrix was heated in a positive temperature gradient until the ambient temperature rose to 180°C; the matrix was kept at 180°C for 24 hours. A model

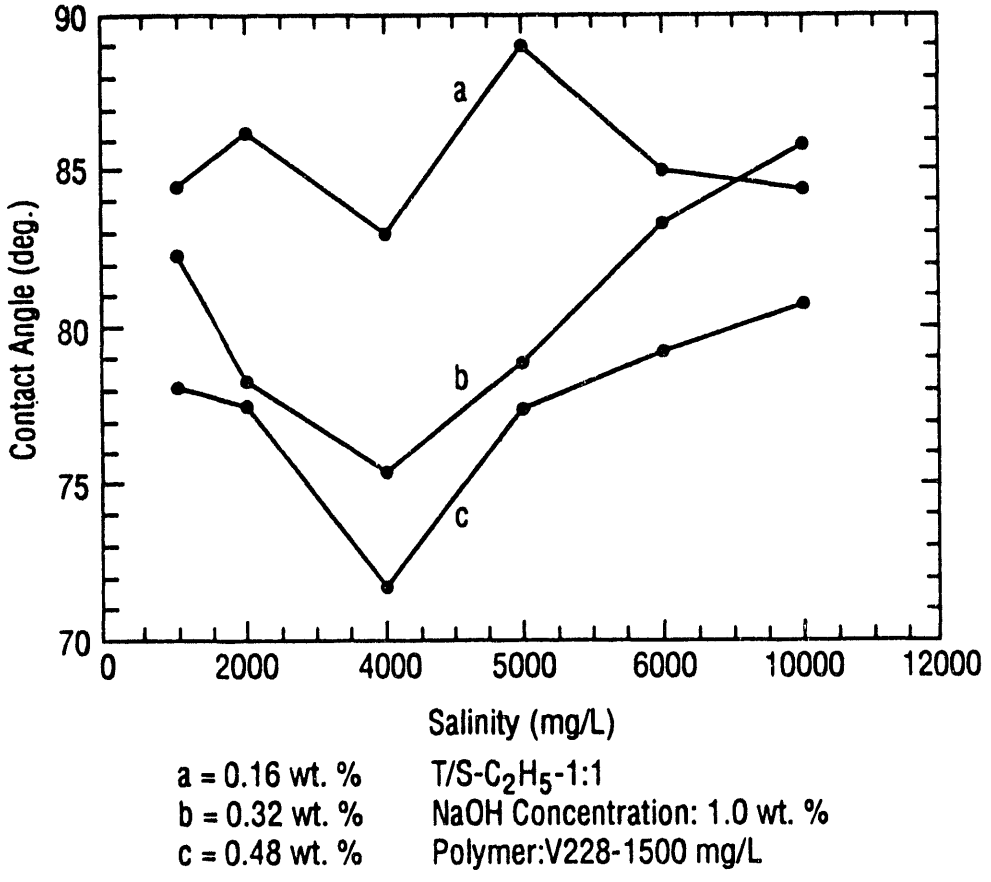


Figure 8. Effect of biosurfactant concentration on contact angle for ASP at 45°C.

CA- A contact angle meter was used to determine the contact angle between the mixture with the biosurfactant droplet and the glass matrix. The results are shown in Figure 7 and Figure 8. Figure 7 demonstrates that the contact angle decreases with an increase of salinity when the salinity is less than 4000 mg/L (the minimum is at 4000 mg/L). After that, the angle rises gradually. This figure also shows that the contact angle could reach a minimum at 0.3 wt% of total concentration of surfactant. This result is consistent with that of minimum interfacial tension which resulted from the salinity requirement diagram (SRD). Figure 8 shows that the concentration of trehalolipids/sophorolipids-C_2H_5 affects the contact angle. The angle diminishes with an increase of the total concentration of biosurfactant.

3.6. The salinity requirement diagram for the biosurfactant combination of ASP

To derive the optimum formulation to be used for oil displacement, the physical and chemical changes that occur when the chemical slug moves through porous medium must be carefully studied; such data could also explain the phenomena occurring during oil displacement because the changes in interfacial tension between a chemical slug and oil usually result from the variations in salinity and in a drop in chemical concentration due to dilution. This problem is dealt with in the salinity requirement diagram.

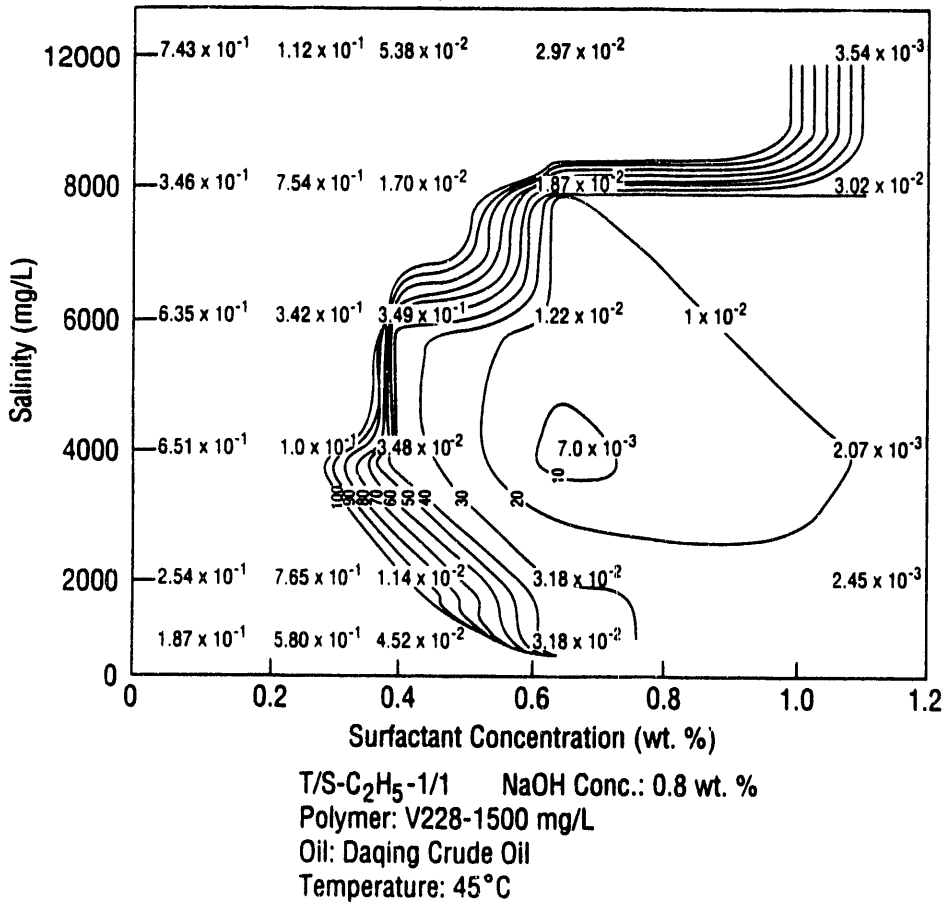


Figure 9. Salinity requirement diagram for ASP of T/S-C_2H_5.

The salinity requirement diagram for the combination of trehalolipids/sophorolipids-C_2H_5 shows that the ultralow interfacial tension of 10^{-1} mN/m, occurs at biosurfactant concentrations which vary from 0.7 to 1.0 wt%, and 3000 to 5500 mg/L in salinity (Figure 9), while for the combination of trehalolipids/sophorolipids-alkaline-polymer, the ultralow value occurs from 0.1 to 0.6 wt% surfactant concentration, and 0.0 to 12000 mg/L in salinity (Figure 10).

3.7. Evaluation of oil displacement of the ASP system on Daqing reservoir core

The coreflooding tests to evaluate the combination of biosurfactant-alkaline-polymer were implemented on the column cores from Daqing reservior (250 mm long and 40 mm in diameter) in a negative salinity gradient.

The procedure for coreflooding is as follows. The core is waterflooded to build up the residual oil. In general, this process can be ended when the water cut reaches 98% while about 2 PV of injection water is pumped. After that the chemical slug can also be ended followed by the injection water until the water cut reaches 98% while the injection is about 2 PV.

The chemical slug, which is prepared with injection water is composed of:

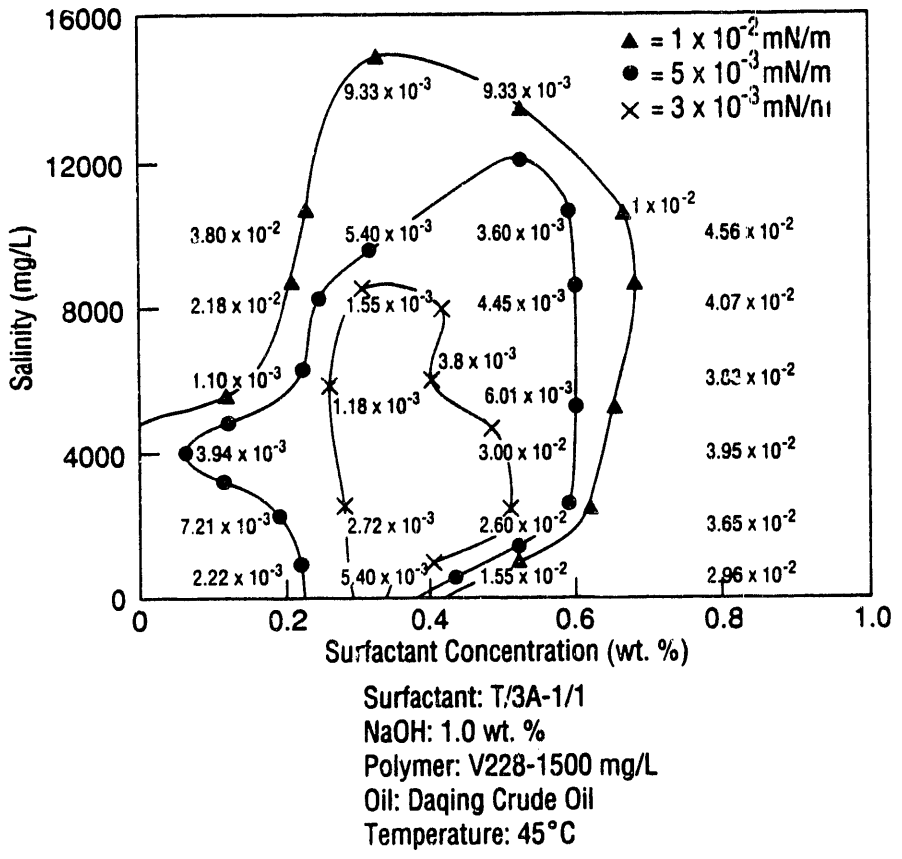


Figure 10. Salinity requirement diagram for ASP.

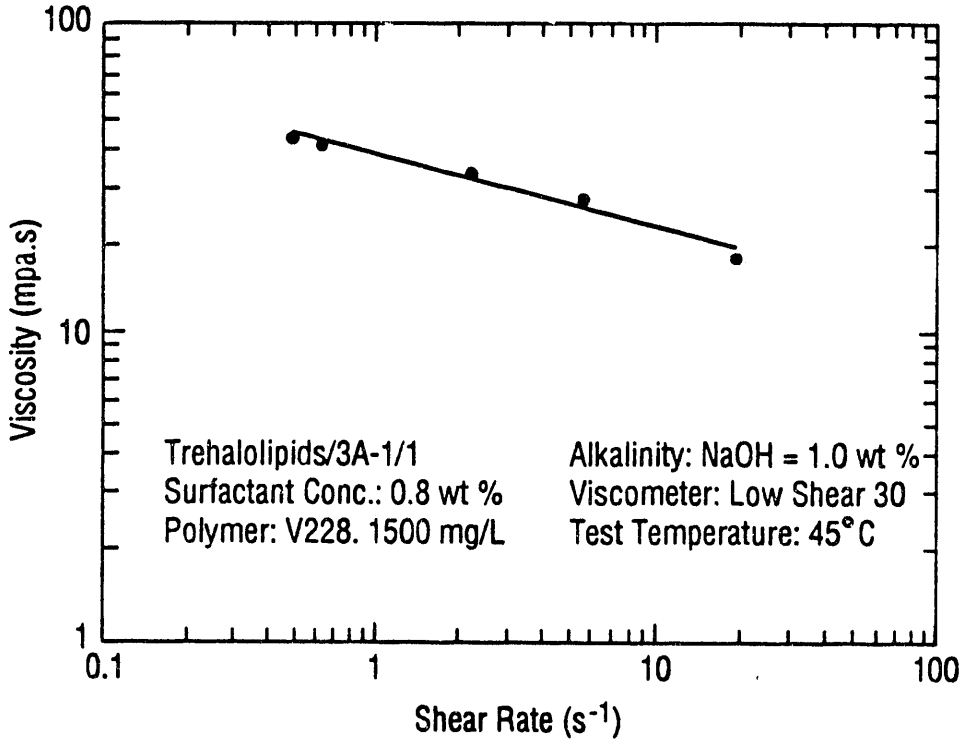


Figure 11. Function of viscosity as shear rate for the ASP system.

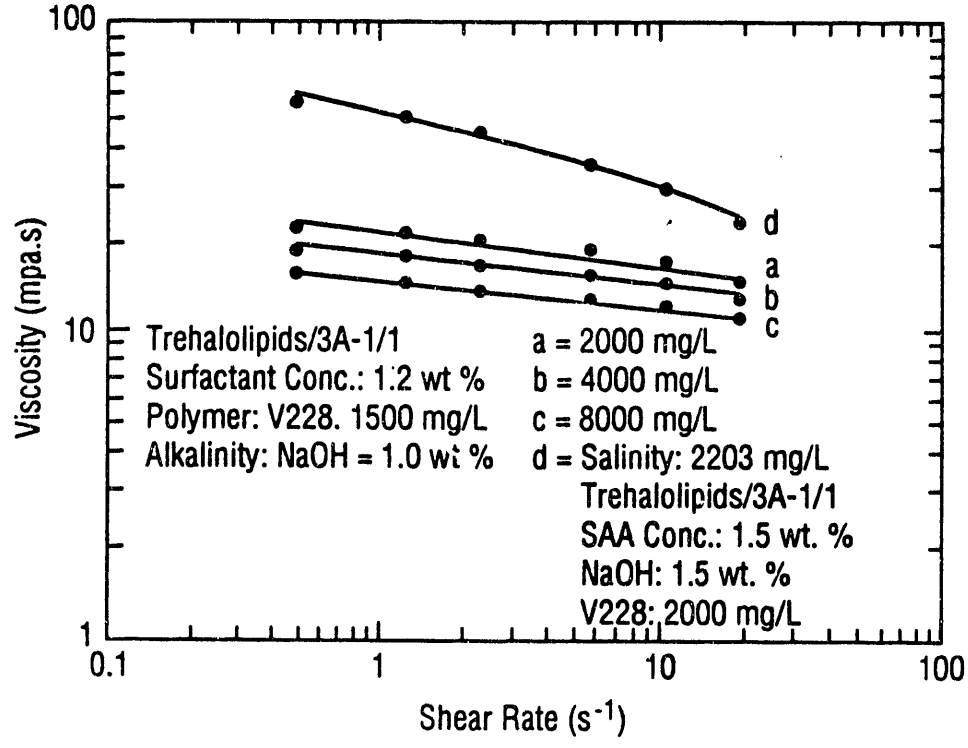


Figure 12. Effect of salinity on viscosity for ASP system at 45°C.

Trehalolipids/Sulfonate 3A: 1/1
Total surfactant concentration: 0.80 wt%.
NaOH: 1.0 wt%
Polyacrylamide V228: 1500 mg/L

The rheology of the slug is shown in Figure 11.

It is apparent that 22 mpa.s and shear rate is 10 s^{-1}. Figure 12 shows the sensitivity of the chemical slug to the salinity of the reservoir water. It illustrates that the viscosity decreases slightly with the increase of shear rate, but strongly with the increase of the salinity. The viscosity is 14.6 mpa.s at 4000 mg/L in salinity and the shear rate is 10 s^{-1}.

Coreflooding Well 901 (Figures 13 and 14) demonstrates that the oil recovery is 11.3% (OOIP) while the water cut drops from 98% to 74% for the combination of trehalolipids/sulfonate 3A-alkaline-polymer. The oil bank does not occur during displacement.

During coreflooding, the pressure drop across the core was monitored with transducers. They showed that the pressure gradient fell rapidly at 0.7 PV injection, and the control of mobility was not favorable.

After coreflooding, the effluents were analyzed including the content of alkaline, surfactant, and polymer while the interfacial tension between oil and effluent was measured. The latter reached 4-5 mN/m, although the concentration of surfactant is very low. This finding demonstrates that a little surfactant still remains in the effluent and that the surfactant cannot be adsorped thoroughly. Next, the two-phase titration method was used to determine the amount of petroleum sulfonate [7]. The alkaline concentration in the effluent shows that a maximum was reached at 1.0 PV. After that, the concentration started to decrease. The result agrees with that of Schuler [8]. The results for the polymer content are the same as those for the alkaline content with a maximum concentration in effluent of 390 mg/L.

Coreflooding Well No. 902 (Figures 15 and 16) shows that oil recovery could be enhanced by 11.3% (OOIP) while the water cut could be decreased from 98% to 74%. The minimum interfacial tension between effluent and oil produced is 0.009 mN/m at about 1.2 PV injection; the lowest value was observed after the chemical slug was broken through. The maximum concentration of petroleum sulfonate in the effluent analyzed was 800 mg/g at 1.2 PV.

4. CONCLUSIONS

1. If a biosurfactant is added to a brine, the surface tension can be lowered. For example, the surface tension of the rhamnolipids brine (4456 mg/L in salinity) could be lowered to 27 mN/m, and the CMC is 0.0016 wt%. For the mixture of sophorolipids/trehalolipids, the value is 27 mN/m and the CMC is 0.0015 wt%. At the Daqing reservoir, where salinity is 4456 mg/L, and temperature is 45°C, the interfacial tension between a biosurfactant brine and Daqing degassed oil varies from 0.4 to 0.6 mN/m.

2. For combinations of a biosurfactant with the others or with a petroleum sulfonate, two favorable formulations were obtained, in which trehalolipids were combined with sophorolipids or with sulfonate 3A. The interfacial tension between both formulations and Daqing degassed oil can drop to 0.1 mN/m.

3. The interfacial tension between the Daqing degassed oil and the combination of trehalolipids and NaOH can be reduced to 0.03 mN/m at 0.8 wt% NaOH. The combination of trehalolipids with petroleum sulfonate 3A evidently acts synergistically. At 0.5 wt% NaOH, the interfacial tension between the

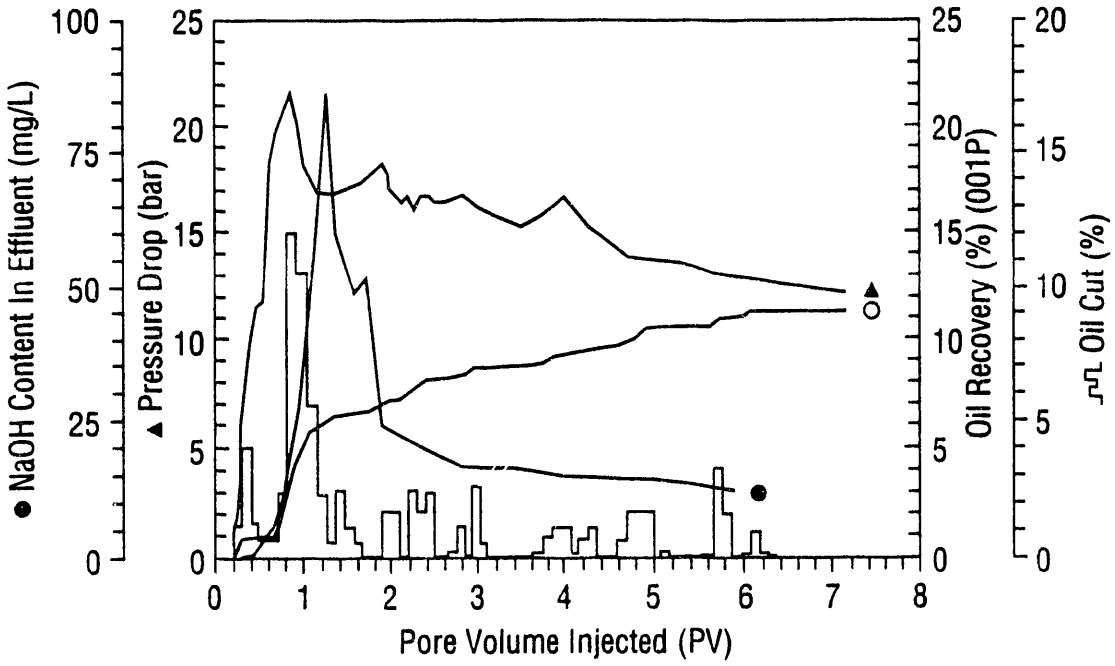


Figure 13. Oil displacement data on ASP on Daqing Reservoir core.

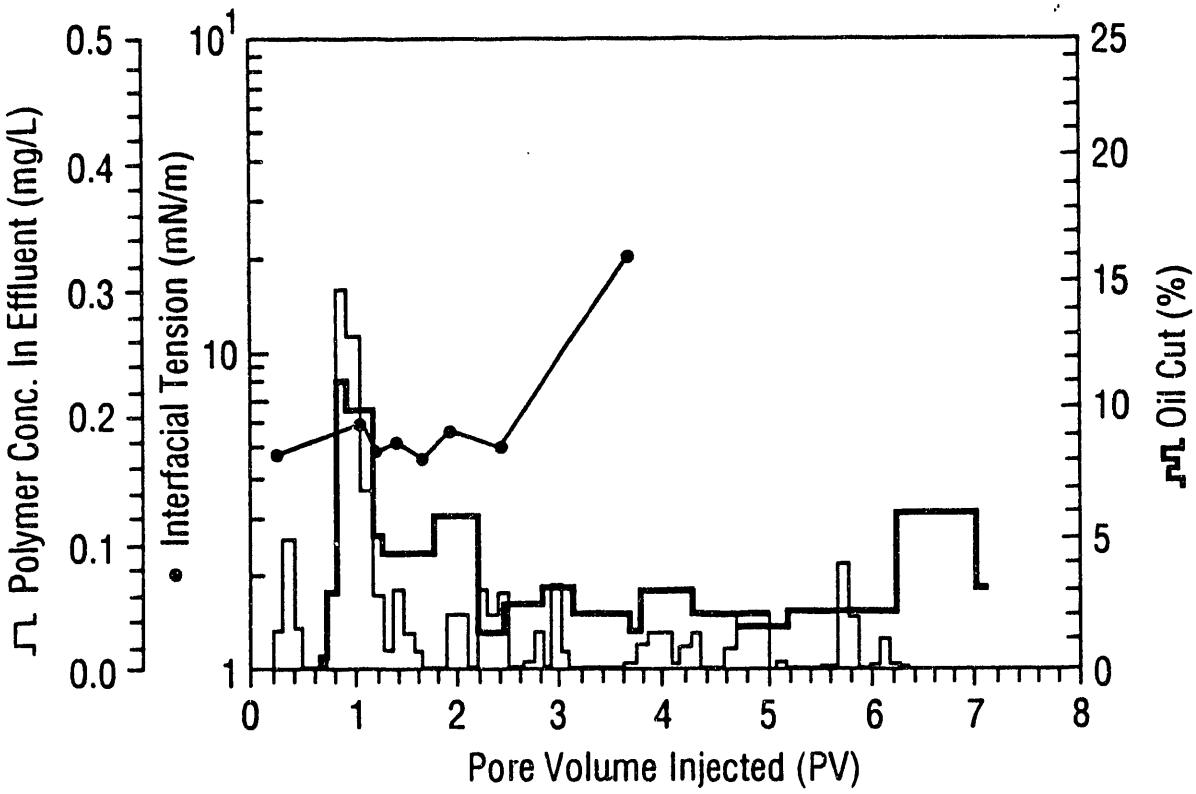


Figure 14. Oil displacement data on ASP on Daqing Reservoir core.

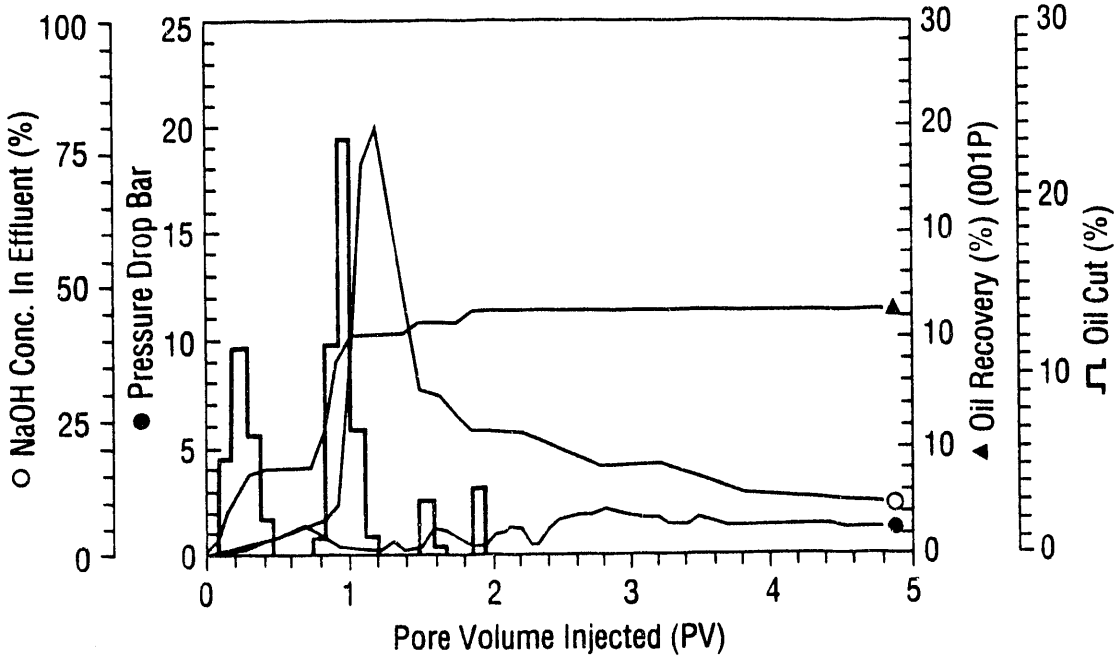


Figure 15. Oil displacement on Daqing Reservoir core for ASP.

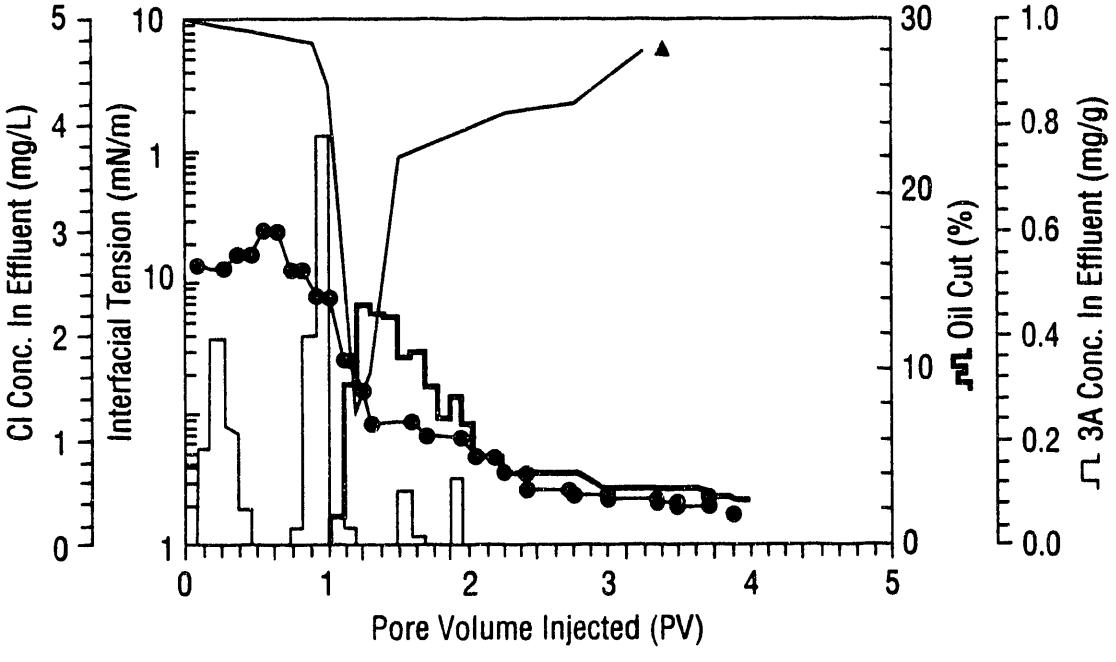


Figure 16. The oil displacement data on ASP on Daqing reservoir core.

combination and oil with low acidity could be lowered to 0.006 mN/m, and to ultralow values when total concentration of surfactant is 0.4 wt%. Similarly, the value is 0.002 mN/m at 1.0 wt% NaOH. The ultralow interfacial tension, 10^{-3}mN/m of the latter can be obtained when the concentration of surfactants varies from 0.2 to 0.6 wt% and salinity from 0 to 1500 m g/L.

4. For the combination of trehalolipids/sulfonate 3A-alkaline polymer, oil recovery was enhanced by 11.3% (OOIP) while the water cut decreased from 98% to 74% at the Daqing reservoir core. The retention of surfactant was less than 1 mg/g core.

5. ACKNOWLEDGEMENTS

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Comparative Analysis of Microbially Mediated Oil Recovery By Surfactants Produced by *Bacillus licheniformis* and *Bacillus subtilis*

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Abstract

Two strains of *Bacillus subtilis* (ATCC 21331 and ATCC 21332) were investigated and compared to *Bacillus licheniformis* JF-2 (ATCC 39307) in the following respects: ability to lower interfacial tension, degrade oil, adhere to hydrocarbons, recover oil, and grow on carbon sources suitable for field application. The different carbon substrates were evaluated based on reduction in surface tension. Both *B. subtilis* strains decreased the surface tension when sucrose, fructose, starch, pyruvate, casitone, glycerol, or yeast extract were used as carbon substrates. Starch was the best carbon substrate for surfactant production.

Cell-free extracts of *B. subtilis* grown in medium E (supplemented with 2.5% NaCl and 1% sucrose) recovered Schuricht crude oil from cores in the laboratory. Interfacial tension (IFT) of *B. licheniformis* JF-2 extracts against Schuricht crude oil was 23.1 mN/m. IFT resulting from *B. subtilis* 21331 under identical conditions was 4.3 mN/m.

1. INTRODUCTION

The microbially enhanced oil recovery (MEOR) research program at the Idaho National Engineering Laboratory (INEL) is focused on fundamental MEOR research and field implementation. The INEL MEOR program involves (1) elucidating microbial mechanisms responsible for oil displacement, (2) developing microbial systems with known recovery mechanisms, (3) applying MEOR systems designed specifically for reservoirs containing moderate/heavy weight crude oils (19.1 to 38.1° API [0.936 to 0.834 g/cm^3 @ 15.6°C]) in industry cost-shared field demonstrations, and (4) controlling microbially mediated souring (biogenic reduction of sulfate).

Approximately 33% of U.S. oil in place will be produced, leaving the remaining 67% for advanced secondary and enhanced recovery technologies [1], which include thermal, chemical, and miscible/immiscible fluid flooding [2]. Microbial flooding, included in the general category of chemical flooding encompasses acid, gas, polymer, solvent, and surfactant flooding techniques, where the flooding agents are generated from fermentation end products. The focus of this paper is a comparative analysis of microbially mediated oil recovery by biosurfactants produced by *B. licheniformis* JF-2 (ATCC 39307) and *B. subtilis* (ATCC 21331 and ATCC 21332) grown on various sources of carbohydrates.

Surfactants are hypothesized to facilitate oil recovery by reducing the interfacial tension (IFT) between the organic and aqueous interfaces. A reduction decreases the pressure required to release oil trapped in the rock pores by capillary forces, which displaces oil from the pores into the mobile liquid phase. Microbially mediated reductions of IFTs have been reviewed by others [3-5].

Historically, molasses has been the feedstock of choice for the *in situ* production of biosurfactants [6]. We propose using alternative carbon substrates (i.e., processing wastes from the agricultural industry) to replace molasses. These wastes are currently disposed of at a cost, and could be employed as viable feedstocks for production of biosurfactants.

2. MATERIALS AND METHODS

2.1. Microbial strains

B. licheniformis (ATCC 39307) and *B. subtilis* (ATCC 21331 and 21332) were obtained from the American Type Culture Collection (ATCC). The organisms were stored according to ATCC protocols [7].

B. licheniformis is a Gram positive, facultatively anaerobic, spore-forming rod, approximately 0.7 by 2.0 μm (width by length) [8]. The organism is capable of using starch as a carbon source, has a maximal growth temperature of 50°C, tolerates salt (as NaCl) to 10% (highest value tested), grows well from pH 4.5 to 8.5, does not adhere to hydrocarbons as determined by the Bacterial Adhesion to Hydrocarbons (BATH) assay [9], and does not degrade Schuricht crude oil.

B. subtilis is a Gram positive, spore-forming rod that also is capable of using starch as a carbon source. Most strains of *B. subtilis* are considered aerobic bacteria; however, both ATCC 21331 and 21332 were observed to be facultative anaerobes. *B. subtilis* 21331 will grow to 50°C and *B. subtilis* 21332 will grow to 55°C. Both strains tolerate NaCl to 10% and are roughly the same size as *B. licheniformis*. Neither strain of *B. subtilis* adheres to hydrocarbons and both slightly degrade Schuricht crude oil.

2.2. Crude oil

Schuricht crude oil from the Minnelusa formation in the Powder River Basin of Wyoming was selected for experimentation. Schuricht crude is 25.4° API, has a viscosity of 0.054 Pa•s at 23°C, and a density of 0.9081 g/cm^3 at 15.6°C. The Schuricht well is on primary production and has not soured. Potential application of MEOR technologies in the Powder River Basin was discussed [10].

2.3. Growth conditions

Three media were used for microbial cultivation: (a) medium E (ATCC 1502) supplemented with 2.5% NaCl and 1% carbon source [11], (b) Trypticase Soy Broth (TSB), and (c) Potato Dextrose Yeast (PDY) (ATCC 336) [12]. Carbon sources used to supplement medium E to determine the feasibility of using alternative carbon substrates were sucrose, fructose, galactose, dextrose, molasses, starch, glycerol, pyruvate, acetate, citrate, casitone, and yeast extract. All carbon substrates were 1% (v/v), except starch (0.5% v/v). Cultures were initiated with a 1% inoculum of fresh culture. The cultures were incubated in shake flasks at 30°C until the stationary phase of growth (≈72 hours). Optical density was monitored at 550 nm. After 72 hours of growth, cells were removed by centrifugation (6,000 x g, 20 min, 10°C).

2.4. Microbial physiology

Growth curves were derived for all microorganisms. Cells were inoculated into TSB and incubated at 37°C. Samples for optical density and surface tension were obtained every 12 hours.

Optimal growth temperature was determined by incubating cultures containing TSB at five temperatures (22°, 30°, 35°, 45°, and 55°C). Optical density was

monitored for each flask and the growth rate was calculated. Surface tensions of the final (72-hour) cell-free extracts were measured.

2.5. Interfacial tension measurements

Interfacial tensions (liquid-liquid) and surface tensions (air-liquid) were measured by the inverse pendent drop method using an interfacial tensiometer designed at the INEL [13]. NANO-pure water was used to calibrate the system and measurements were taken in replicates of 7. The reported values are expressed as the means and standard deviations. All IFT measurements were made at room temperature.

2.6. Coreflood procedure

Berea sandstone cores 1 inch in diameter by 6 inches long, coated with epoxy and fitted with endplates were used for coreflood experiments. Cores were flushed with nitrogen and then saturated with a 2.5% NaCl/0.5% $CaCl_2$ degassed brine. Schuricht crude was injected into the cores. Initial water (S_{wi}) and oil (S_{oi}) saturations were determined, and the cores were waterflooded (15 pore volumes [PV]) to residual oil saturation (S_{orwf}). One PV of cell-free extracts, prepared from 72-hour incubation in medium E and sucrose, was injected into each waterflood residual core followed immediately by a 15 PV waterflood. Three cores were injected with cell-free supernatants. A control core (sterile medium E) also was performed. Surface tensions of the cell-free extracts were measured before injection into the cores. All coreflood experiments were carried out at ambient temperature and pressure. Flow rates were 5 ft/day. Oil recoveries as percent original oil in place (%OOIP) and percent residual oil in place (%ROIP) were determined.

3. RESULTS AND DISCUSSION

3.1. Growth curve

Figure 1 shows the absorbance (growth) and surface tension (surfactant production) plotted versus time for *B. subtilis* 21332. Data for TSB medium indicate a typical growth curve having lag, log, and stationary growth phases. Surfactant production was directly proportional to cell growth; as the cell density increased the surface tension decreased. The greatest decrease in surface tension was observed during log phase (within 24 hours). This curve shows that a 72-hour incubation is sufficient time for surfactant production in shake flask experiments. Surface tension dropped from 57.10 mN/m to 30.17 mN/m within the first 24 hours and stabilized over the next 48 hours to a value of 28.49 mN/m. Data (not shown) indicate similar trends for *B. subtilis* 21331; the surface tension dropped from 57.10 mN/m to 29.91 mN/m in 72 hours. The largest decrease in surface tension occurred during the log phase. Likewise, *B. licheniformis* showed a decrease in surface tension within 24 hours. However, the surface tension for *B. licheniformis* did not drop as low as the *B. subtilis* strains (57.10 to 45.65 mN/m).

3.2. Temperature effects on surfactant production

The growth rate (absorbance + time) for each organism was determined and plotted versus temperature to determine optimal growth temperature. The results indicate that 35°C is optimal for all strains (Figure 2). It was hypothesized that maximum surfactant production would occur at the optimal growth temperature.

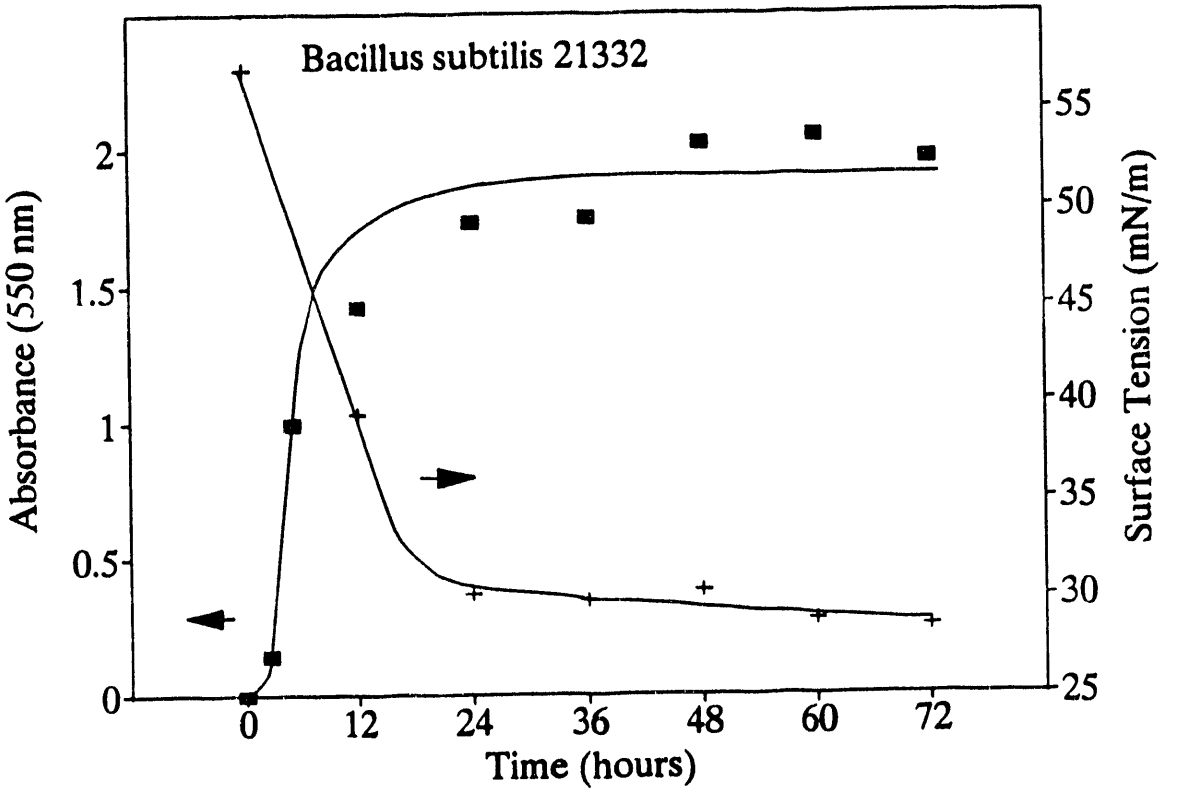


Figure 1. Reduction of surface tension (▪) as a function of cell growth (+).

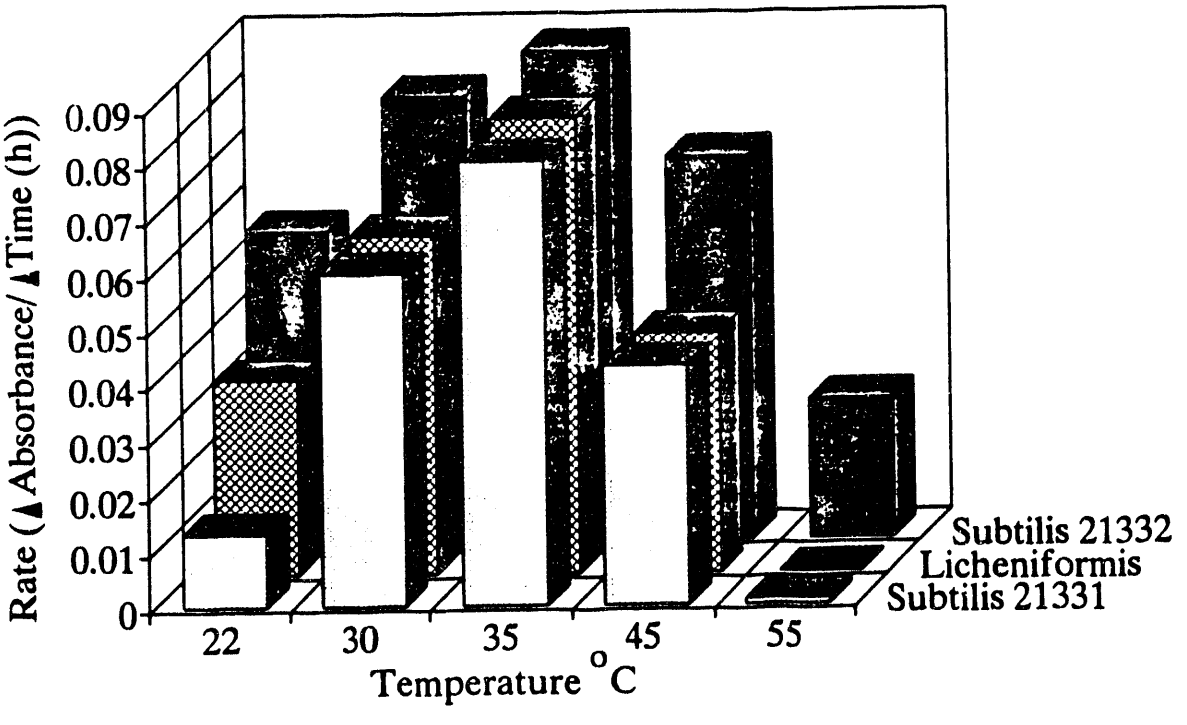


Figure 2. Growth rate as a function of temperature.

Table 1
Interfacial tensions between medium E, trypticase soy broth, and potato dextrose yeast medium cell extracts and Schuricht crude oil (n = 7)

Media	Abiotic Control	*Bacillus licheniformis*	*Bacillus subtilis* 21331	*Bacillus subtilis* 21332
Medium E	32.37 ± 0.07	23.10 ± 0.07	4.24 ± 0.31	18.03 ± 0.18
TSB	18.72 ± 0.24	7.65 ± 0.32	2.73 ± 0.12	7.71 ± 0.14
PDY	17.18 ± 0.15	9.12 ± 0.03	14.24 ± 0.05	8.07 ± 0.13

However, when surface tension is plotted against temperature (Figure 3), it appears that this is not true for all strains. *B. subtilis* strains 21331 and 21332 produce maximum surfactant at 35°C. However, surfactant production by *B.licheniformis* appears to be better at 22°C.

3.3. Utilization of different carbon sources

Figure 4 shows reductions in surface tension as a result of growth on various carbon substrates for *B. licheniformis*, *B. subtilis* 21331, and *B. subtilis* 21332. Growth was observed on all carbon sources. Yeast extract was the only suitable carbon source for a reduction in surface tension by *B. licheniformis*. However, growth of *B. subtilis* 21331 on sucrose, fructose, starch, pyruvate, casitone, or yeast extract resulted in surface tensions lower than 27 mN/m. Likewise, *B.subtilis* 21332 facilitated a significant decrease in surface tension when sucrose, fructose, starch, glycerol, pyruvate, casitone, and yeast extract were the carbon sources. The reduction in surface tension indicates at least six potential carbon substrates for surfactant production.

Although the lowest IFT was observed using the complete TSB medium (*B. subtilis* 21331, 2.73 mN/m), the decrease noted for minimal medium E with the same organism was 4.24 mN/m. This reduction in surface tension represents an 87% reduction in medium E versus 85% in TSB. Interfacial tensions for *B. subtilis* 21332 grown on PDY medium dropped from 17.18 mN/m to 8.07 mN/m. The data supports the idea of using starchy wastes from the potato processing industry as viable carbon substrates for surfactant production.

Table 1 shows IFTs between media E, TSB, and PDY cell-free supernatants of all strains and Schuricht crude oil. Sucrose was chosen because it represents the major carbohydrate constituent of beet molasses. Sucrose represents 63.5% of total solids in beet molasses and 95.4% of all carbohydrate present [11], TSB was selected as a complete growth medium for comparison, and PDY was chosen based on the results of Figure 4.

3.4. Oil recovery potential

Table 2 shows results from coreflood experiments. The surface tension of *B.licheniformis* cell-free extract before injecting into the core was 66.94 mN/m. The surface tensions for *B. subtilis* 21331 and 21332 extracts were 28.89 mN/m and 28.45 mN/m, respectively. The surface tensions reported are not the lowest measured values for these organisms, and these results suggest that the lower the surface tension, the higher the oil recovery. Perhaps the oil recovery would have been increased if the optimal carbon source was used for this coreflood experiment. One could expect that if yeast extract was used as the carbon

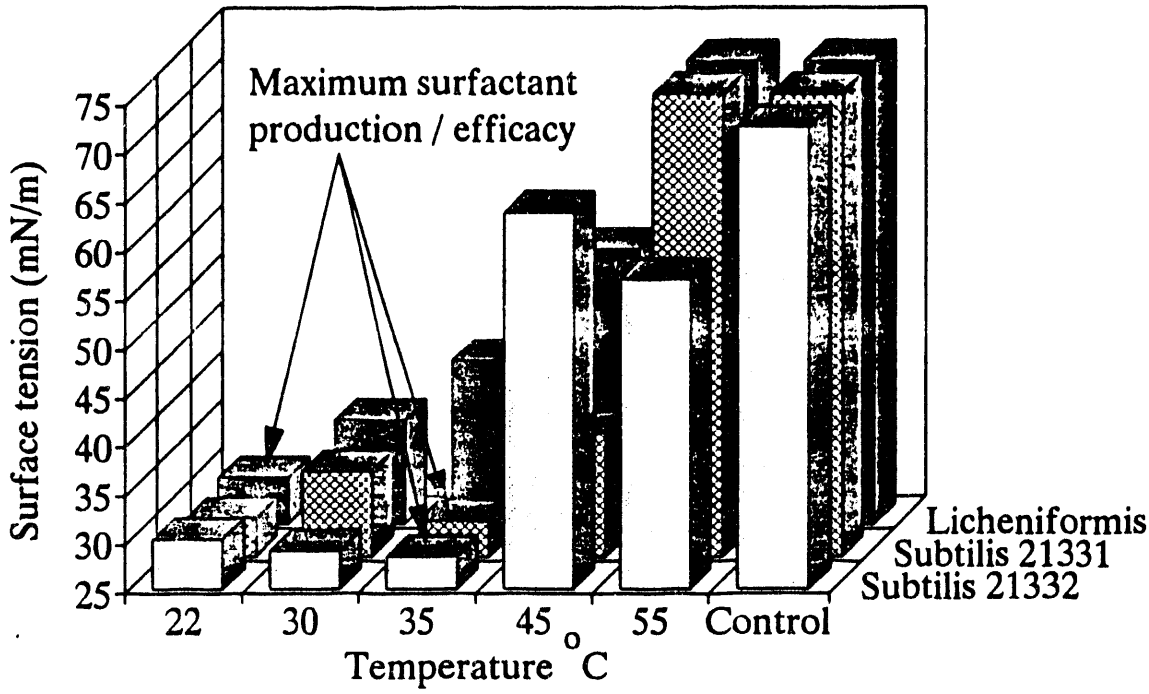


Figure 3. Surface tension as a function of temperature in 72 hour cultures.

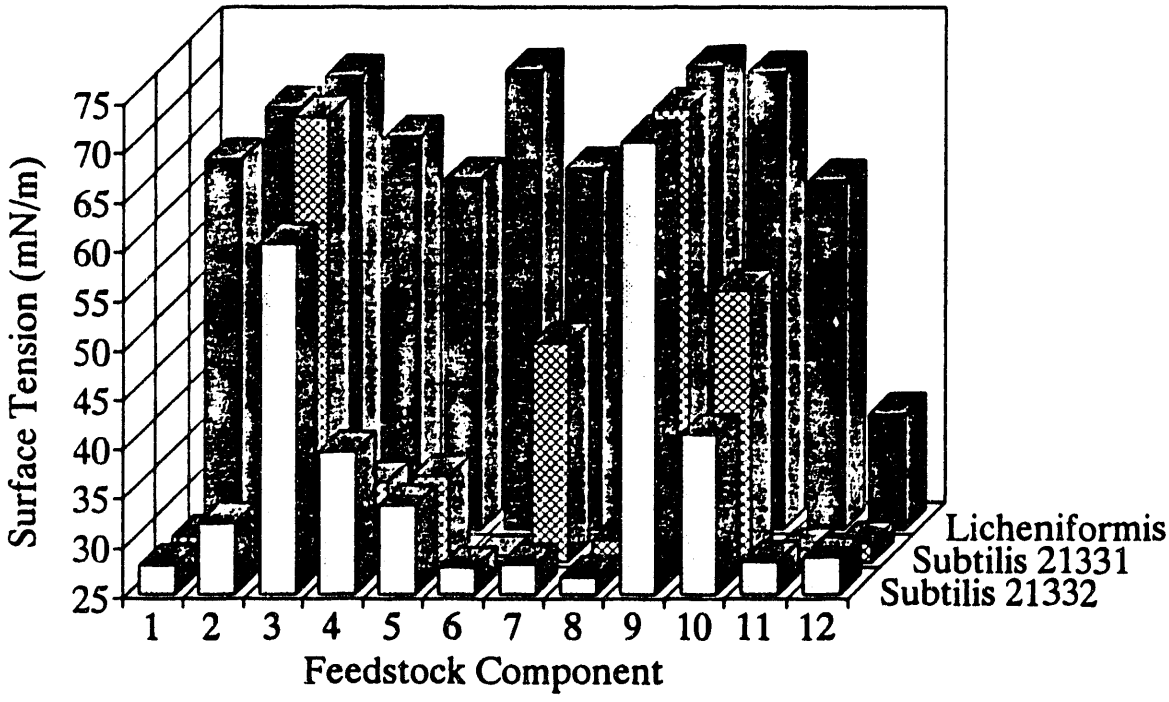


Figure 4. Reductions in surface tension as a result of growth on various carbon substrates. Substrates are 1) sucrose, 2) fructose, 3) galactose, 4) dextrose, 5) molasses, 6) starch, 7) glycerol, 8) pyruvate, 9) acetate, 10) citrate, 11) casitone, and 12) yeast extract. Control values for all substrates were ≈71 mN/m except casitone (≈65 mN/m) and yeast extract (≈68 mN/m).

Table 2
Oil recovery mediated by 1 PV of cell-free extracts

Organism	Surface Tension (mN/m)[a]	% Original Oil in Place	% Residual Oil in Place
Abiotic control	72.02	2.5	3.7
B. licheniformis	66.94	0.2	0.5
B. subtilis 21331	28.89	3.8	5.4
B. subtilis 21332	28.45	4.3	6.9

a) Medium E extract

source, oil recovery by *B. licheniformis* would have been greater. Similarly, if potato starch was used to cultivate the *B. subtilis* strains, the oil recovery may have increased. Experiments are underway to investigate this hypothesis.

4. CONCLUSIONS

This study indicates that surfactant production is directly proportional to cell growth for *B. licheniformis* and *B. subtilis* 21331 and 21332. Surfactant production by *B. licheniformis* and *B. subtilis* 21331 and 21332 is optimal using specific temperatures and carbon sources. Carbon substrates that are suitable for production of surfactants by *B. subtilis* are sucrose, fructose, starch, glycerol, casitone, and yeast extract. Starch is the best carbon substrate for production of surfactants by *B. subtilis* 21331 and 21332.

5. ACKNOWLEDGEMENTS

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Noninvasive Methodology to Study the Kinetics of Microbial Growth and Metabolism in Subsurface Porous Materials

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Abstract

The effect of pore size on the growth of *Escherichia coli* through anaerobic, nutrient-saturated cores packed with different sizes of spherical glass beads (75-150 m to 710-1180 m) under static conditions was determined by following, noninvasively, the rate of gas production. The rate of hydrogen production and the final amount of hydrogen produced decreased with a decrease in pore size. Kinetic parameters describing microbial processes were estimated by nonlinear regression using modified versions of the integrated Monod and Michaelis-Menten equations. This new mathematical approach accurately estimated kinetic parameters from ideal and experimentally obtained data sets. The data suggest that the reduced rate of bacterial growth observed in cores with smaller pore sizes may be due to a restriction of bacterial cell division.

1. INTRODUCTION

Mathematical approaches to predict microbial growth during microbial enhancement of oil recovery [1-3] or the movement of microorganisms and use of organic carbon in groundwater aquifers [4-6] assume that exponential growth of bacteria occurs as described by the Monod equation. However, the applicability of Monod kinetics to growth in subsurface formations has not been established. The structure and the size of pores in subsurface material could limit the availability of nutrients, which will influence microbial growth kinetics or possibly physically restrict bacterial cell division. In addition, the ability to obtain reliable kinetic parameter estimates is an important constraint limiting the accuracy of predictions from models for microbial processes. Standard methods for performing measurements to determine these kinetic parameters are impractical for use with porous materials. Parameter estimates derived from experiments in liquid cultures may not faithfully reflect rates occurring within pore spaces. Thus, it is important to develop a simple experimental system and the necessary mathematical approaches to verify whether exponential growth does occur in porous materials. Here, we describe a noninvasive method to measure microbial activity in porous media. The approach measures the kinetics of gas production by monitoring the change in pressure with a computer-controlled data collection system. Progress curves of the gas production are then analyzed by nonlinear regression analysis to obtain the kinetic parameter estimates.

Mathematical methodology was developed which permits the use of pressure measurements to assess microbial activity occurring within glass bead-packs. The newly developed methodology provided estimates of kinetic parameters needed to

quantitatively characterize microbial growth and metabolism. Estimated parameter values were obtained by converting pressure measurements into product concentrations, and then performing nonlinear regressional analyses using expressions for Monod and Michaelis-Menten kinetics. Kinetic parameters generated by analyses of data from laboratory models can be used to reliably predict microbial activity in porous materials.

2. MATERIALS AND METHODS

2.1. Bacterial strain and growth conditions

The chemotactic, motile, *Escherichia coli* strain RW262 (Genotype; F^+ *mel*-1 *tan*A sup F58 lambda$^-$) was grown anaerobically in motility growth medium (pH 7.0) as described previously [7]. Anaerobic media and solutions were prepared and used as described previously [8,9]. The gas phase of all anaerobically prepared media and solutions was that of the anaerooic chamber, about 1 to 5% H_2 with the balance being N_2.

2.2. Core preparation

Acid-washed glass beads (Sigma Chemical Co., Inc., MO) were used as the porous material. Five different sizes were used in this study. The glass core chamber (1.25 by 11 cm) was prepared and packed with one of the sizes of glass beads, as described previously [7].

2.3. *In situ* bacterial growth

The *in situ* production of the fermentation end product (H_2) was determined by monitoring the gas pressure of the glass bead-packed core with time. Duplicate core chambers packed with a given bead size were each inoculated with 0.1 ml (about 10^7) of exponentially growing culture. Core chambers filled with the growth medium, but not with glass beads, served as the positive controls.

The gas pressure of each core chamber was monitored by the method described by DeWeered et al. [10], except that the transducer system was modified to provide a 1 ml headspace between the core chamber and the pressure transducer. The pressure transducer (Omega Series PX136; Omega Engineering Inc., Stanford, CT) was attached to a 1 ml syringe, and the joint was sealed with a coating of epoxy to make the apparatus leak-free. An 18-gauge needle was attached to the other end of the syringe. The tip of the needle was bent manually at an angle of about 20° to prevent coring of the stopper and plugging of the needle by the glass beads. The syringe was then flushed with 100% N_2. The needle was sterilized with ethyl alcohol just before use and aseptically inserted through one end of the core into porous material. Each core chamber was placed in a vertical position in a test tube rack and incubated at 37° for 100 hours.

The headspace gas pressure was electronically monitored every 24 or 60 min by a computer-controlled device [10]. The pressure transducer used for the experiment had an electrical output that was proportional to the gas pressure in the core chamber (1 mV/kPa). The changes in transducer output were processed through a switching circuit and digital-analog input-output module of the computer. The computer was used to start the time schedule for recording the electrical output from each transducer and for collecting data. Transducer response in mV to H_2 injected into a serum bottle was linear up to 100 kPa of excess pressure. The amount of H_2 production in mmoles was determined by converting the gas pressure measurements into the molar quantity of H_2 produced using the ideal gas law [11].

2.4. Development and testing of mathematical methods

Development and testing of new mathematical methods necessitated the use of ideal data sets having well-characterized kinetic properties. The initial step in creating the ideal data sets was to generate data points consisting of concentrations of galactose substrate at uniform increments of time. Sets of concentrations of galactose substrate simulating consumption according to Monod kinetics were constructed using the integrated Monod equation. Similar sets representing Michaelis-Menten kinetics were obtained from the integrated Michaelis-Menten equation. Substrate concentrations were then re-expressed as total amounts and converted into moles of the gaseous product H_2 using a simple mass balance expression (eq. 1):

$$P = \alpha (S_o - S) + P_o \qquad (1)$$

The amounts of substrate and product in the mass balance expression are denoted by S and P, respectively. The initial amounts of substrate and product are shown as S_o and P_o. The stoichiometric coefficient α in equation 1 describes the rate at which H_2 was formed from the limiting substrate, galactose. Pressure measurements for ideal data sets were derived by substituting moles of product H_2 into the ideal gas law. Values for kinetic parameters and constants used during the generation of ideal data sets were representative of values in literature [12] and preliminary growth curve experiments. Ideal data sets were then used to assess alternative methods to analyze experimental data.

2.5. Kinetic parameter estimation from pressure data sets

The ideal gas law was used to calculate moles of the gaseous product, H_2, from pressures contained in ideal data sets or observed during the experiments. Amounts of H_2 were then related to galactose consumption by solving equation 1 for S and substituting amounts of product for P. Plots of substrate consumption data and sensitivity equations were examined graphically to identify mixed order regions required for estimating parameters.

Following the conversion of pressure measurement data to the corresponding profiles of substrate concentrations, initial kinetic parameter estimates were calculated by linear regressional analyses using model equations presented in Robinson and Tiedje [13] and Robinson [14]. Initial parameter estimates were then used as starting values in nonlinear regressional analyses to obtain the final kinetic parameter estimates.

Nonlinear regressional analyses were performed by rewriting the integrated Monod and Michaelis-Menten equations explicitly in terms of the independent variable, time. Although theoretically invalid, this approach avoids the need to use a complex numerical method to approximate values for the dependent variable. Previous authors have demonstrated that nonlinear regressional estimation of kinetic parameters using a similarly transformed model equation gave parameter estimates which were as accurate as those provided by other techniques [15]. Parameter estimates provided by the transformed model were found to be unbiased over a substantial range of initial reactant concentrations.

The Levenberg-Marquardt algorithm [16] was used to iteratively solve the explicit versions of the model equations for values of u_{max} and K_s, or v_{max} and K_m. Final parameter estimates were values which minimized the sum of the squared error between predicted times for measurements and times when measurements were actually recorded. Plots of residuals from all nonlinear procedures were examined to determine whether errors were independent of model variables, had a zero mean and followed a normal distribution [17].

3. RESULTS

3.1. Core system

Core chambers packed with different glass bead sizes all had very similar porosities, with an average value of 38%. This was expected because the variation of the diameter of the spherical particle should alter the pore size, but not porosity, regardless of whether the packing was cubic or rhombic [18]. Permeability of the packed cores depended on bead size. Core chambers packed with 75-150 μm glass beads had a permeability of 0.05 μm^2 while those packed with 710-1150 μm sized beads had a permeability of 12.7 μm^2. Calculated pore sizes increased linearly from 10 to 80 μm for chambers packed with the smallest to the largest bead sizes, respectively. Hence, the porous experimental system can be used to delineate the effects of pore size from closely related physical factors, such as pore liquid volume, on microbial growth.

3.2. *In situ* bacterial growth

The *in situ* rate of metabolism of strain RW262 was monitored during its growth inside the cores (Figure 1). The rate of H_2 production in core chambers packed with beads of 710-1180 μm was much slower than that observed in the chambers filled only with liquid medium. Very little gas production was observed in chambers packed with the 75-150 μm bead size. The resultant data were used to calculate the total amount of H_2 produced inside the porous media. In core chambers packed with beads with a size range of 710-1180 μm, about 36 mmoles of H_2 were produced by strain RW262. This value is comparable to that in core

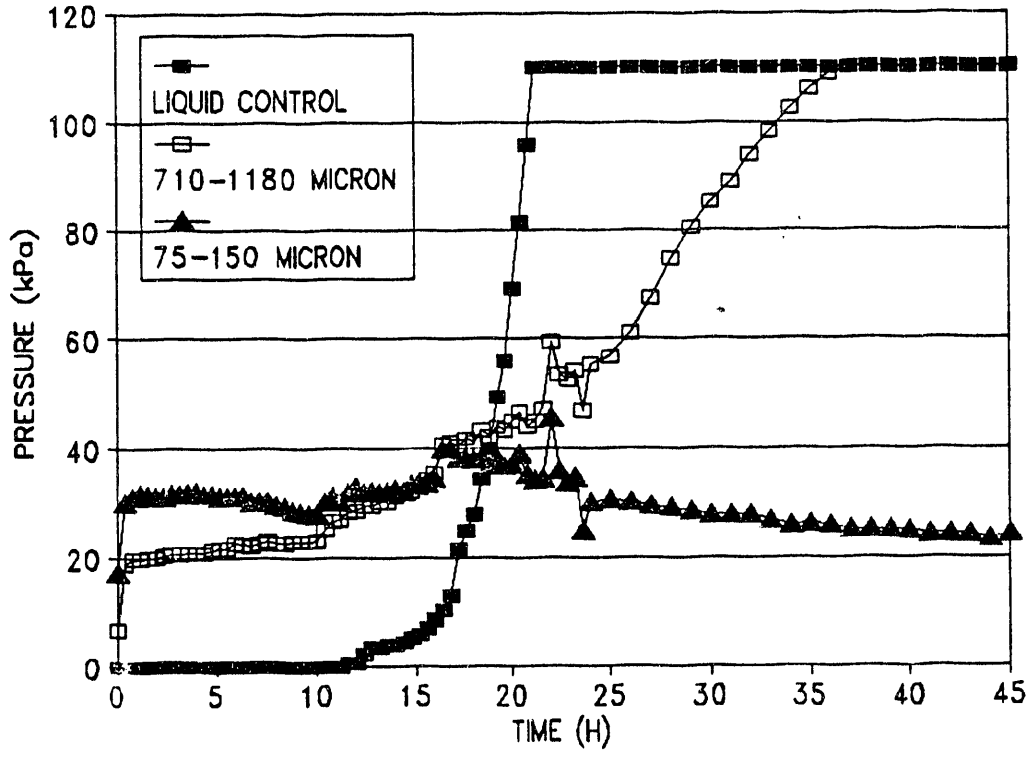


Figure 1. Changes in pressure with time.

Table 1
Kinetic parameter estimates generated from nonlinear regressional analyses of ideal pressure data sets[a]

Source	Parameter Estimates			
	Monod Kinetics		Michaelis-Menten Kinetics	
	U_{max}	K_s	V_{max}	K_m
	hr^{-1}	mM	hr^{-1}	mM
Actual	0.693	2.78	10.0	5.6
Estimate	0.691	2.70	10.8	6.9

[a]Ideal data sets obtained by substitution of substrate concentrations in the integrated forms of the Monod and Michaelis-Menten equations containing known parameter values.

chambers filled only with liquid medium. This suggests that the grain size, and consequently, the pore size of both porous materials was large enough not to affect the extent of the bacterial growth. However, in core chambers packed with the smallest bead size of 75-150 μm, only 4 mmoles of H_2 were produced. No further change in pressure was observed with extended incubations up to 100 hours. This suggests that the extent of microbial activity was controlled by the physical properties of the system. Since the porosity and the liquid pore volume in all of the cores was the same [18], it appears that the pore size was the physical factor governing the *in situ* microbial activity.

3.3. Kinetic parameter estimation

Analysis of sample data sets yielded parameter estimates which were very similar to actual values (Table 1). Error plots indicated that residuals were randomly distributed about a mean of zero. Thus, the nonlinear mathematical method was able to accurately predict the kinetic parameters from known data sets.

Pressure data from laboratory experiments conducted to examine the effects of bead size upon microbial growth and metabolism were analyzed using the newly developed mathematical methodology (Table 2). The integrated Monod model was successfully fit to data obtained from chambers filled with liquid medium. The μ_{max} value was very close to the value, 0.46 per hour, for the growth rate of the culture determined from absorbance change. Usually, K_s values for *E. coli* growth with glucose are in μm range. The relatively high value obtained by the nonlinear regression method may have been caused by the truncation of the pressure measurements as the transducer reading approached 110 kPa. This occurred during late log phase of growth where substrate concentrations became limiting. This is the region of the progress curve that is critical for the K_s determination. Attempts to fit the integrated Monod model to data from other chambers filled with any size range of beads were not successful. The integrated Michaelis-Menten model was successfully fit to the data collected from the 710-1180 μm bead size treatment (Table 2). Residuals in error plots had a mean close to zero, but demonstrated abnormal behavior near boundary values when plotted against the predictor variables or predicted values of the dependent variable.

Table 2
Kinetic parameter estimates generated from nonlinear regressional analyses of experimental pressure data

Treatments	Parameter Estimates			
	Monod Kinetics		Michaelis-Menten Kinetics	
	U_{max}	K_s	V_{max}	K_m
	hr^{-1}	mM	hr^{-1}	mM
Control (liquid)	0.3	1.78		
710-1180 μm bead size			106	2.83

4. DISCUSSION

4.1. Why bacterial growth was restricted by small pore spaces

Since all core chambers were packed with evenly shaped spherical glass beads, continuous pore channels would be expected in each core. Cells are able to move towards the nutrient rich regions of the core chamber at a rate of 0.52 cm/h [19]. The rate of diffusion of galactose in the core is estimated to be about 0.15 cm/h [20]. Thus, it appears unlikely that the bacterial growth inside of the core chambers ceased after 40 h because of the non-availability of nutrients. We hypothesize that the decrease in microbial activity in core chambers packed with small bead sizes must have been due to a restriction of bacterial cell division by the small pore space. This hypothesis is in agreement with early studies [21-23] on the bacterial growth in small liquid-filled capillaries. These studies show that the rate, but not the extent of growth of methane-oxidizing bacteria, was independent of the diameter of the capillary tube used. The number of cell divisions of methane-oxidizing bacteria decreased with a decrease in the diameter of the capillary [21]. The decrease in the number of cell divisions was not related to a mass transfer limitation of nutrients, because the same phenomenon was observed under fluid-flow conditions [21-23]. Thus, it appears that small pore sizes restrict bacterial growth by restricting bacterial cell division.

4.2. Kinetic parameter estimation

Accurate estimation of kinetic parameters requires a large number of data points in the mixed order regions of the progress curves. This is very difficult to obtain for experiments on microbial growth and metabolism in porous materials because the small liquid-pore volumes of the porous material prevent intensive sampling. A parameter of microbial growth activity that can be measured accurately and continuously in porous materials is the gas production. Our experiments show that this approach has promise as a method for determining kinetic parameter estimates of microbial growth and metabolism in porous materials. However, problems, such as the truncation in pressure measurements, may have introduced errors in the estimation of the K_s and K_m values. Also, further work is required to determine the effect of mass transfer limitations on

these estimates, particularly the liquid to gas transition. The fact that Monod kinetics could not be fit to the transformed pressure data suggests that some factor other than growth may have controlled the rate of reaction. This may have been the liquid to gas transfer rate, or the rate of substrate diffusion to the cells.

The integrated Monod equation has frequently been used to model the metabolism of microbial substrates under culture conditions which support balanced growth. Similarly, alternate forms of the Michaelis-Menten equation have commonly been used to model reactions in nongrowing cultures. To avoid using a complex numerical method to approximate substrate concentrations, the model equations were rewritten explicitly in terms of the independent variable, time. Mathematical tests using ideal data sets showed that the mathematical methodology was capable of accurately estimating values for kinetic parameters.

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Adhesion of Microbial Cells to Porous Hydrophilic and Hydrophobic Solid Substrata

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Abstract

Adhesion of microbial cells to solid surfaces is thought to significantly influence the growth and transport of microbes in porous geological materials. The feasibility of cell adhesion to a porous solid substratum can be predicted as a function of the hydrophobicity of the substratum according to the following equation derived from the balance of surface and interfacial free energy in relation to adhesion under conditions where the electrical charge interaction is negligible:

$$\frac{d(\Delta G_{adh})}{d\gamma_{sv}} = (\gamma^{p}_{sv})^{-1/2} (\gamma^{p}_{lv})^{1/2} - (\gamma^{p}_{wv})^{1/2}$$

This thermodynamic approach provides us with the following predictions: (1) the more hydrophilic microbes may be expected to adhere more favorably to the hydrophilic surfaces of a solid substratum, and 2) the more hydrophobic microbes will adhere more favorably to the hydrophobic surfaces of a solid substratum.

To experimentally verify the theoretical predictions, a series of experiments were carried out to investigate the influence of hydrophobicity of solid substrata upon the adhesion of growing cells of *Penicillium spiculisporum* ATCC 16071 using either a typically hydrophobic or hydrophilic solid substratum. The results revealed that nearly 100% of the growing cells of *P. spiculisporum* adhered to the hydrophilic substrata; by contrast, most of the cells were suspended freely in the culture broth when hydrophobic polyurethane foam was used as an adhesive substrate.

These results are in reasonable agreement with the results of the theoretical prediction, and we conclude that the thermodynamic approach offers a powerful tool to predict microbial adhesion onto solid substrates.

1. INTRODUCTION

Adhesion or adsorption phenomenon of microbial cells to solid surfaces is thought to significantly influence the growth and transport of microbes in or through porous geologic materials. Such a phenomenon may be regarded as a prerequisite for microbial plugging, particularly in the initial stage, associated with conventional waterflooding operations for oil recovery from reservoirs.

Adhesion of bacteria or other microorganisms to surfaces of geological materials is thought to involve the physiocochemical surface characteristics of the solid surface, such as charge and hydrophobicity. A thermodynamic approach offers a powerful tool to predict microbial adhesion to a solid substratum under conditions where electrical charge interaction can be neglected.

In this study, a theoretical prediction was made on the basis of thermodynamics about the possibility of particular microbial cells adhering onto both hydrophilic and hydrophobic solid substrata. To verify the theoretical predictions, a series of adhesion experiments were made using either typically hydrophilic or hydrophobic solid substrata to investigate the influence of hydrophobicity, and surface and interfacial free energy of the solid substrata upon the numbers of microbial cells adhering to the substrata during their growth.

2. THEORETICAL CONSIDERATIONS

Suppose a material, M, suspended or dispersed freely in an aqueous liquid, L, adheres to the surface of a solid substratum, S. A change in the interfacial free energy during the process of adhesion of material M to the surface of solid substratum S, ΔG_{adh}, can then be written as equation (1) based on the interfacial free energy balance before and after adhesion under conditions where the electrical charge interaction is negligible.

$$\Delta G_{adh} = \gamma_{SM} - \gamma_{ML} - \gamma_{SL} \tag{1}$$

where γ_{SM}, γ_{ML}, and γ_{SL} are interfacial free energies between solid S and material M, material M and liquid L, and solid S and liquid L, respectively. Equation (1) predicts that adhesion may be expected if ΔG_{adh} is less than zero ($\Delta G_{adh} < 0$), whereas adhesion is energetically unfavorable if ΔG_{adh} is greater than zero ($\Delta G_{adh} > 0$).

To estimate ΔG_{adh}, the change in free energy in the adhesion process, it is necessary to determine the interfacial free energies, three terms found on the right-hand side of equation (1) according to the surface chemical approaches. One useful approach is based on separation of the surface free energy into two components--a dispersion component γ^d, and a polar component γ^p. Surface free energy is essentially caused by attractive force acting between molecules of the material. This intermolecular attractive force can be separated into the following two components: (1) a dispersion force proposed by London in 1930, γ^d, [1] and (2) the attractive forces due to the polarity of the molecules involved, γ^p. Thus surface free energy of, for example, material A, γ_{AV}, can be expressed as:

$$\gamma_{AV} = \gamma^d_{AV} + \gamma^p_{AV} \tag{2a}$$

Similarly, the surface free energy of material B, γ_{BV}, can be written as:

$$\gamma_{BV} = \gamma^d_{BV} + \gamma^p_{BV} \tag{2b}$$

According to Fowkes' approach [2] the dispersion and polar components of interfacial free energy between any two surfaces A and B can be expressed as follows on the basis of the geometric mean equation:

$$\gamma_{AB} = \gamma_{AV} + \gamma_{BV} - 2(\gamma^d_{AV}\,\gamma^d_{BV})^{1/2} - 2(\gamma^p_{AV}\,\gamma^p_{BV})^{1/2}$$

Consequently, ΔG_{adh} in equation (1) can be written as follows by using equation:

$$\begin{aligned}\Delta G_{adh} &= \gamma_{SV} + \gamma_{MV} - 2(\gamma^d_{SV}\,\gamma^d_{MV})^{1/2} - 2(\gamma^p_{SV}\,\gamma^p_{MV})^{1/2} \\ &\quad - \gamma_{SV} - \gamma_{LV} + 2(\gamma^d_{SV}\,\gamma^d_{LV})^{1/2} + 2(\gamma^p_{SV}\,\gamma^p_{LV})^{1/2} \\ &\quad - \gamma_{MV} - \gamma_{LV} + 2(\gamma^d_{MV}\,\gamma^d_{LV})^{1/2} + 2(\gamma^p_{MV}\,\gamma^p_{LV})^{1/2}\end{aligned} \tag{3}$$

or rearranged:

$$\begin{aligned}\Delta G_{adh} &= -2\gamma_{LV} - 2(\gamma^d_{SV}\,\gamma^d_{MV})^{1/2} - 2(\gamma^p_{SV}\,\gamma^p_{MV})^{1/2} + 2(\gamma^d_{SV}\,\gamma^d_{LV})^{1/2} \\ &\quad + 2(\gamma^p_{SV}\,\gamma^p_{LV})^{1/2} + 2(\gamma^d_{MV}\,\gamma^d_{LV})^{1/2} + 2(\gamma^p_{MV}\,\gamma^p_{LV})^{1/2}\end{aligned} \tag{4}$$

The influence of the surface characteristics of the solid substratum upon the adhesion of material M is subsequently described by the following equation obtained by differentiating equation (4) with respect to γ^d_{SV} and γ^p_{SV}:

$$\begin{aligned}d(\Delta G_{adh}) &= -(\gamma^d_{MV})^{1/2}\,(\gamma^d_{SV})^{-1/2}\,d\gamma^d_{SV} - (\gamma^p_{MV})^{1/2}\,(\gamma^p_{SV})^{-1/2}\,d\gamma^p_{SV} \\ &\quad + (\gamma^d_{LV})^{1/2}\,(\gamma^d_{SV})^{-1/2}\,d\gamma^d_{SV} + (\gamma^p_{LV})^{1/2}\,(\gamma^p_{SV})^{-1/2}\,d\gamma^p_{SV} \\ &= (\gamma^d_{SV})^{-1/2}\left[(\gamma^d_{LV})^{1/2} - (\gamma^d_{MV})^{1/2}\right] d\gamma^d_{SV} \\ &\quad + (\gamma^p_{SV})^{-1/2}\left[(\gamma^p_{LV})^{1/2} - (\gamma^p_{MV})^{1/2}\right] d\gamma^p_{SV}\end{aligned} \tag{5}$$

Our experimental determination showed the value of γ^d_{SV} to be approximately 40 mN/m for almost all solid substrata we used. Therefore, we can assume $d\gamma^d_{SV}$ to be zero, and equation (5) can be simplified to equation (6):

Since, $d\gamma_{SV} = d(\gamma^d_{SV} + \gamma^p_{SV}) = d\gamma^d_{SV} + d\gamma^p_{SV} = d\gamma^p_{SV}$,

$$\frac{d(\Delta G_{adh})}{d\gamma_{SV}} = (\gamma^p_{SV})^{-1/2}\left[(\gamma^p_{LV})^{1/2} - (\gamma^p_{MV})^{1/2}\right] \tag{6}$$

Equation (6) is a useful theoretical expression for predicting microbial adhesion onto the solid substrata when material M is regarded as microbial cell. From equation (6), it appears [3]:

1) If $\gamma^p_{MV} > \gamma^p_{LV}$, (namely, if the microbial cell is more hydrophilic): The left-hand side of the equation (6) becomes less than zero as γ_{SV} increases because $(\gamma^p_{LV})^{1/2}$ is less than $(\gamma^p_{MV})^{1/2}$. Therefore, ΔG_{adh} becomes less than zero. This finding means that the adhesion of microbial cells onto surfaces of the solid substrata may become energetically more favorable as γ_{SV} increases. In other words, adhesion may be expected as the surface of the solid substratum becomes more hydrophilic. Therefore, more hydrophilic microbes may be expected to adhere more favorably to the surface of the solid substratum if the surface

Table 1
Variations of parameters influencing ΔG_{adh} · γSV was variable throughout

	γ_{LV}	γ_{MV}
Simulation I	44 mN/m	(1) 105 mN/m
		(2) 86 mN/m
		(3) 69 mN/m
		(4) 55 mN/m
Simulation II	56 mN/m	(1) 105 mN/m
		(2) 86 mN/m
		(3) 69 mN/m
		(4) 55 mN/m
Simulation III	68 mN/m	(1) 105 mN/m
		(2) 86 mN/m
		(3) 69 mN/m
		(4) 55 mN/m

becomes more hydrophilic and vice versa. Therefore, we conclude that more hydrophilic microbes may be expected to adhere more favorably to hydrophilic surfaces of solid substrata.

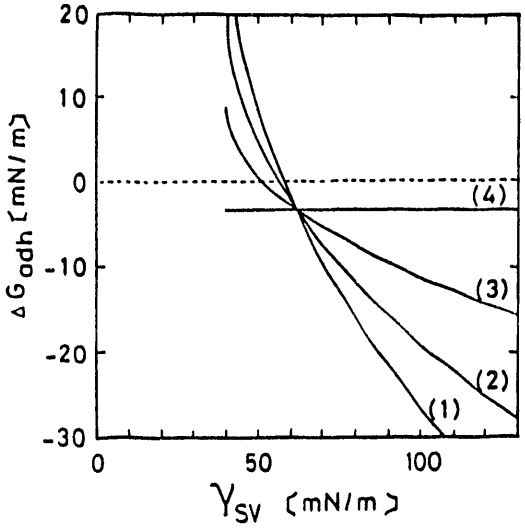
2) By contrast, if $\gamma^p_{MV} < \gamma^p_{LV}$, namely, if the microbial cell is more hydrophobic, then $d(\Delta G_{adh}) / d\gamma_{SV}$ becomes more than zero as γ_{SV} increases. Therefore, more hydrophobic microbes can be expected to adhere more favorably to hydrophobic surfaces of the solid substrata.

To clarify the results of the prediction from equation (6), a simulation was made to investigate the factors influencing adhesion of microbial cells suspended freely in an aqueous liquid to surfaces of solid substrata having different surface properties. The three major parameters in equation (6) which may affect the adhesion of microbial cells are the surface free energies of solid, γ_{SV}, the microbial cell, γ_{MV}, and the liquid, γ_{LV}. Simulations were made under conditions where these three parameters were independently varied to demonstrate the influence of each parameter upon microbial adhesion. Table 1 shows range of variation of each parameter for the simulation. The results of the simulation are illustrated schematically in Figure 1 through Figure 3 as plots of ΔG_{adh} against γ_{SV} with the parameter γ_{MV}.

Simulation I was made under the conditions where 1) the surface free energy of the liquid, γ_{LV}, was 44 mN/m, and 2) the surface free energy of the microbial cells, γ_{MV}, ranged between 105 mN/m and 55 mN/m, which covers microbial cells having surface properties from extremely hydrophilic to hydrophobic.

The results are illustrated schematically in Figure 1, where ΔG_{adh} is plotted against γ_{SV}. This figure shows that the area where γ_{SV} is lower than 50 mN/m, or the surface of the solid substrata is relatively hydrophobic, ΔG_{adh} becomes greater than zero for cells (1), (2), and (3), which covers cells having hydrophilic surfaces. Thus, adhesion does not occur spontaneously in this area. As γ_{SV} increases, adhesion may become energetically more favorable since ΔG_{adh} becomes less than zero.

Simulation II was made at γ_{LV} = 52 mN/m; in other words, γ_{LV} is greater than that of Simulation I, but other conditions are the same as before. So far as

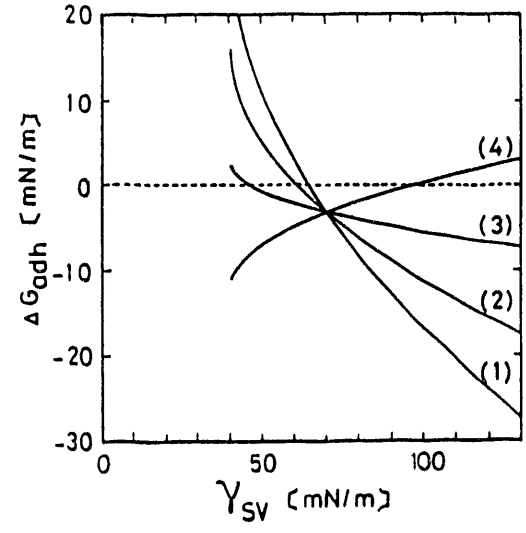


conditions :

γ_{LV} = 44 mN/m

γ_{MV} = (1) 105 mN/m
(2) 86 mN/m
(3) 60 mN/m
(4) 55 mN/m

Figure 1.
Simulation I.

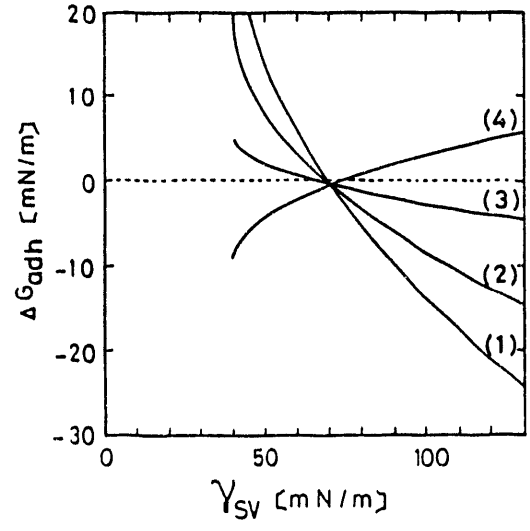


conditions :

γ_{LV} = 52 mN/m

γ_{MV} = (1) 105 mN/m
(2) 86 mN/m
(3) 69 mN/m
(4) 55 mN/m

Figure 2.
Simulation II.



conditions :

γ_{LV} = 60 mN/m

γ_{MV} = (1) 105 mN/m
(2) 86 mN/m
(3) 69 mN/m
(4) 55 mN/m

Figure 3.
Simulation III.

Table 2
The size and porosity of the carriers

	Size	Porosity
hydrophilic carrier - 1	2.5 x 2.5 x 2.5 mm cubic	97%
hydrophilic carrier	5 mm diameter, spherical	97%

hydrophilic microbial cells, or cells (1), (2), and (3) are concerned, similar trends are observed as those in Simulation I (Figure 2).

Figure 3 indicates the result of Simulation III made at γ_{LV} = 60 mN/m, which means that the surface tension of the liquid medium is greater than those of Simulations I and II, but other conditions are the same as before. Almost completely similar curves of ΔG_{adh} as function of γ_{SV} were obtained as those of Simulation II. In this case, it is also apparent that for hydrophilic cells with a γ_{MV} to be greater than 70 mN/m, adhesion may become energetically more favorable than an increase in the surface free energy of a solid surface.

3. EXPERIMENTS

To verify the prediction made from the thermodynamic considerations in equation (6), an experimental study was made to investigate the influence of hydrophobicity, or surface and interfacial free energy of solid substrata upon the number of microbial cells adhered to the substrata during microbial growth.

3.1. Microorganism

Penicillium spiculisporum ATCC 16071, a fungus, was used in this experimental study. This microbe produces 4-hydroxy-4,5-dicarboxypentadecanoic acid, one of the microbial biosurfactants, during aerobic growth on glucose [4]. While developing a bioreactor system for producing the biosurfactant using immobilized *P. spiculisporum* and selecting porous carriers onto which the microbial cells adhered and became immobilized, a focus of attention was given to the theoretical analysis of adhesion phenomenon of microbial cells onto solid substrata.

3.2. Composition of growth medium

The growth medium used for *P. spiculisporum* consisted of 10% glucose, 0.1% NH_4Cl, 0.1% KH_2PO_4, 0.02% Mg $SO_4 7H_2O$, 0.1% peptone, and 0.1% yeast extract.

3.3. Porous carriers

Two types of porous polymers were used as solid substrata or microbe adhesion carriers for *P. spiculisporum*. One was a commercial polyurethane foam, which is a typical hydrophobic porous carrier, hereafter called the hydrophobic carrier. The other was a typical hydrophilic porous carrier prepared from natural cellulose, and referred to as the hydrophilic carrier - 1. The size and porosity of both the above porous carriers are shown in Table 2.

Gelatin-treated polyurethane foam also was used as a hydrophilic carrier, which was prepared by coating the surface of the commercial polyurethane foam with gelatin film, and thus giving a relatively hydrophilic surface: We called this hydrophilic carrier - 2.

3.4. Determination of surface free energy

3.4.1. Surface free energy of liquid

The surface free energy, or surface tension of liquid, γ_{LV}, can be easily and directly measured by using a proper tensiometer. In this study we used Wilhelmy's vertical plate method.

3.4.2. Surface free energy of hydrophobic solid substratum

The surface free energy, or surface tension of the hydrophobic solid substratum (the hydrophobic carrier), γ_{SV}, was determined as critical surface tension, γ_C, estimated from a Zisman plot [5] by measuring the contact angle.

3.4.3. Surface free energy of hydrophilic solid substrata and microbial cells

Determination of the surface free energy of both the hydrophilic solid substrata, γ_{SV}, and the microbial cells, γ_{MV}, was made using the method developed by Busscher and Arends [6] on the basis of the following equation:

$$\cos\theta = -16.74\,(\gamma_{SV}^{p})^{1/2}\,D_L^2 + 2(\gamma_{SV}^{d})^{1/2} + 3.996\,(\gamma_{SV}^{p})^{1/2}\,D_L + 0.002\,(\gamma_{SV}^{p})^{1/2} - 1 \qquad (7)$$

where,

$$D_L = (\gamma_{LV}^{d})^{1/2}\,\gamma_{LV}^{-1} \qquad (8)$$

3.5. Growth and adhesion experiments

Growth experiments with *P. spiculisporum* were made using a 500 ml flask containing 100 ml of growth medium and a specified amount of pieces of either hydrophobic or hydrophilic carrier. The flask was autoclaved, inoculated, and then placed on a reciprocal shaker operated at 120 strokes per minute at 30°C to allow the microbe to grow.

After reaching almost stationary phase of growth, after 12 days cultivation, the amount of cells adhering to the carriers, and those suspended freely in the culture broth were weighed.

4. EXPERIMENTAL RESULTS

4.1. Experimental determination of surface free energies

4.1.1. Zisman plot

Figure 4 shows Zisman plots of the hydrophobic carrier used in this experiment and of several commercial synthetic polymers including polyethylene, which is a typical non-polar compound. The value of the surface free energy, γ_{SV}, of the hydrophobic carrier obtained from the Zisman plot, in other words, the value of its critical surface free energy, γ_C, was estimated to be 44 mN/m.

4.1.2. Results of experimentally determined surface free energies of liquid and solid materials

Table 3 shows the experimentally determined surface free energies of the culture broths, of the solid substrata including hydrophilic carriers, and of the microbial cells.

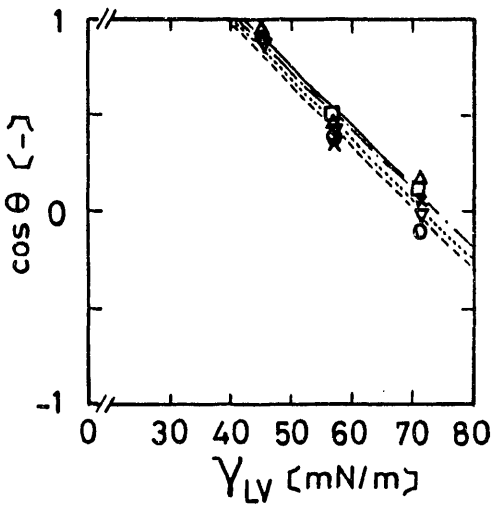
Surface free energies of culture broth at the initial and final stages of cultivation measured directly by using Wilhelmy's vertical plate method were 60 mN/m and 44 mN/m, respectively.

Table 3
Experimentally determined surface free energies

Culture Broth	(1) initial stage	γ_{LV} = 60 mN/m
	(2) final stage	γ_{LV} = 44 mN/m
Hydrophobic Carrier		γ_{SV} = 44 mN/m
Hydrophilic Carrier 1		γ_{SV} = 123 mN/m
Hydrophilic Carrier 2		γ_{SV} = 110 mN/m
Microbial Cells		γ_{MV} = 101 mN/m

The γ_{SV} values of the hydrophilic carriers 1 and 2 were determined from equation (7) by measuring the contact angle, and were 123 mN/m, and 110 mN/m, respectively.

The γ_{SV} value of the hydrophobic carrier was much less than those of hydrophilic carriers, and was estimated to be 44 mN/m from the Zisman plot (Figure 4).



polyethylene (γ_{SV} = 39 mN/m
polyvinyl chloride (γ_{SV} = 40 mN/m
polyacryl resin (γ_{SV} = 41 mN/m
polycarbonate (γ_{SV} = 43 mN/m
hydrophobic carrier (γ_{SV} = 44 mN/m

Figure 4. Zisman plots of various hydrophobic polymers including the one used in this study.

The surface free energy of the microbial cells used in this study was 101 mN/m, as determined by measurement of the contact angle. Thus, the cells of *P. spiculisporum* are extremely hydrophilic.

4.2. Simulation

By using the experimentally determined values of surface free energies, a theoretical prediction of the feasibility of adhesion of the microbial cells to both hydrophobic and hydrophilic carriers was made. The result of the simulation is illustrated in Figure 5.

The shaded curve indicates the resultant prediction under our experimental conditions. Thus, ΔG_{adh} for the hydrophobic carrier (γ_{SV} = 44 mN/m) is estimated to be greater than zero. Therefore, the microbial cells may be expected to adhere less successfully to the hydrophobic carrier.

However, in the case of both hydrophilic carriers, the surface free energies were 123 mN/m and 110 mN/m, respectively, and thus ΔG_{adh} becomes much lower than zero for both carriers, and the adhesion of the microbe becomes energetically more favorable.

A series of growth and adhesion experiments were carried out to verify the above theoretical prediction experimentally.

4.3. Results of the growth and adhesion experiment

The growth of *P. spiculisporum* and adhesion of the microbial cells to hydrophobic or hydrophilic carriers are illustrated in Figure 6, which gives the % adhesion of cells on either carrier. The % adhesion is plotted as a function of the amount of carrier added in the culture broth before starting cultivation.

The % adhesion in defined in the following equation:

$$\% \; adhesion = \frac{M_{adh}}{M_{adh} + M_{free}} X\, 100 = \frac{M_{adh}}{M_{total}} X\, 100 \qquad (9)$$

where, M_{adh} is the amount of microbial cells adhered to the carrier, M_{free} is the amount of microbial cells suspended freely in the broth, and M_{total} is the total amount of cells grown, expressed as: $M_{total} = M_{adh} + M_{free}$.

As the amount of carrier added to the culture broth increased, the adhesion increased steadily, finally approaching 10%. Under these conditions, almost all the microbial cells adhere to the hydrophilic carriers, and very few cells remain suspended in the culture broth when there is more than 10g of carrier per liter of the medium.

For the hydrophobic carrier, the % adhesion similarly increased as the amount of carrier increased. However, it was interesting to note that the amount of microbial cells adhering to the carrier was restricted, and significant number of microbial cells were suspended freely in the culture broth.

From these adhesion experiments we conclude that the experimental results are in good agreement with the theoretical predictions.

5. CONCLUSIONS

While developing a bioreactor system to produce a microbial biosurfactant using immobilized *P. spiculisporum*, particularly selecting porous carriers to

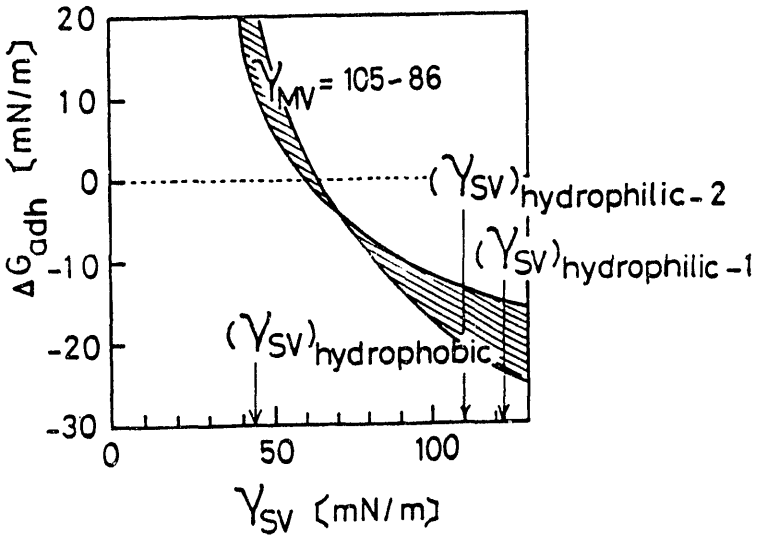


Figure 5. Theoretical prediction on the feasibility of adhesion of the cells of *Penicillum spiculisporum* to the hydrophobic and hydrophilic carriers.

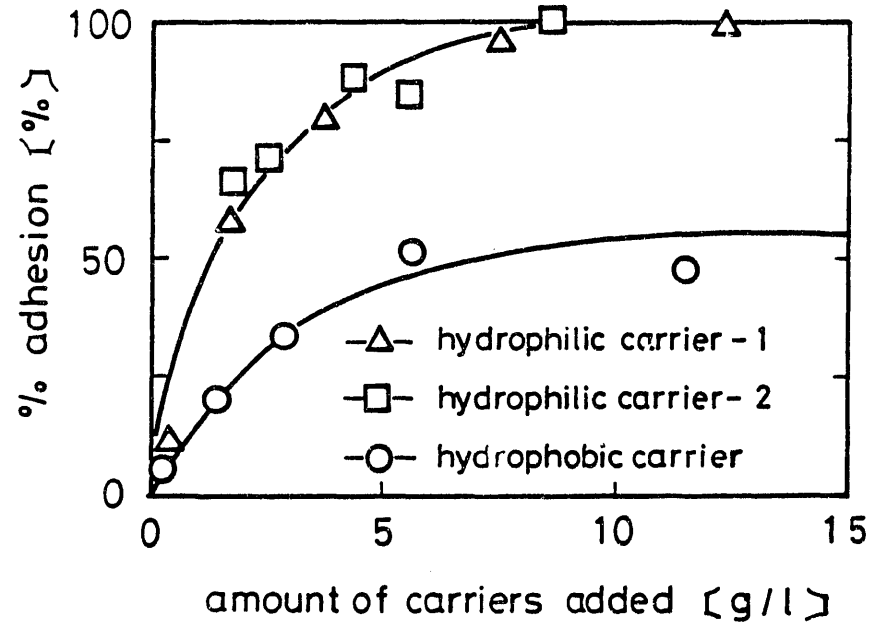


Figure 6. Experimental results of cell adhesion onto both hydrophobic and hydrophilic carriers.

which the microbial cells can adhere and become immobilized, we gave particular attention to the theoretical analysis of the adhesion.

The feasibility of cell adhesion or immobilization to porous solid carriers could be predicted as a function of the hydrophobicity of the solid surface according to the equation (6); this equation is derived from surface and interfacial free energy balance in relation to the adhesion under conditions where electrical charge interaction is negligible. We believe that the thermodynamic approach may offer a powerful tool to predict adhesion or adsorption of microbial cells onto porous geological materials encountered in common waterflooding operations.

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A Mathematical Model for Microbially Enhanced Oil Recovery Process

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Abstract

A three-phase, multiple-species, one-dimension model has been developed to simulate bacterial transport, growth, and metabolism processes involved in microbially enhanced oil recovery (MEOR) and to predict the modification in permeability that results from these microbial activities in porous media. Convection-dispersion equations and microbial kinetics are incorporated in the model system to characterize and quantify biomass production, product formation, and nutrient utilization in the MEOR process. Modification of permeability is assumed to be due to both pore-surface retention and pore-throat plugging by bacterial cells.

The model has been applied to static (sand packs) and core-flooding (sandstone cores) experiments to describe microbial movement, metabolite production, and nutrient consumption during growth and metabolism and to estimate reduction in permeability. Comparison between numerical solutions and experimental results indicated that the model does simulate the essential microbial kinetics of laboratory experiments and that it can be extended to provide numerical predictions for the design and evaluation of MEOR field projects.

1. INTRODUCTION

The transport, growth, and metabolism of viable cells in subsurface formations are governed by many complicated physical, chemical, and biological phenomena. Many experimental studies [1-3] have shown (1) that viable bacteria and nutrients required for growth can be transported through Berea sandstone cores, (2) that the in situ growth of bacteria results in significant reduction in permeability, (3) that the reduction is selective for high permeability cores and improves sweep efficiency, and (4) that additional oil is recovered as a result of improved microscopic displacement and sweep efficiencies.

Associated with laboratory investigations, mathematical simulation has been introduced to help understand the mechanisms involved in the MEOR process [4-8].

This paper presents a three-phase, multiple-species, one-dimension mathematical model to simulate biomass growth, product formation, and substrate consumption during in situ microbial growth, and to predict the reduction in permeability as a result of in situ growth and metabolism in porous media. This model differs from the previously published models in that the kinetics with two growth-limiting substrates is incorporated in the model and that both pore-surface retention and pore-throat plugging by biomass are considered in the modifications of permeability.

2. MODEL FORMULATION

The mathematical representations developed in this study include (1) convection-dispersion equations for species transport, (2) microbial kinetics for growth and metabolism, (3) an empirical formula for permeability reduction, and (4) continuity equations for pressure and saturations.

The materials used in the experimental studies were bacteria ($CH_{1.8}O_{0.5}N_{0.2}$), glucose ($C_6H_{12}O_6$), and ammonium nitrate (NH_4NO_3). The metabolic products were acetate (CH_3COO^-), carbon dioxide (CO_2), and nitrogen (N_2). A mass balance for metabolite production can be expressed as:

$$5C_6H_{12}O_6 + 8NO_3^- \rightarrow 10CH_3COO^- + 10CO_2 + 4N_2 + 14H_2O + 2H^+ \quad (1)$$

An empirical equation for cell growth and metabolism by consumption of both glucose (carbon source) and ammonium (nitrogen source) is given [9]:

$$C_6H_{12}O_6 + 1.2NH_4^+ \rightarrow 6CH_{1.8}O_{0.5}N_{0.2} + 3H_2O \quad (2)$$

2.1. Assumptions

The major assumptions for developing the model equations were:

1. One-dimension horizontal linear flow;
2. Homogeneous, isotropic, and incompressible porous media;
3. Three-phase Newtonian fluids: oil, water, and gas;
4. Both glucose and ammonium nitrate are growth-limiting;
5. Bacteria are partitioned into a planktonic phase, consisting of cells suspended in the flowing aqueous phase, and into a sessile phase composed of cells retained on pore surfaces;
6. Anaerobic microbial growth and metabolism occur in both the planktonic phase and sessile phase.

2.2. Basic equations

The mathematical relationships for species convection, dispersion, and production, for permeability modification, and for pressure and saturations are presented below.

2.2.1. Species transport

Material balance equations are written for the planktonic bacteria, limiting nutrients, and metabolic products in the flowing water phase [8]:

$$\frac{\partial(\phi S_w C_i)}{\partial t} = -\frac{\partial(u_f C_i)}{\partial x} + \frac{\partial}{\partial x}(\phi S_w K_D \frac{\partial C_i}{\partial x}) + (\phi S_w) R_i \quad (3)$$

The three terms on the right-hand side of Eq. (3) correspond to convection, dispersion, and source terms. Eq. (3) can be applied to species such as biomass (B), acetate (A), carbon dioxide (C), nitrogen (N), glucose (S1), ammonium ($S2^+$), and nitrate ($S2^-$).

2.2.2. Biomass retention

The accumulation of the biomass attached on the pore surface is assumed to be a net result of biomass retention, detachment, and growth. Retention and detachment are two biomass exchange processes between the planktonic phase and

sessile phase. Hence, a conservation equation for the sessile phase can be written:

$$\frac{\partial(\rho_B \sigma)}{\partial t} = R_r - R_d + R_{Bs} \tag{4}$$

where, R_r, R_d, and R_{Bs} are the rates for retention, detachment, and growth, respectively. ρ_B is the density of the biomass, and σ is the pore fraction occupied by the retained cell bodies.

2.2.3. Sources and rates

A typical source term (R_B) for viable cells suspended in the flowing water phase is expressed as:

$$R_B = R_d - R_r + R_{Bf} \tag{5}$$

The biomass detachment rate (R_d) is a function of the biomass "$\sigma\rho_B$" attached on the pore surfaces, and the shear force "$u_f\mu_w/K$" applied between the fluids and sessile phase [10]:

$$R_d = K_d(\sigma\rho_B)u_f \frac{\mu_w}{K} \tag{6}$$

The biomass retention rate (R_r) is considered to be proportional to the biomass "$u_f C_B$" entering a given area, and to the applicable plugging capacity "$(1-\sigma)\rho_B$" of the porous medium [10]:

$$R_r = K_r(u_f C_B)(1-\sigma)\rho_B \tag{7}$$

Since it is assumed that the two substrates (glucose and ammonium) are simultaneously growth-limiting in the MEOR process, a Monod growth rate, dependent on both limiting nutrients has been adopted [9]:

$$\mu_B = \mu_{Bm}\frac{C_{S1}}{K_{B/S1} + C_{S1}}\frac{C_{S2^+}}{K_{B/S2^+} + C_{S2^+}} \tag{8}$$

where, μ_{Bm} is maximum specific growth rate; $K_{B/S1}$ and $K_{B/S2+}$ are saturation constants for biomass growth by consuming glucose and ammonium, respectively. Therefore, the production rates (R_{Bf} and R_{Bs}) of biomass in both the planktonic phase and sessile phase can be computed:

$$R_{Bf} = \mu_B C_B \ , \qquad R_{Bs} = \mu_B(\sigma\rho_B) \tag{9}$$

An empirical model has been proposed for estimating the production rate of metabolic products [11]:

Table 1
Rate equations for MEOR processes

Retention	$R_r = K_r(u_f C_B)(1 - \sigma)\rho_B$
Detachment	$R_d = K_d(\sigma\rho_B)(u_f \frac{\mu_w}{K})$
Bacteria	$R_{Bf} = \mu_{Bm}\frac{C_{S1}}{K_{B/S1} + C_{S1}}\frac{C_{S2^+}}{K_{B/S2^+} + C_{S2^+}}C_B$
Sessile	$R_{Bs} = \mu_{Bm}\frac{C_{S1}}{K_{B/S1} + C_{S1}}\frac{C_{S2^+}}{K_{B/S2^+} + C_{S2^+}}(\sigma\rho_B)$
Acetate	$R_A = \mu_{Am}\frac{C_{S1} - C^*_{S1}}{K_{A/S1} + C_{S1} - C^*_{S1}}(C_B + \sigma\rho_B)$
CO_2	$R_C = \mu_{Cm}\frac{C_{S1} - C^*_{S1}}{K_{C/S1} + C_{S1} - C^*_{S1}}(C_B + \sigma\rho_B)$
N_2	$R_N = \mu_{Nm}\frac{C_{S2^-} - C^*_{S2^-}}{K_{N/S2^-} + C_{S2^-} - C^*_{S2^-}}(C_B + \sigma\rho_B)$
Glucose	$R_{S1} = -\frac{R_{Bf} + R_{Bs}}{Y_{B/S1}} - \frac{R_A + R_C}{Y_{(A+C)/S1}} - m_{S1}(C_B + \sigma\rho_B)$
Ammonium	$R_{S2^+} = -\frac{R_{Bf} + R_{Bs}}{Y_{B/S2^+}}$
Nitrate	$R_{S2^-} = -\frac{R_N}{Y_{N/S2^-}} - m_{S2^-}(C_B + \sigma\rho_B)$

$$R_P = \mu_{Pm}\frac{C_S - C^*_S}{K_{P/S} + C_S - C^*_S}(C_B + \sigma\rho_B) \tag{10}$$

where, μ_{Pm} is the maximum specific production rate; $K_{P/S}$ is the saturation constant for product P by consumption of substrate S; C^*_S is the critical concentration of substrate S for metabolic production.

In general, utilization of substrates for microbial growth and metabolism can be described [9]:

$$R_S = -\frac{R_B}{Y_{B/S}} - \frac{R_P}{Y_{P/S}} - M_S(C_B + \sigma\rho_B) \tag{11}$$

where, $Y_{B/S}$ and $Y_{P/S}$ are yield coefficients for biomass B and product P per unit substrate S, respectively; m_S is a constant for energy maintenance by substrate S.

Table 1 lists the set of equations describing biomass retention, detachment, and growth, product formation, and substrate consumption in porous media.

2.2.4. Modification of permeability

The permeability reduction factor has previously been defined as [6]:

$$\frac{K}{K_0} = \left(\frac{\phi}{\phi_0}\right)^3, \qquad \phi = \phi_0(1 - \sigma) \tag{12}$$

where, K_0 and ϕ_0 are initial permeability and porosity, respectively; K and ϕ are the instantaneous permeability and porosity.

Equation 12 states that the reduction in permeability is proportional to the cubic power of porosity reduction by fine particles retained on pore surfaces. However, when the pore throats of a porous medium are blocked, the porosity of the medium may not markedly change, but permeability may decrease dramatically because the connection between the pores are closed. Therefore, a flow-efficiency coefficient (f) has been introduced into the permeability-porosity correlation to account for the plugging of the pore-throat [12]:

$$\frac{K}{K_0} = f\left(\frac{\phi}{\phi_0}\right)^3 \tag{13}$$

From a probabilistic standpoint, pore-throat size usually distributes over finite ranges and the distribution may have more than one peak. Thus, a bimodal function was proposed to represent the distribution for pore-throat size (Figure 1). The general form of the bimodal distribution function is given [13]:

$$f(x) = wf_1(x) + (1 - w)f_2(x) \tag{14}$$

where, x is the pore-throat size; $f_1(x)$ and $f_2(x)$ are two unimodal distribution functions; w is weighting factor, $0 \le w \le 1$.

An empirical expression [12] has been proposed as a criterion for pore-throat plugging for the given values of particle concentration C_p and flux u_f/ϕ:

$$\frac{X_{pt}}{X_p} \le A\left[1 - \exp\left(-B\frac{C_p u_f}{\phi}\right)\right] \tag{15}$$

where, X_{pt}/X_p is ratio of pore-throat size to particle size; A and B are empirical parameters.

However, bacterial cells differ from other particles in some important characteristics. Once viable cells are retained on pore surfaces and form a sessile phase (biofilm), the cells may multiply and occupy more pore space. Such multiplication will make pore throats smaller so that plugging of the pore-throat becomes more effective, even if the concentration of cells in the planktonic bacterial phase may be much lower. Hence, Equation 15 is modified to consider the contribution of the sessile bacterial phase to pore-throat plugging. The critical pore-throat diameter (X_{ptcr}) necessary for plugging by cells can be computed:

$$X_{ptcr} = X_p A[1 - \exp(-B\frac{C_B u_t}{\phi} - C\sigma)] \tag{16}$$

where, C is an empirical parameter and σ is pore fraction occupied by cells retained on pore surfaces.

Therefore, the flow-efficiency coefficient can be determined by estimating the likelihood of pore-throat plugging [12]:

$$f = 1 - \frac{\int_{X_{ptmin}}^{X_{ptcr}} f(x)\,dx}{\int_{X_{ptmin}}^{X_{ptmax}} f(x)\,dx} \tag{17}$$

where, x_{ptmin} and x_{ptmax} are the minimum and maximum sizes of the pore throat, respectively.

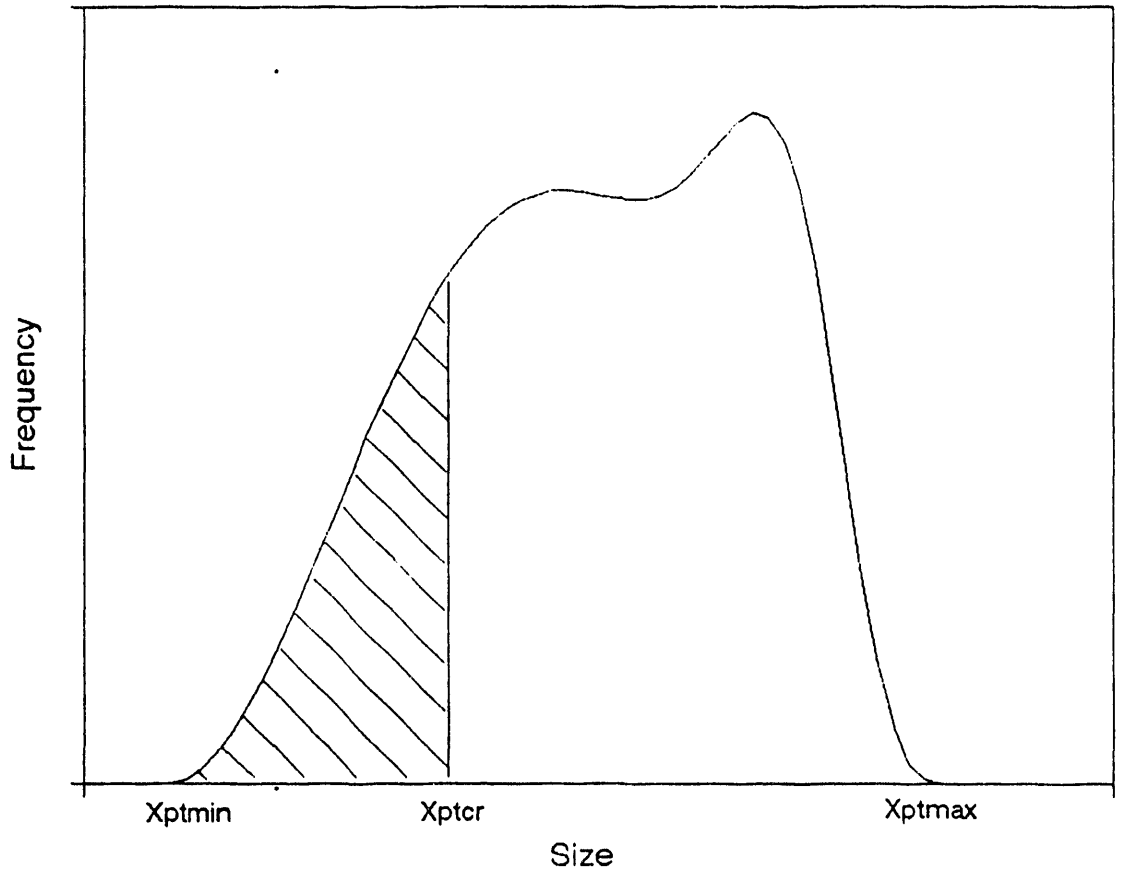


Figure 1. Bimodal distribution for pore throat.

2.2.5. Pressure and saturations

After successful MEOR treatments, the simultaneous flow of oil, water, and gas phases may occur in the porous medium during the post-flush period. A three-phase transport model can be developed to describe this process:

$$-\frac{\partial}{\partial x}\left(\frac{\rho}{B}u\right)_l + q_l = \frac{\partial}{\partial t}\left(\frac{\phi\rho S}{B}\right)_l \tag{18}$$

where, l = oil, water, and gas phases.

Since the deposited biomass (sessile phase) occupies a portion of the pore space during the MEOR process, the saturation balance equation becomes:

$$S_o + S_w + S_g + \sigma = 1 \tag{19}$$

Thus, pressure and saturations can be solved from the three-phase continuity equations applicable in an environment of black oil reservoirs [14].

Through the systematic derivation above, a complete set of model equations describing the general MEOR process was obtained, which is summarized in Table 2.

2.3. Numerical solution

The first-order spatial derivative corresponding to the convection transport for species i at node j is replaced by a third-order upwinding formula [15]:

$$\left(\frac{C_i}{\partial x}\right)_j = \frac{(C_i)_{j-2} - 6(C_i)_{j-1} + 3(C_i)_j + 2(C_i)_{j+1}}{6\Delta x} \tag{20}$$

The third-order upstream weighting is the lowest order method possessing the properties needed for a stable and accurate difference operator for the convection term.

The dispersion term, a second-order spatial derivative, is approximated by the second-order central finite difference scheme:

$$\frac{\partial}{\partial x}\left(\phi K_D \frac{\partial C_i}{\partial x}\right)_j = \frac{(\phi K_D)_{j+\frac{1}{2}}\left[(C_i)_{j+1} - (C_i)_j\right] - (\phi K_D)_{j-\frac{1}{2}}\left[(C_i)_j - (C_i)_{j-1}\right]}{\Delta x^2} \tag{21}$$

Through separation in space using the finite difference form, the model system is translated into a set of ordinary differential equations corresponding to each node j along the one-dimensional porous medium. The resulting equations were solved using the Runge-Kutta-Fehlberg method.

3. APPLICATIONS

The mathematical model that we developed was tested using experimental data from laboratory cases of bacterial growth in sand packs (static) and a MEOR core flood. Because quantities representing biomass growth, product formation, and substrate use are primary variables affecting the MEOR process, the numerical simulation is based on a reduced one-phase, seven-species system.

Table 2
Model equations for MEOR processes

Species	$\frac{\partial C_i}{\partial t} = -\frac{u_t}{\phi S_w}\frac{\partial C_i}{\partial x} + \frac{1}{\phi}\frac{\partial}{\partial x}(\phi K_D \frac{\partial C_i}{\partial x}) + \phi_0 C_i \frac{\partial \sigma}{\partial t} + R_i$
Sessile	$\frac{\partial \sigma}{\partial t} = K_r\, u_t\, C_B(1 - \sigma) - K_d\, \sigma\, u_t \frac{\mu_w}{K} + \mu_B \sigma$
Pressure	$\phi C_t \frac{\partial p}{\partial t} = \beta_o \frac{\partial}{\partial x}(\psi_o \frac{\partial p}{\partial x}) + \beta_w \frac{\partial}{\partial x}(\psi_w \frac{\partial p}{\partial x}) + \beta_g [\frac{\partial}{\partial x}(\psi_g \frac{\partial p}{\partial x}) + q_g] + \phi \frac{\partial \sigma}{\partial t}$
Saturations	$\frac{\partial}{\partial t}(\phi \frac{S_o}{B_o}) = \frac{\partial}{\partial x}(\psi_o \frac{\partial p}{\partial x})$ $\frac{\partial}{\partial t}(\phi \frac{S_w}{B_w}) = \frac{\partial}{\partial x}(\psi_w \frac{\partial p}{\partial x})$ $\frac{\partial}{\partial t}[\phi(\frac{R_{so}S_o}{B_o} + \frac{R_{sw}S_w}{B_w} + \frac{S_g}{B_g}] = \frac{\partial}{\partial x}(\psi_g \frac{\partial p}{\partial x}) + q_g$

3.1. Static Cores

Microbial growth and activity under anaerobic, nutrient-saturated, static conditions have been studied in sand-packed cores [16]. The medium contained galactose as the sole added carbon source. Nitrate served as an external electron acceptor. The growth rate for *Escherichia coli* RW262 was determined in the sand packs. The galactose consumption and formation of end-products were measured at various intervals.

The maximum specific growth rate for the growth of biomass was obtained from the experiments (Table 3). Yield coefficients (Table 3) for bacterial growth and product formation were calculated from the chemical balances described in Equations 1 and 2. Other kinetic parameters were estimated to match the experimental results. Comparison of the results of simulation with the experimental data indicated that the best match with 1.5% of an average relative error was achieved for nutrient consumption (Figure 2). The predicted metabolite productions were a little lower than measured (Figures 3 and 4). Bacterial growth showed a relatively large discrepancy between simulation and experiment (Figure 5).

3.2. MEOR core flooding

Experimental studies [17] were conducted to investigate microbial mechanisms involved in recovery of the residual oil after waterflooding. Indigeneous bacteria and nutrients (glucose and ammonium nitrate) were injected into Berea sandstone cores after the cores had been waterflooded to residual oil saturation. The effective reduction in permeability, production of acetate, CO_2, and N_2, and the residual oil recovery from one of the cores were used to test the simulator.

Table 3
Yield coefficients and growth rate

Symbols	Definitions	values	Units
$Y_{B/S1}$	yield coeff. for biomass on S1	0.820	mg/mg
$Y_{B/S2+}$	yield coeff. for biomass on $S2^+$	6.825	mg/mg
$Y_{(A+C)/S1}$	yield coeff. for acetate and CO_2	1.144	mg/mg
$Y_{N/S2-}$	yield coeff. for N_2	0.226	mg/mg
μ_{Bm}	max. specific growth rate	0.602	1/hr

The input data needed for the simulation are given in Tables 3 to 5. Comparisons between modeling and experimental results are given in Figures 6 to 9. No significant metabolic products were observed during the first four treatments (Figures 6 and 7). During this time, microbial populations were probably too small to result in detectable production of metabolites. CO_2 recovery in the effluent was much lower than the simulated value (Figure 7); this might be due to the solubility of CO_2 in the brine (in the form of H_2CO_3 or HCO^-_3) and in the oil. Predicted consumption of glucose and nitrate are close to that

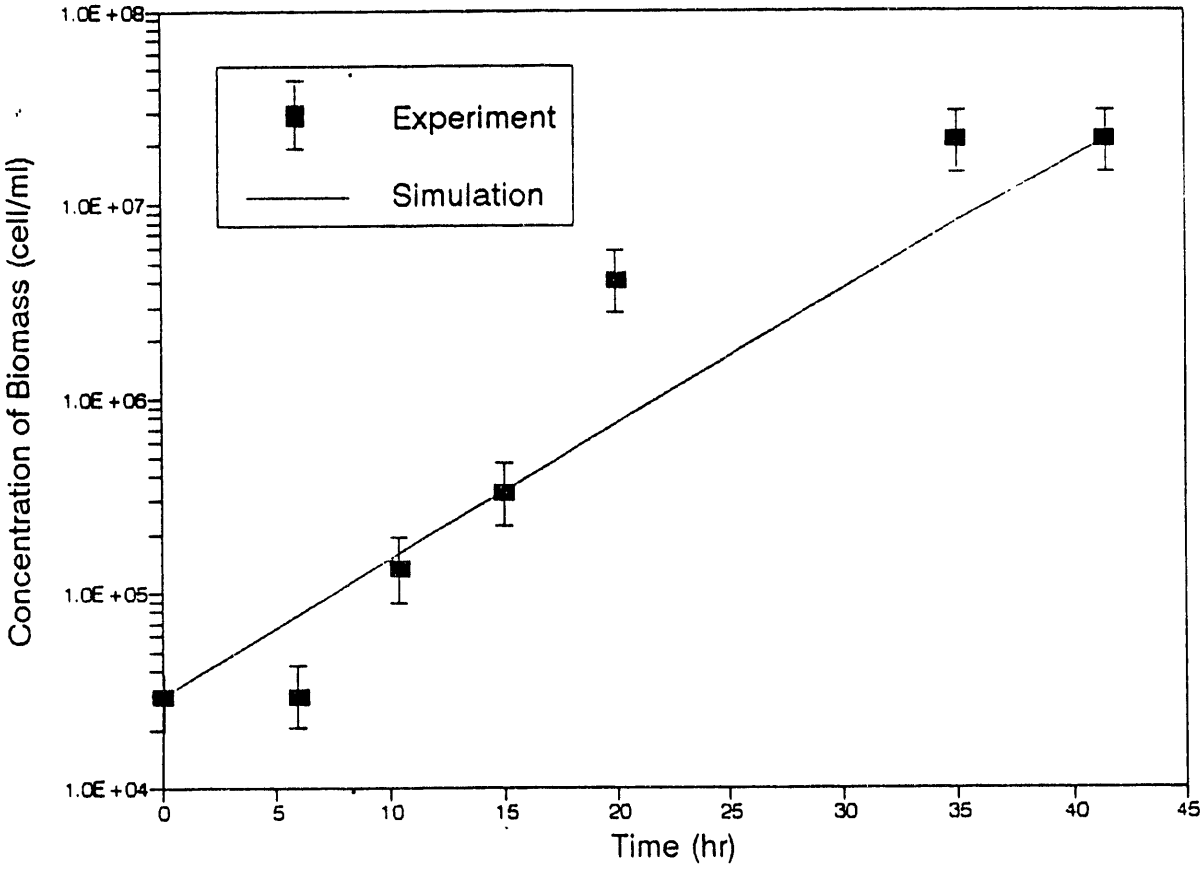


Figure 2. Nutrient consumption in static cores.

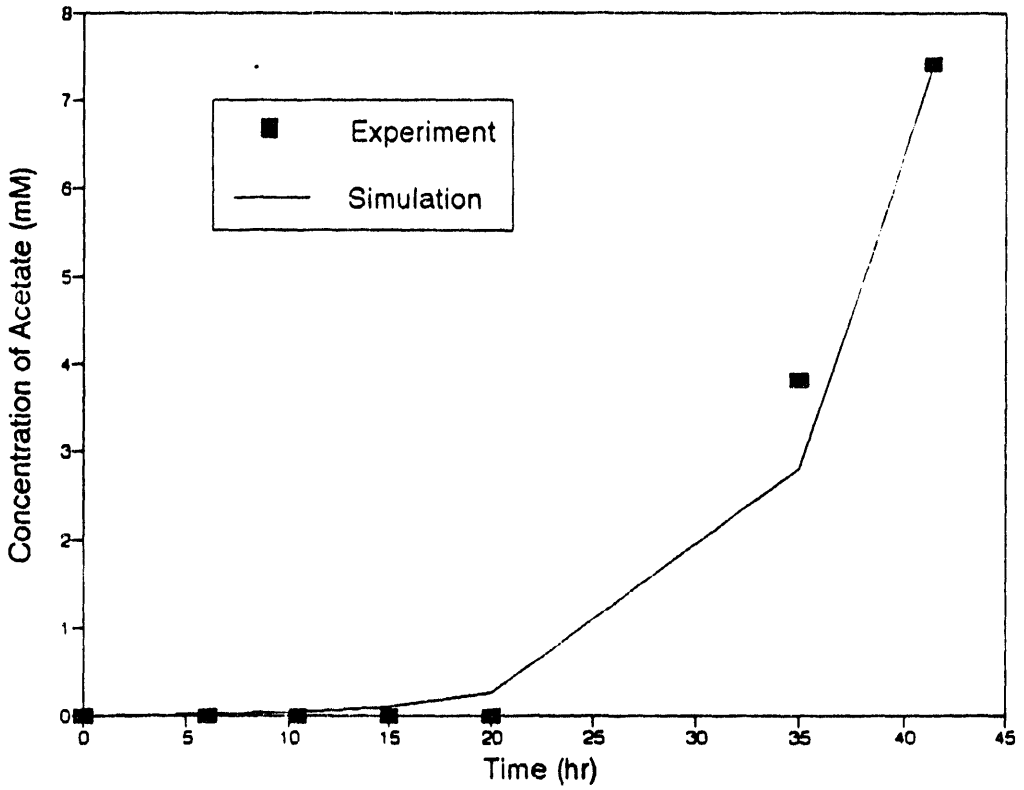


Figure 3. Acetate production in static cores.

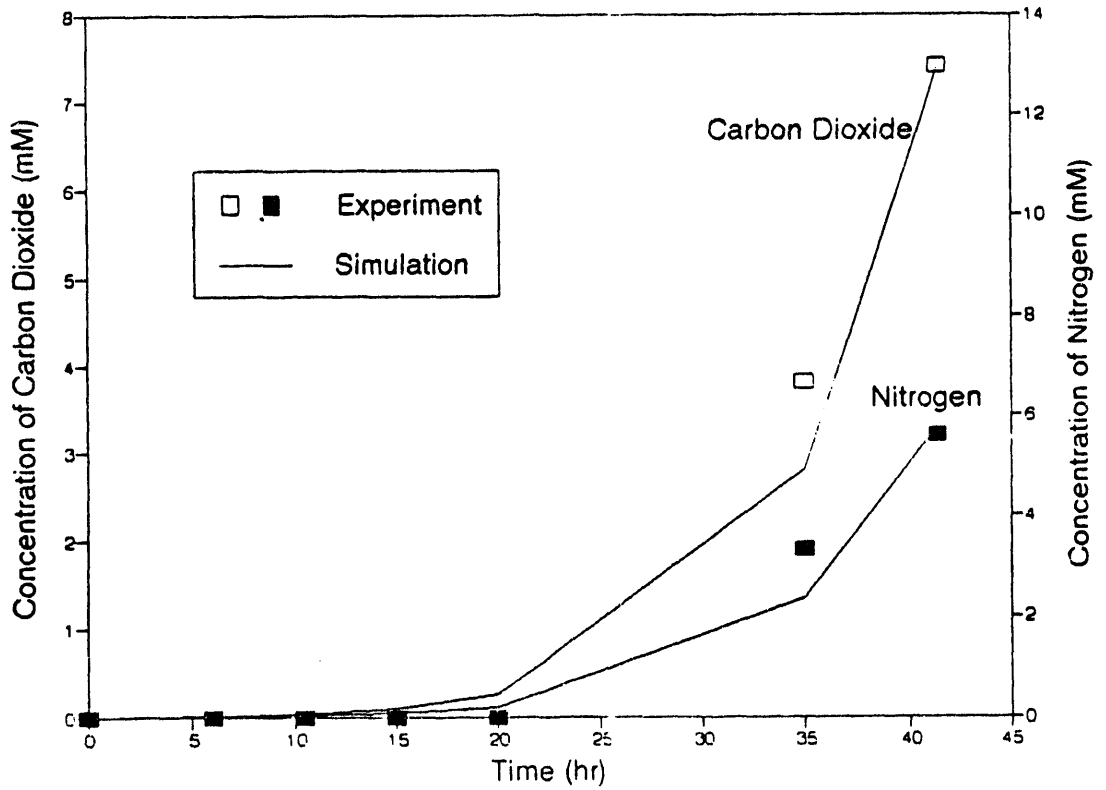


Figure 4. Gas production in static cores.

measured in the experiment (Figure 8). As shown in Figure 8, nutrient consumption slowed after treatments 5 and 6 which suggested that microbial growth and metabolism was controlled by the two substrates.

Figure 9 compares the predicted and measured permeability reduction factors. The top solid line (f=1) describes permeability reduction due only to pore-surface retention of biomass. For the middle line (f<1, C=0), both pore-surface retention and pore-throat plugging are considered, but the effect of the sessile phase on pore-throat plugging is ignored. The bottom line (f<1, C>0) represents the new permeability modification model used in this study. The model appears to predict the basic trend of permeability reduction observed in the experiment. Since viable cells were injected into the core system during the first four treatments, the relatively large portion of the bacteria remaining in the flowing water phase could cause significant plugging in pore throats. Injection of the bacteria into the core was stopped in the later treatments. However, the continuous decrease in permeability during later treatments could be explained as a result of plugging due to in situ microbial growth.

4. CONCLUSIONS

1. A model was developed that can predict biomass growth, metabolic product formation, substrate consumption, and permeability reduction in a MEOR process.

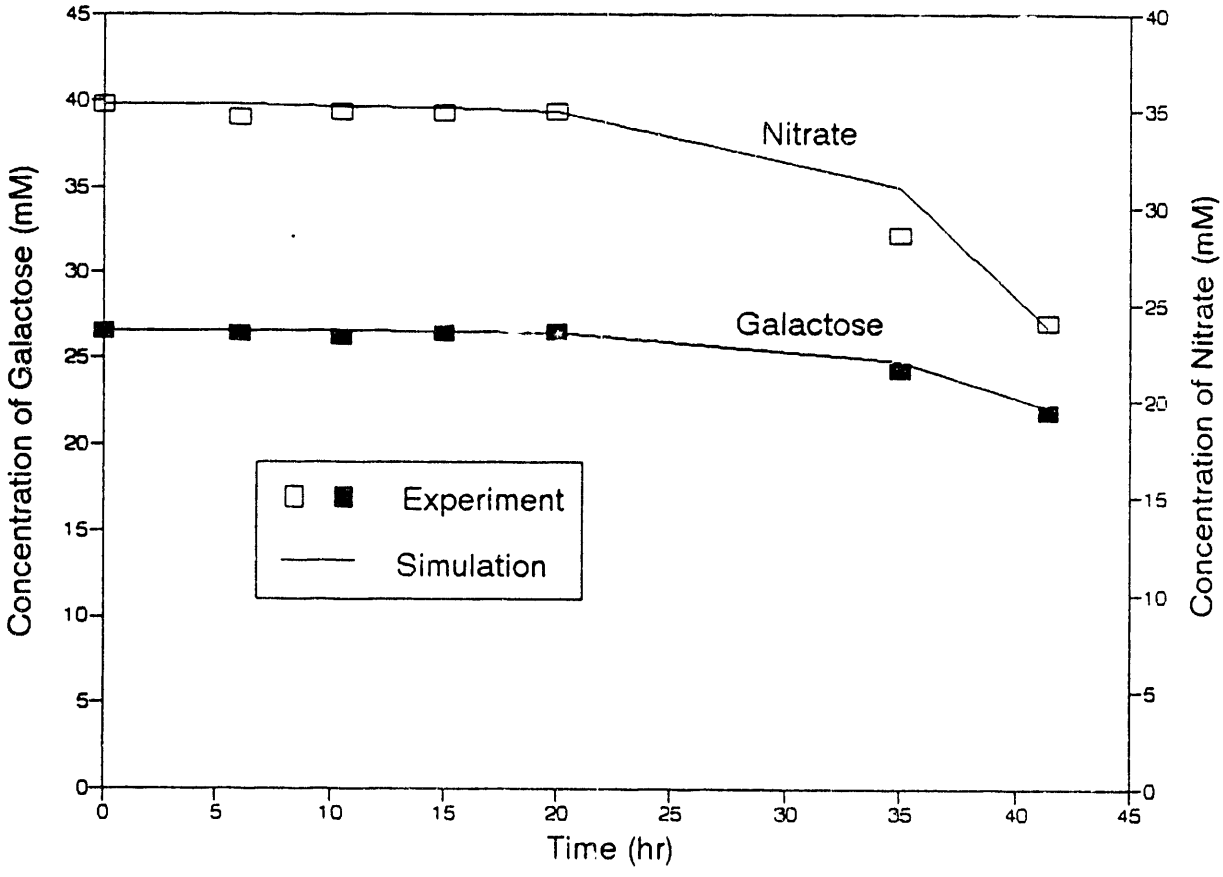


Figure 5. Biomass growth in static cores.

Table 4
Core and injection data

Symbols	Definition	Values	Units
D	core diameter	5.100	cm
L	core length	15.200	cm
ϕ_0	initial porosity	0.220	cm^3/cm^3
K_{w0}	initial permeability to water	55.000	md
u_f	injection rate	2.291	cm/hr
C_B	injected bacterial conc.	9.0×10^8	$cell/cm^3$
C_{S1}	injected glucose conc.	59.000	mM
C_{S2+}	injected ammonium conc.	47.500	mM
C_{S2-}	injected nitrate conc.	37.500	mM

2. Considering both pore-surface retention and pore-throat plugging due to planktonic bacteria and in situ microbial growth is a more effective approach to model modifications in permeability.

3. The comparison between numerical solutions and laboratory results indicated that the model simulated the basic species transport phenomena and microbial kinetics observed in laboratory experiments.

Table 5
Pore throat plugging data

Symbols	Definition	Values	Units
X_{ptmin}	min. pore throat diameter	1.000	μm
X_{ptmax}	max. pore throat diameter	100.000	μm
X_p	cell body size	10.000	μm
A	empirical const. for plugging	4.000	
B	empirical const. for plugging	0.049	
C	empirical const. for plugging	11.500	

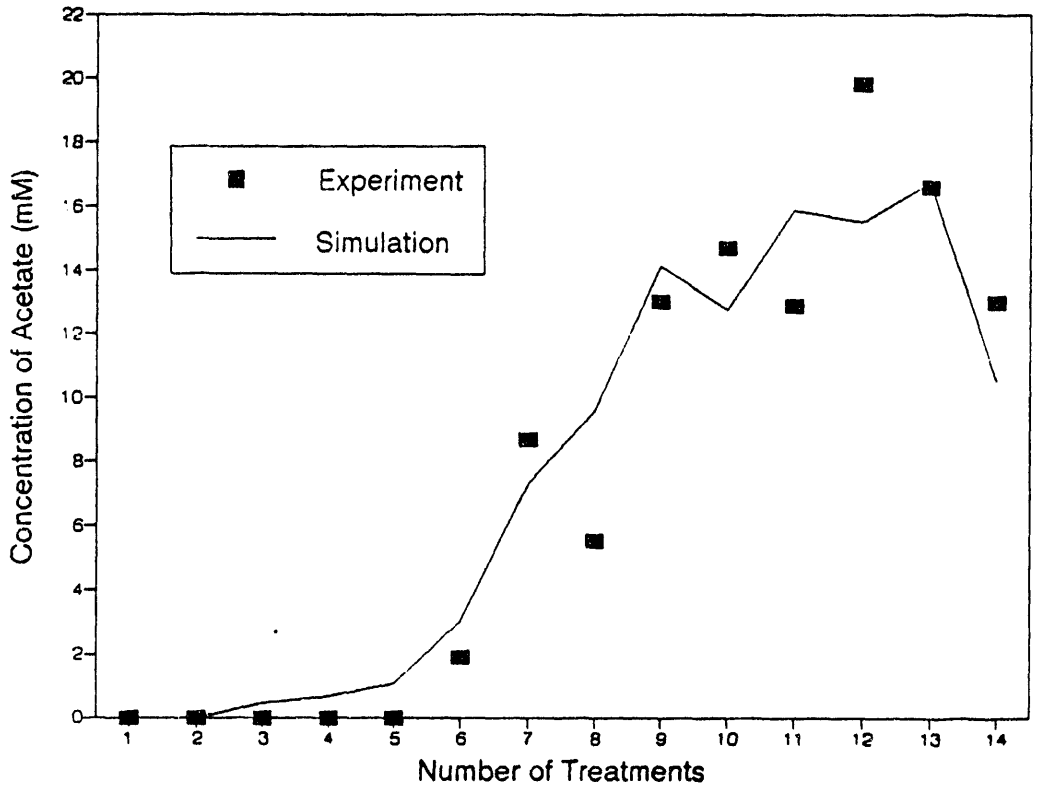


Figure 6. Acetate production in MEOR processes.

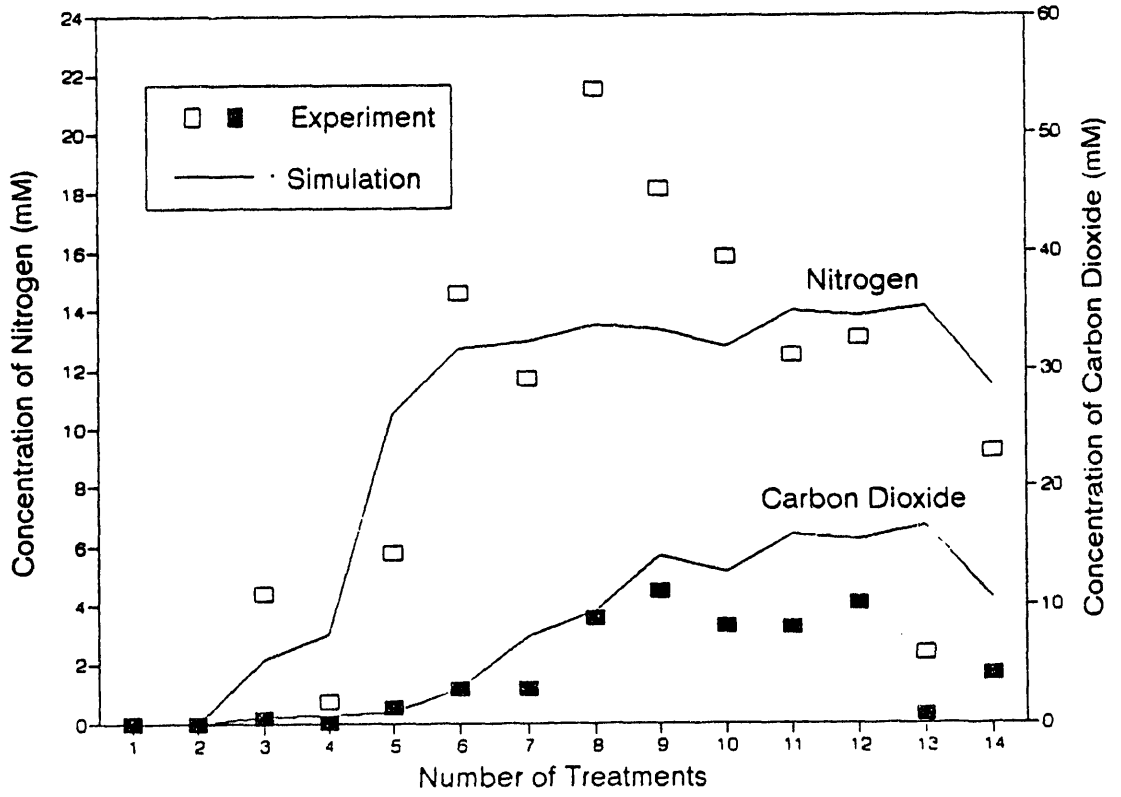


Figure 7. Gas production in MEOR processes.

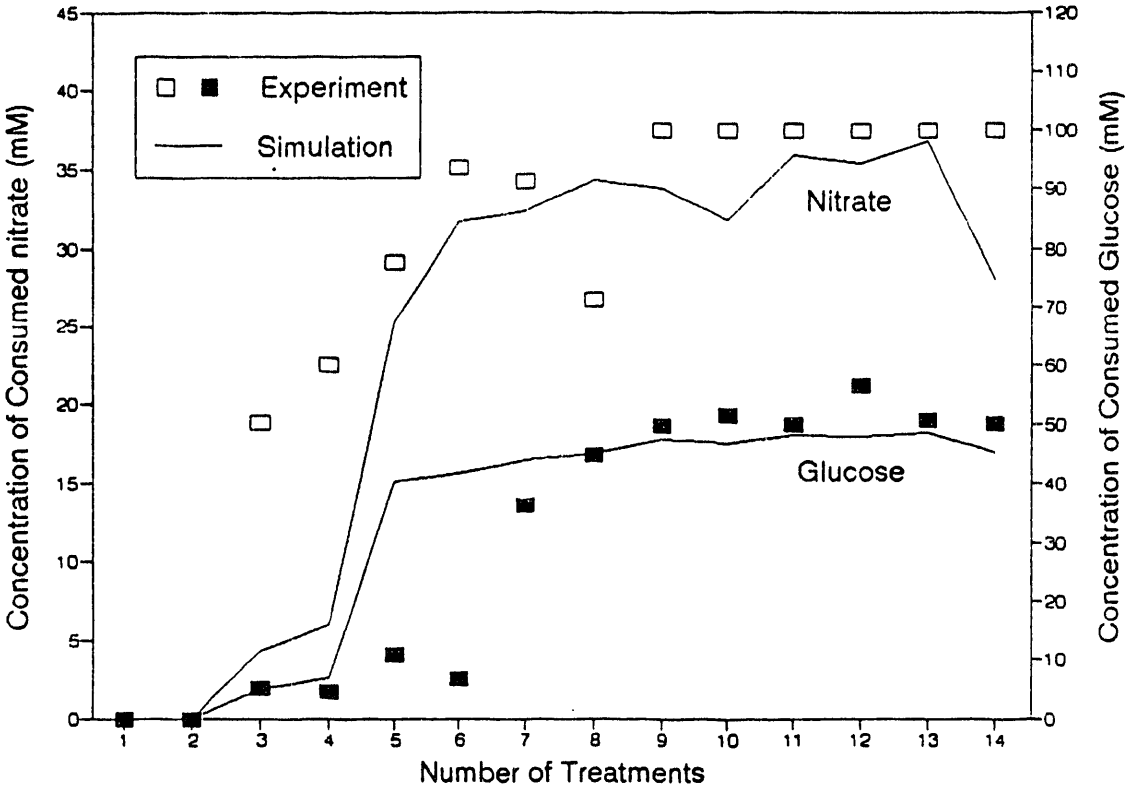


Figure 8. Nutrient consumption in MEOR processes.

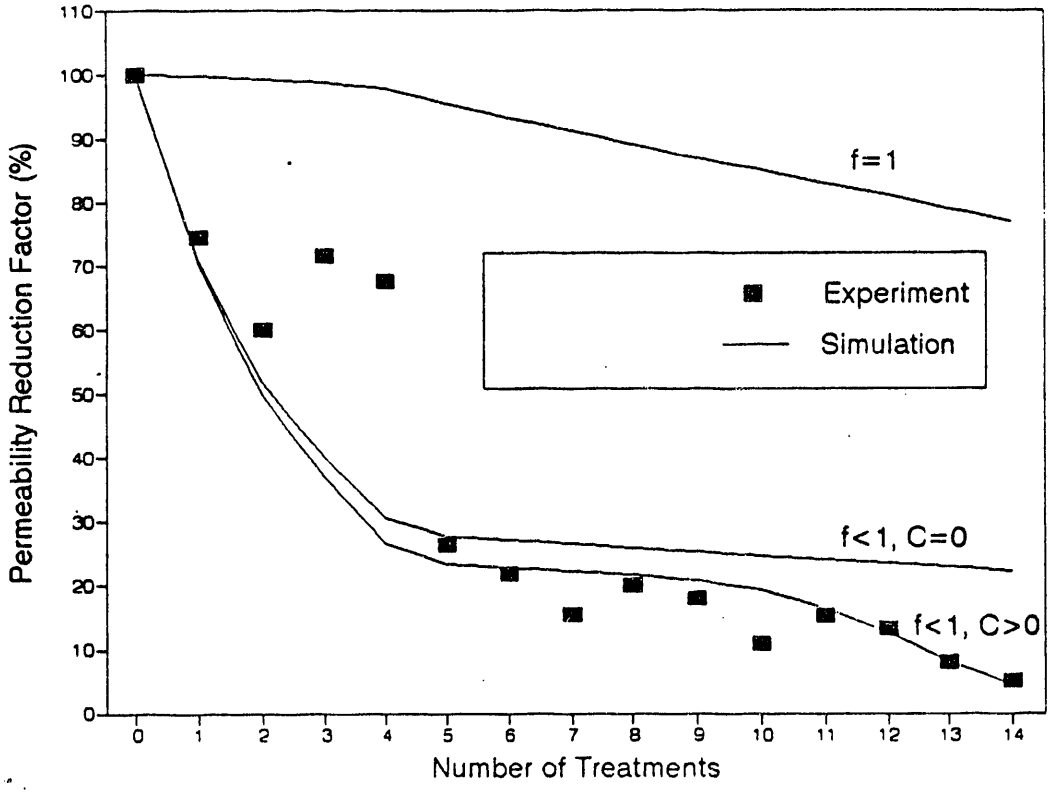


Figure 9. Permeability reduction in MEOR processes.

5. NOMENCLATURE

A - const. for pore throat plugging
B - const. for pore throat plugging
C - const. for pore throat plugging
B_l - formation vol. factor of phase l
C_i - conc. of species i (mg/cm^3)
C^*_S - crit. conc. of substrate S (mg/cm^3)
K_D - dispersion coeff. (cm^2/hr)
K_d - cell detachment coeff. (cm^2hr/mg)
K_r - cell retention coeff. (cm^2/mg)
K_0 - initial permeability (md)
K - instantaneous permeability (md)
K_{rl} - relative permeability to phase l
$K_{P/S}$ - satuation const. for product P on substrate S (mg/cm^3)
$K_{B/S}$ - satuation const. for biomass B on substrate S (mg/cm^3)
m_S - coeff. for energy maintenance by substrate S (mg/mg.hr)
R_d - cell detachment rate (mg/cm^3hr)
R_r - cell retention rate (mg/cm^3hr)
R_{Bf} - production rate for biomass in flowing phase (mg/cm^3hr)
R_{Bs} - production rate for biomass in sessile phase (mg/cm^3hr)
R_P - production rate for product P (mg/cm^3hr)
R_S - consumption rate for substrate S (mg/cm^3hr)
p - pressure (psi)
S_l - satuation for phase l (cm^3/cm^3)
u_f - Darcy flux (cm/hr)
X_p - viable cell size (μm)
X_{ptmin} - minimum pore throat size (μm)
X_{ptmax} - maximum pore throat size (μm)
X_{ptcr} - critical pore throat size for plugging (μm)
$Y_{B/S}$ - yield coeff. for biomass B on substrate S (mg/mg)
$Y_{P/S}$ - yield coeff. for product P on substrate S (mg/mg)

Greek Symbols

ϕ_0 - initial porosity (cm^3/cm^3)
ϕ - instantaneous porosity (cm^3/cm^3)
σ - pore fraction occupied by sessile phase (cm^3/cm^3)
ρ_B - density of biomass (mg/cm^3)
ρ_l - density of phase l (mg/cm^3)
μ_l - viscosity of phase l (cp)
μ_B - Monod growth rate of biomass (1/hr)
μ_{Bm} - max. specific growth rate (1/hr)
μ_{Pm} - max. specific production rate (1/hr)

Subscript

i - B (biomass), A (acetate), C (CO_2), N (N_2), S1 (glucose), $S2^+$ (ammonium), and $S2^-$ (nitrate)
l - o (oil), w (water), and g (gas)
P - A (acetate), C (CO_2), and N (N_2)
S - S1 (glucose), $S2^+$ (ammonium), and $S2^-$ (nitrate)

6. ACKNOWLEDGEMENTS

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Effect of Hydrophobicity of the Solid Substratum on Oil Displacement in the Hele-Shaw Model

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Abstract

A study was made to assess the influence of surface free energies of oil, solid substratum, and displacing aqueous liquid, γ_{OV}, γ_{SV}, and γ_{LV}, on oil displacement in the Hele-Shaw model [1,2] which was used as a simple model of the oil reservoir.

From the simulation made according to equation (2) which was derived thermodynamically, the adsorption-desorption phenomena of oil on or from the surface of a solid substratum can be related to the surface free energies of both the solid substratum, γ_{SV}, and the aqueous liquid as a continuous phase of the system, γ_{LV}. It can be predicted that oil adhered to the surface of a solid substratum may become energetically more difficult to remove and displaced with water, if the surface becomes more hydrophobic. However, when an aqueous surfactant solution, exhibiting extremely low surface tension, is used as a displacing fluid in place of water, oil displacement may become more effective, if the surface of the solid substratum becomes more hydrophobic.

A series of oil displacement experiments were carried out to verify the theoretical prediction. The results were in reasonable agreement with the results of the theoretical prediction made from equation (2). The thermodynamic approach used in this study may offer a powerful tool to predict the potential of oil recovery from porous media such as sandstone formations.

1. INTRODUCTION

Various laboratory models have been developed for evaluating oil removal from porous media. This study uses a Hele-Shaw model to investigate the factors influencing displacement of oil entrapped in the Hele-Shaw cell with a displacing fluid and the potential for enhanced oil recovery.

The Hele-Shaw model does not purport to be a method of simulating conditions present in a reservoir, but represents a simple method of evaluating and comparing solutions for their potential for enhanced oil recovery using a single-pore system.

Using a thermodynamic approach, an equation was derived which describes the energetical simplicity or difficulty of moving and displacing oil entrapped in a porous media of solid substratum with an aqueous displacing fluid. The equation correlates changes in interfacial free energy before and after the oil adheres to the surface of solid substratum, ΔG_{adh}, with the surface free energies of oil, γ_{OV}, displacing aqueous fluid, γ_{LV}, and solid substratum, γ_{SV}.

From the simulation made from equation (2), in the next section, Theoretical Considerations, we predict that displacing oil trapped in the Hele-Shaw cell with pure water may become energetically more difficult if the surface of the cell becomes more hydrophobic. The theoretical predictions also demonstrate that the use of a displacing fluid having extremely low surface tension remarkably improves oil displacement, if the surface of the Hele-Shaw cell becomes more hydrophobic.

To verify the theoretical predictions, a series of oil displacement experiments were carried out using the Hele-Shaw model.

2. THEORETICAL CONSIDERATIONS

On the basis of an interfacial free-energy balance under conditions where the electrical charge interaction is negligible, adhesion of oil drops, O, dispersed in a continuous phase of aqueous liquid, L, to the surface of a solid substratum, S, may be expected, if:

$$\Delta G_{adh} = \gamma_{OS} = \gamma_{OL} - \gamma_{OL} - \gamma_{SL} < 0 \tag{1}$$

where ΔG_{adh} indicates changes in the interfacial free energy before and after adhesion, and γ_{OS}, γ_{OL}, and γ_{SL} are interfacial free energies at the interfaces of O/S, O/L, and S/L, respectively.

Using Fowkes' approach [3] based on the separation of surface free energy, γ, into a dispersion component, γ^d, and a polar component, γ^p, and assuming the value of dispersion component of solid surface free energy, γ^d_{SV}, to be identical for every solid substrata considered in this study, the following equation can be derived from equation (1):

$$\frac{d(\Delta G_{adh})}{d\gamma_{SV}} = (\gamma^p_{SV})^{-1/2}\left[(\gamma^p_{LV})^{1/2} - (\gamma^p_{OV})^{1/2}\right] \tag{2}$$

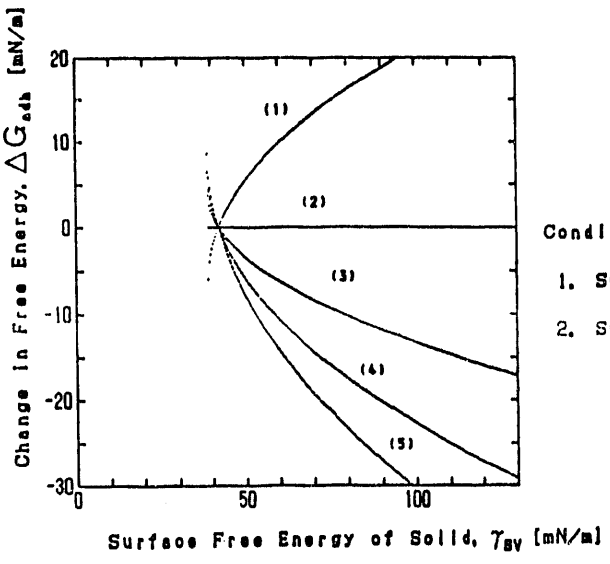
where, γ_{SV}, γ_{LV}, and γ_{OV} are surface free energies, or surface tensions, of solid substratum, aqueous liquid as a continuous phase, and oil dispersed in the continuous phase, respectively.

Usually γ^p_{OV} is less than γ^p_{LV} because the oil phase is much more hydrophobic than the aqueous liquid phase. Therefore, the left-hand side of equation (2) becomes greater than zero, which means that the adhesion of oil drops to the surface of a solid substratum and may become energetically more difficult if the surface of the solid substratum becomes more hydrophilic. In other words, oil adhered to the surface of a solid substratum may be removed from the surface and be displaced with aqueous liquid more easily if the surface becomes more hydrophilic.

By contrast, oil adhered to the surface of a solid substratum may become energetically more difficult to displace with aqueous liquid if the surface becomes more hydrophobic.

To clarify the results of the theoretical predictions made from equation (2), a simulation was made to investigate the factors influencing the ease of displacing the oil adhered to the surface of the solid substratum with aqueous liquid. Major factors involved in equation (2) which may affect adsorption-desorption phenomena of the oil on or from the surface of solid substrata placed in the aqueous continuous phase are the surface free energy of solid substratum, γ_{SV}, and the surface free energy of aqueous liquid as a continuous phase, γ_{LV}.

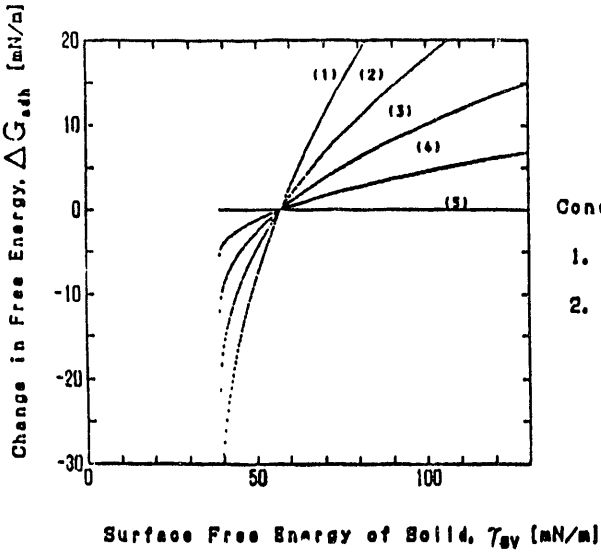
The simulation was made under conditions where these two parameters were independently varied to find how each parameter influenced adsorption-desorption of oil on or from the surface. The results are shown in Figures 1 to 3 as plots of ΔG_{adh} as a function of γ_{SV} with parameter of γ_{MV}.



Conditions:

1. Surface free energy of aqueous liquid, γ_{LV}=25 mN/m
2. Surface free energy of oil, γ_{OV}
   - (1) γ_{OV}=22 mN/m
   - (2) γ_{OV}=25 mN/m
   - (3) γ_{OV}=30 mN/m
   - (4) γ_{OV}=35 mN/m
   - (5) γ_{OV}=40 mN/m

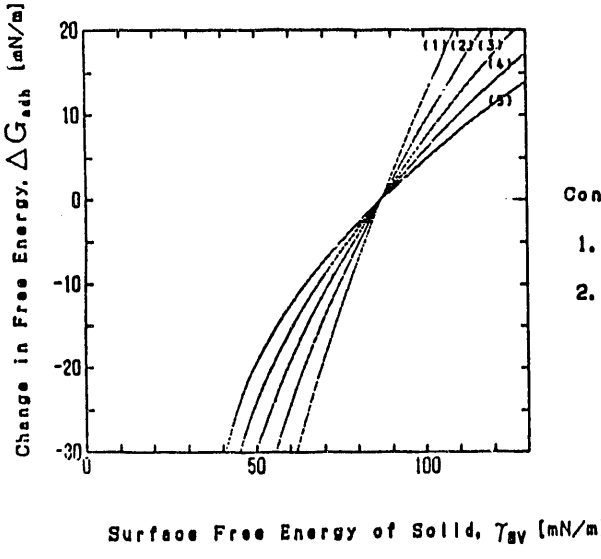
Figure 1. Simulation I.



Conditions:

1. Surface free energy of aqueous liquid, γ_{LV}=40 mN/m
2. Surface free energy of oil, γ_{OV}
   - (1) γ_{OV}=22 mN/m
   - (2) γ_{OV}=25 mN/m
   - (3) γ_{OV}=30 mN/m
   - (4) γ_{OV}=35 mN/m
   - (5) γ_{OV}=40 mN/m

Figure 2. Simulation II.



Conditions:

1. Surface free energy of aqueous liquid, γ_{LV}=70 mN/m
2. Surface free energy of oil, γ_{OV}
   - (1) γ_{OV}=22 mN/m
   - (2) γ_{OV}=25 mN/m
   - (3) γ_{OV}=30 mN/m
   - (4) γ_{OV}=35 mN/m
   - (5) γ_{OV}=40 mN/m

Figure 3. Simulation III.

These results suggest that oil adhered to the surface of solid substratum with pure water may become energetically more difficult to displace if the surface becomes more hydrophobic. However, when an aqueous surfactant solution having extremely low-surface free energy is used instead of water, displacement of oil adhered to the surface of the solid substratum may become more effective if the surface becomes more hydrophobic.

3. EXPERIMENTS

To experimentally verify the results of theoretical predictions, a series of oil displacement experiments were carried out, using both hydrophilic and hydrophobic Hele-Shaw cells.

3.1. Determination of surface free energy

3.1.1. Surface free energy of liquids

The surface free energy, or the surface tension of oil, γ_{OV}, and the aqueous displacing fluid, γ_{LV}, can be directly measured by using a tensiometer. In this study, we followed Wilhelmy's vertical plate method.

3.1.2. Surface free energy of hydrophobic solid substratum

The surface free energy of a hydrophobic solid substratum, γ_{SV}, (a polyacryl resin plate was used as the Hele-Shaw cell material), was determined as critical surface tension, γ_C, obtained from Zisman plot [4] by measuring the contact angle.

3.1.3. Surface free energy of hydrophilic solid substratum

The surface free energy of hydrophilic solid substratum γ_{SV}, (a glass plate was used as the Hele-Shaw cell material) was determined using a method developed by Busscher and Arends [5] by measuring contact angle.

3.2. Oil displacement experiment

3.2.1. Oil

Liquid paraffin was used as the oil to be displaced in the Hele-Shaw model. The viscosity of the liquid paraffin was 59.5 cP. The surface free energy, or surface tension, measured with Wilhelmy's vertical plate tensiometer, was 32.5 mN/m. Operational flow through the Hele-Shaw cell was laminar, with a Reynolds number below 300. Hereafter, the liquid paraffin is referred to as oil.

3.2.2. Displacing fluid

Aqueous sodium alginate was used as the displacing fluid. The viscosity of this solution was 60.0 cP, which is almost identical with that of liquid paraffin. The surface tension of the solution was 69.5 mN/m at 30°C, which is not different from that of pure water. Hereafter, the aqueous sodium alginate solution is called displacing fluid-1. Another displacing fluid used was prepared by dissolving sodium dodecylbenzenesulfonate, a synthetic surfactant, and n-butanol, a co-surfactant, in water. We call this aqueous solution displacing fluid-2. The surface tension was 25.0 mN/m.

3.2.3. Apparatus

The Hele-Shaw apparatus, shown in Figure 4, consisted of (a) a storage vessel with a spigot containing the displacing fluid, silicone tubing, and glass tubing; (b) the Hele-Shaw cell; (c) a graduated cylinder, and (d) a platform for

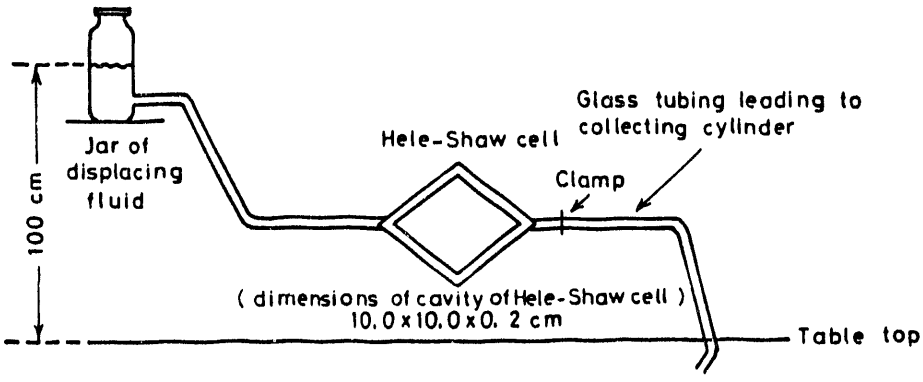


Figure 4. Experimental set up of the Hele-Shaw model.

adjusting the height of the displacing fluid to achieve a constant head pressure. The height was adjusted to 100 cm in this experiment.

The Hele-Shaw model consists of two closely spaced plates (2 mm spacing) with provisions for displacing fluid from the cell. Spacing of the plates was accomplished by placing silicone tubings at the edges of the plates and gluing them together with a sealant.

Two materials were used in this experiment as the plates; a glass plate with a hydrophilic surface, and a polyacryl resin plate, which is typical of hydrophobic, synthetic polymer materials. Hydrophilic and hydrophobic Hele-Shaw cells were assembled by using a glass plate and polyacryl resin plate, respectively.

Stainless steel tubes were placed at opposite corners of the model for inlet and outlet flow. The internal dimensions of the cells were arbitrarily chosen to be 10.0 cm x 10.0 cm x 0.2 cm.

3.2.4. Experimental procedure

The spigoted displacement jar was filled with 200 ml of the test fluid and then raised to a height of 100 cm. We carefully removed all air bubbles in the tubing leading from the jar. The Hele-Shaw cell was then filled with oil (liquid paraffin) by connecting it to the second spigoted displacement jar filled with oil. After being filled with oil, the Hele-Shaw cell was connected to the jar containing the test fluid. The oil-water system was held in place with a clamp at the position shown in Figure 4. We used a 100 ml graduated cylinder to collect oil and oil-water flowing through the model.

To start the test, the clamp was removed and flow started. As expected, the initial liquid displaced was oil. At the point of water breakthrough, the flow was stopped, and the % oil displacement was determined as the fraction of oil displaced and collected in the cylinder to the total amount of oil initially in the Hele-Shaw cell, as defined by equation (3):

$$\% \text{ oil displacement} = \frac{\text{amount of oil displaced at the point of water breakthrough}}{\text{amount of oil initially in the Hele-Shaw cell}} \times 100 \qquad (3)$$

Table 1
Experimentally determined surface free energies

Oil (liquid paraffin)	γ_{OV} = 32.5 mN/m
Displacing fluid-1 (aqueous sodium alginate solution)	γ_{LV} = 69.5 mN/m
Displacing fluid-2 (aqueous surfactant and co-surfactant solution)	γ_{LV} = 25.0 mN/m
Solid surface of hydrophobic Hele-Shaw cell (polyacryl resin plate)	γ_{LV} = 41.0 mN/m
Solid surface of hydrophilic Hele-Shaw cell (glass plate)	γ_{LV} > 70 mN/m

Photographs were taken during each displacement run at appropriate intervals to monitor changes in the fraction of oil remaining in the Hele-Shaw cell.

4. EXPERIMENTAL RESULTS

4.1. Determination of surface free energies

Table 1 shows experimental values of surface free energies of (a) oil, (b) displacing fluid - 1, and - 2, (c) the glass plate, and (d) the polyacryl resin plate given.

4.2. Simulation according to equation (2)

By using the surface free energies summarized in Table 1, theoretical predictions were made on the ease or difficulty of oil displacement. Figures 5 and 6 show the results of the simulation. Shaded curves indicate the change in ΔG_{ads} as a function of γ_{SV} when liquid paraffin is the oil to be displaced.

Figure 5 shows the result of the simulation made when aqueous sodium alginate solution was used as the displacing fluid (its surface free energy is approximately 70 mN/m, almost identical to that of pure water). Figure 5 indicates that displacement of oil trapped in the Hele-Shaw cell with displacing fluid-1 may become energetically more difficult if the surface of the Hele-Shaw cell plate becomes more hydrophobic.

Figure 6 shows the result of the simulation made when an aqueous surfactant solution having extremely low surface tension is used as a displacing fluid in place of pure water. The surface tension of the displacing fluid-2 was determined to be 25.0 mN/m. Under these conditions, displacement of oil trapped in the Hele-Shaw cell with this fluid may energetically become more effective if the surface of the cell becomes more hydrophobic.

4.3. Results of oil displacement experiments

4.3.1. Oil displacement experiments with displacing fluid-1

Fractions of oil remaining in the Hele-Shaw cell composed of (a) glass plates (hydrophilic Hele-Shaw cell), and (b) polyacryl resin plates (hydrophobic Hele-

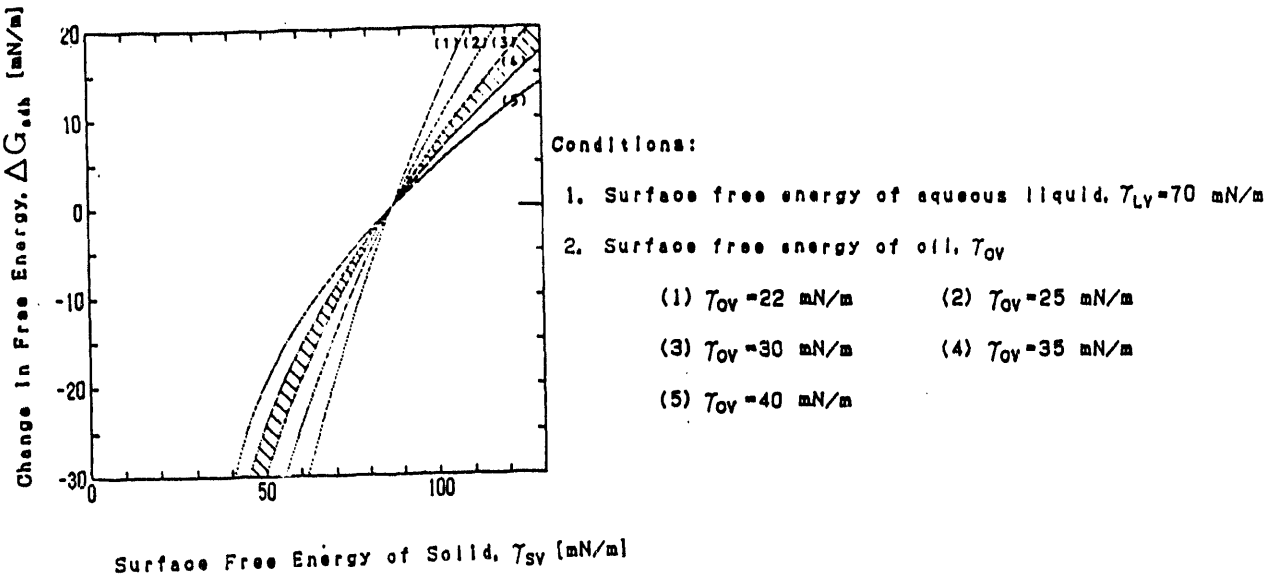


Figure 5. Simulation of oil displacement with displacing fluid-1.

Shaw cell) as the function of time are shown in Figure 7. The shaded area indicates oil remaining in the cell.

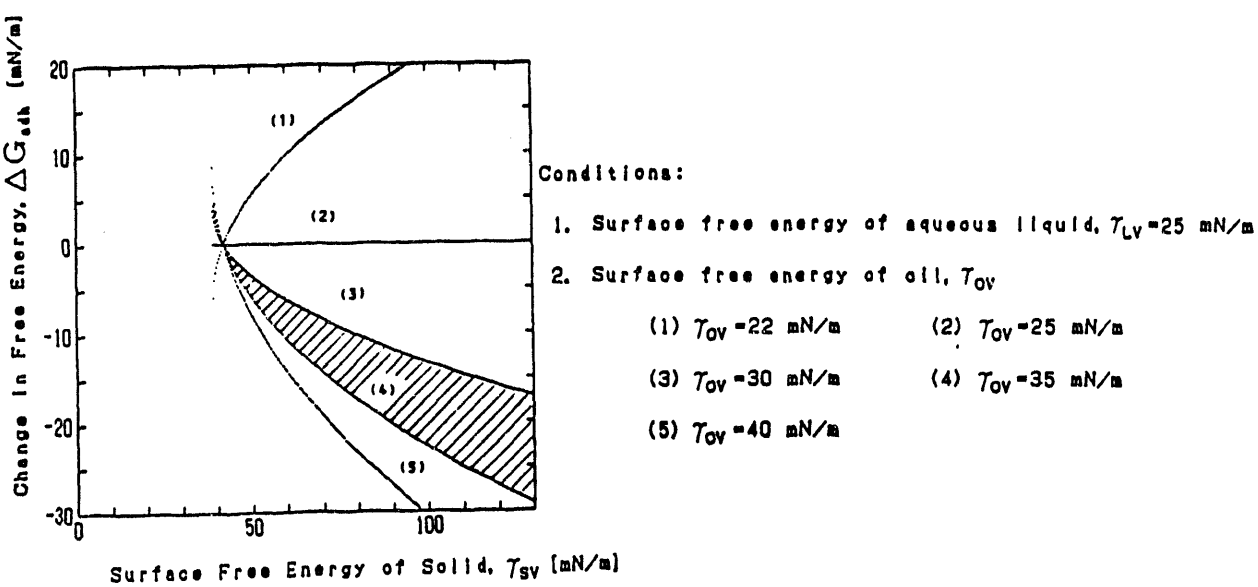
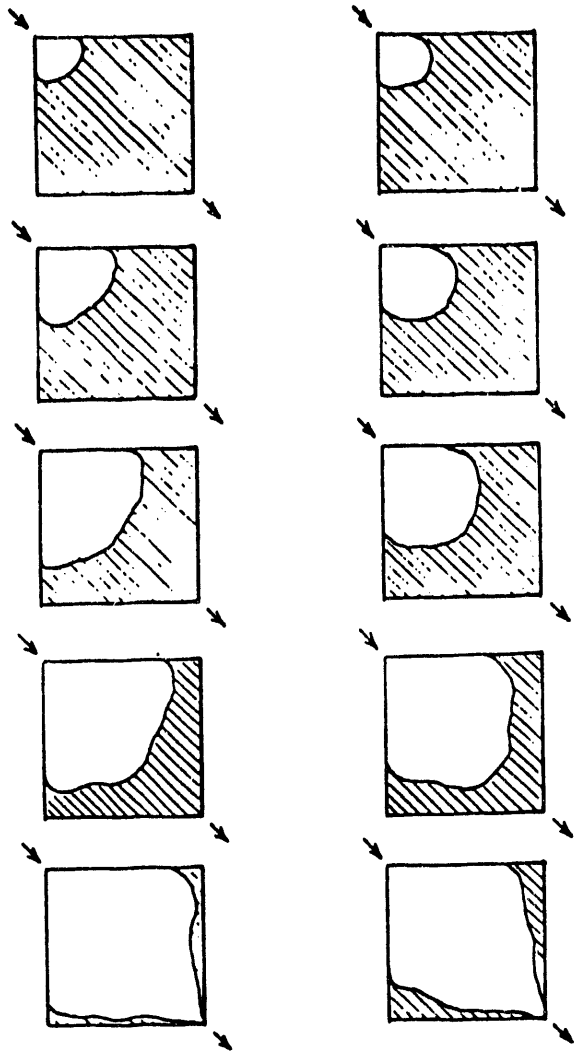
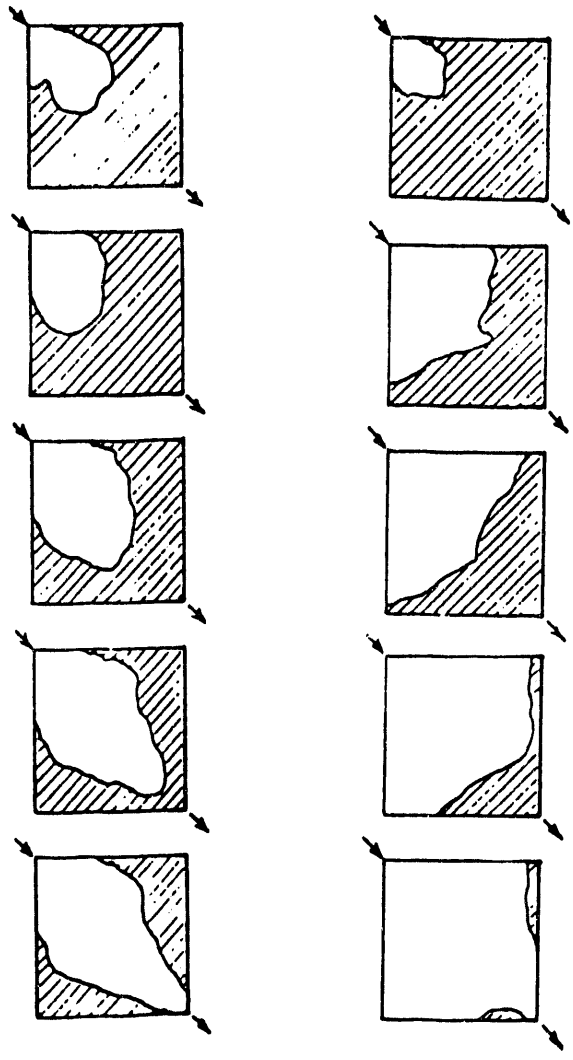


Figure 6. Simulation of oil displacement with displacing fluid-2.



(a)Hydrophilic cell (b)Hydrophobic cell

Figure 7. Result of oil displacement experiment with displacing fluid-1 in both hydrophilic and hydrophobic Hele-Shaw cells.



(a)Hydrophilic cell (b)Hydrophobic cell

Figure 8. Result of oil displacememt with displacing fluid-2 in both hydrophilic and hydrophobic Hele-Shaw cells.

The % oil displacement, which is defined by equation (3), was 88.2% in the glass plate cell, and 78.8% in the polyacryl resin plate cell, respectively. There is a significant difference in the results of % oil displacement between hydrophilic and hydrophobic Hele-Shaw cells, and the result is in reasonable agreement with the theoretical prediction made from the simulation shown in Figure 5.

4.3.2. Oil displacement experiments with displacing fluid-2

Figure 8 shows the change in the fraction of oil remaining in the hydrophilic and hydrophobic Hele-Shaw model as a function of time. As shown in Figure 8, oil entrapped in the Hele-Shaw cell composed of the hydrophobic surface of polyacryl resin plates can be displaced, almost completely, with displacing fluid that has

extremely low-surface free energy. By contrast, in the hydrophilic Hele-Shaw cell composed of glass plates, the % oil displacement was approximately 60%, much less than that of the hydrophobic cell. This result may support the theoretical considerations made on the use of displacing fluid having extremely low surface tension, which predict that the displacement of oil entrapped in the Hele-Shaw cell may become more effective if the surface of the cell becomes more hydrophobic (Figure 6).

5. CONCLUSIONS

A thermodynamic approach may offer a tool for predicting the ease of oil recovery from the Hele-Shaw model. From theoretical predictions, and from oil displacement experiments using both hydrophilic and hydrophobic Hele-Shaw cells, we presume that oil recovery from the reservoir by waterflooding becomes less effective if the reservoir is composed of more hydrophobic material and is oil-wetted. By contrast, oil recovery by a low-tension waterflooding technique may become more effective when the reservoir is oil-wetted.

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Microbially Enhanced Oil Recovery Field Pilot, Payne County, Oklahoma

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Abstract

A multi-well MEOR field study was performed in the Vassar Vertz Sand Unit in Oklahoma. The purpose of the field trial was to determine whether microorganisms could be used to preferentially plug high permeability zones to improve waterflood sweep efficiency. Laboratory studies determined that a nutrient system based on molasses and ammonium nitrate would induce the growth of nitrate-utilizing bacteria indigenous to the Vertz unit reservoir while limiting sulfate reduction. Tracer studies were done to determine the predominant flow pattern from the pilot injection well within the reservoir. The molasses and ammonium nitrate nutrients were injected over a four-month period. Samples were routinely analyzed for sulfate-reducing bacteria, molasses-nitrate utilizing bacteria, general fermentative bacteria, microbial metabolites, carbohydrates, sulfide, sulfate, nitrite, and nitrate. Over the period of nutrient injection, 56.2 tonnes of molasses and 18.8 tonnes of ammonium nitrate were injected into the pilot area. The results show that large-scale injection of readily metabolizable carbohydrates did not detrimentally affect the ongoing operation in the field but did result in an alteration of the existing flow patterns and reduction in transmissibility within the pilot area.

1. INTRODUCTION

The United States remains dependent on crude oil and crude oil products to fuel its economy and although it is one of the world's largest producers of oil, its imports constitute over 40% of current U.S. consumption. Nearly two-thirds of all of the known U.S. oil (330 billion barrels) will be abandoned after conventional primary and secondary recovery techniques have been exhausted (Department of Energy, 1985). Enhanced oil recovery (EOR) methods increase the total recovery of oil beyond that achievable with primary and secondary methods by increasing the proportion of the reservoir affected (sweep efficiency), reducing the amount of residual oil in the swept zones, and reducing the viscosity of heavy oils. Unfortunately, conventional EOR technologies have only been marginally effective and the strong dependence of their economics on the price of oil has limited widespread application. Microbially enhanced oil recovery (MEOR) is a newer technology that has evolved in recent years, with a great potential for cost-effective recovery of residual oil. Crawford [1,2] hypothesized that injected bacteria could preferentially plug high-permeability zones, and that this plugging could correct variations in permeability in oil

reservoirs. Recent results from field pilot studies on interwell MEOR floods have been encouraging [3-6].

The Department of Botany and Microbiology and the School of Petroleum and Geological Engineering at University of Oklahoma have been involved in a field pilot study at the Southeast Vassar Vertz Sand Unit (SEVVSU), Payne County, OK. The objective of this study was to determine whether stimulation of indigenous bacteria in the reservoir by injecting molasses and ammonium nitrate could selectively plug high-permeability regions of a reservoir and improve volumetric sweep efficiency.

2. DESCRIPTION OF STUDY AREA AND THE FIELD PILOT TEST

The Southeast Vassar Vertz Sand Unit (SEVVSU) is located 10 miles southwest of Stillwater, in Payne County, Oklahoma. A plat map of the reservoir is shown in Figure 1. The field is currently operated using a line drive waterflood with wells 3-2, 3-3, 7-3, 7-5, and 9-2 active as the brine injection wells. The flood front is believed to move in a northeasterly direction. The field pilot was performed in the southwest quarter of section 13, behind the line of active brine injection wells. Wells 5-1, 5-2, and 7-1 were converted to production wells, and well 7-2 served as the injector for the pilot trial. Daily rates of fluid production in the pilot area were set at 150 barrels per day (bbl/d) each for wells 5-1 and 5-2, and at 300 bbl/d for well 7-1. About 300 bbl/d of brine was injected into 7-2. The production of 300 bbl/d more from wells 5-1, 5-2, and 7-1 than injected into 7-2 was done to ensure that the fluids injected into well 7-2 would mostly flow to the pilot production wells.

Previous studies determined that the flood water from the reservoir was highly saline containing 11 to 19% NaCl and 1 to 2% calcium and magnesium ions. Diverse populations of anaerobic bacteria capable of growing with simple nutrient additions at these salinities are present throughout the reservoir. An active sulfate-reducing population was present in the waterflooded areas of the reservoir and in the water tanks at the field tank battery. Both sulfate-reducing and nitrate-reducing bacteria were isolated and characterized. Of these, five nitrate-reducing bacteria were chosen for more extensive study. These were shown to be new obligate halophiles with a definite requirement for salt of concentrations greater than 6%. Of the various carbohydrate and inorganic nutrient mixtures tested, a molasses-ammonium nitrate mixture best stimulated their growth [7]. Several core experiments were performed in which permeability was altered and residual oil was recovered as a result of in-situ microbial growth at the temperature and pressure of the field using oil, brine, core material, and bacteria from the SEVVSU [8].

3. MATERIALS AND METHODS

3.1. Nutrient injection

Nutrient injection into well 7-2 was initiated at the end of August 1991. In total, 56.2 tonnes of molasses and 18.8 tonnes of ammonium nitrate were injected in four phases over four months. On August 29, 2.6 tonnes of ammonium nitrate were injected into the 7-2 well as previous studies had shown inhibition of sulfide production in both oil field samples and sewage sludge samples by high

samples by high nitrate concentrations [9, 10]. Beginning on September 27, 10.9 tonnes of molasses and 3.2 tonnes of ammonium nitrate were injected into well 7-2. Injection was stopped on October 4 for a 30-day incubation period to allow metabolism of the nutrients injected in the pilot area. A third nutrient treatment was initiated on November 9, during which 22.7 tonnes of molasses and 6.3 tonnes of ammonium nitrate were injected into the pilot area. This injection was completed on November 25. A fourth and final nutrient treatment of 22.7 tonnes of molasses and 6.7 tonnes of ammonium nitrate was injected from December 3 to 20. Injection into well 7-2 and production from wells 5-1, 5-2, and 7-1 was stopped for a second incubation period of 14 days on January 28, 1992.

3.2. Biochemical analysis

Brine samples were routinely collected from wells 5-1, 5-2, 7-1, 1A-1, and 1A-9. Nitrate and nitrite were analysed using chemical test kits (Spectroquant). Hydrogen sulfide was determined colorimetrically [11]. Sulfate concentration was

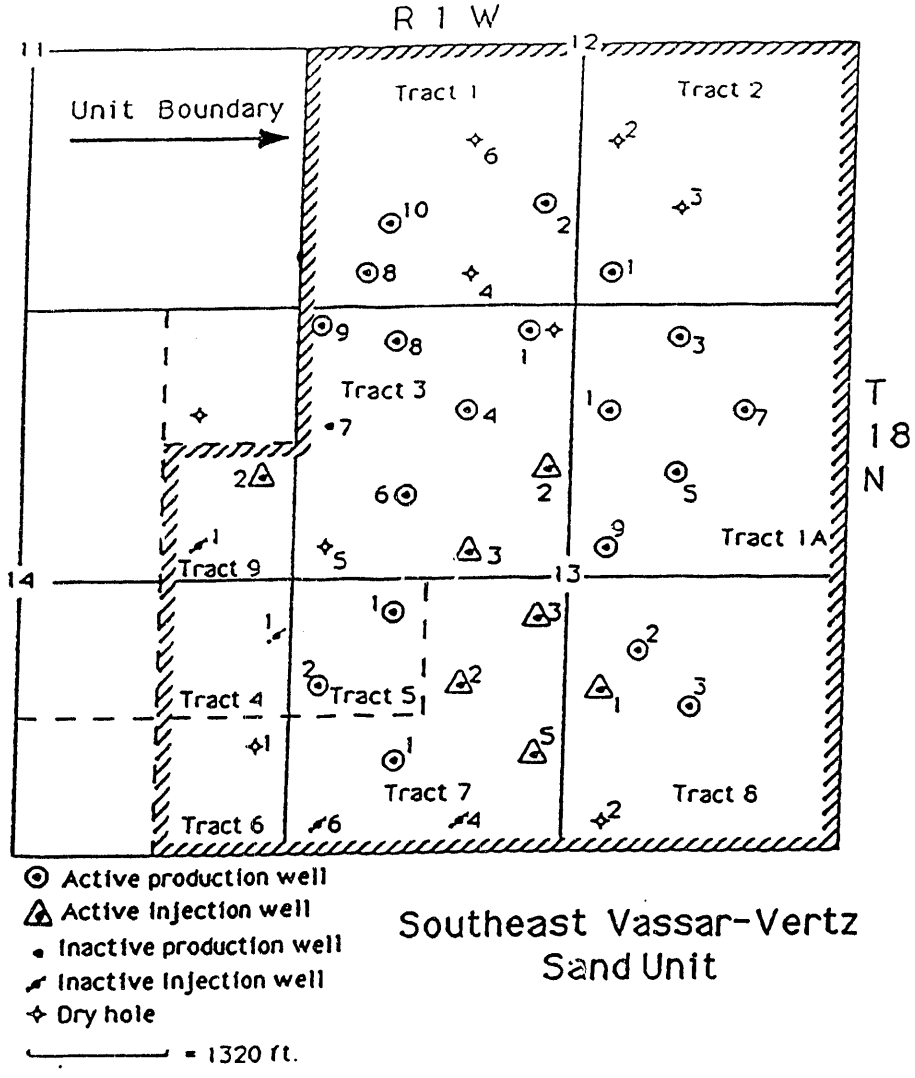


Figure 1. Plat Map of the Southeast Vassar Vertz Sand Unit (SEVVSU).

determined by high-performance liquid chromatography using an ion exchange column and a conductivity detector. Volatile fatty acids were determined by gas chromatography. Alkalinity was determined as outlined by Clesceri et al. [12] and total carbohydrates were determined by the phenol-sulfuric acid method [13].

3.3. Bacteriological analyses

Enumeration studies were done by the three-tube most probable number (MPN) technique. Aerobic heterotrophic bacteria, anaerobic heterotrophic bacteria, anaerobic bacteria using molasses-nitrate, and sulfate-reducing bacteria were enumerated using media containing 15% NaCl as previously outlined [14].

4. RESULTS AND DISCUSSION

4.1. Pre-treatment data

Before treating the pilot area, baseline concentrations of salinity, alkalinity, sulfide, sulfate, nitrite, nitrate, carbohydrate, and volatile fatty acids were determined on a regular basis. From February 1990 to August 1991, the sulfide concentration in the reservoir increased from an average of 11 mg/l to over 52 mg/l. This increase in souring was the result of an increase in the volume of make-up water used, which has a sulfate concentration of 2,500 mg/l.

Two fluorescein tracer studies were also completed before nutrient injection to determine the predominant flow path of the injected brine in the pilot area. The results indicated a significant flow channel existed from 7-2 to the 1A section of the reservoir, although it was originally believed that the preferential flow path was from well 7-2 to 7-1. On both occasions, the tracer was observed in wells 1A-1, 1A-5, and 1A-9, a distance of 1870 feet from 7-2, within 18 days of initial injection, thus, traveling over 100 feet per day. The calculated rate of travel of the tracer through the reservoir suggested the existence of a fracture system.

4.2. Microbial activation by nutrient treatment

Following the first injection on August 29 of 2.6 tonnes of ammonium nitrate, the nitrate concentration produced in the brine of the pilot production wells increased from undetectable levels to about 5 to 10 mg/l, 50 days later. Coproduced brine sulfide concentrations in the pilot area decreased from an average of 52 mg/l to 28 mg/l during the same period (Table 1). However, because of the high sulfide concentrations in the original brine and the high sulfate concentration in the make-up water, the amount of ammonium nitrate added was not sufficient to inhibit transient increases in the sulfide concentration of the coproduced brines due to molasses metabolism. About 56 days after the second nutrient injection, the sulfide concentrations increased and peaked at 203 mg/l for 5-1 and 5-2, respectively, and 334 mg/l for 7-1 before returning to 65 mg/l. A similar peak in sulfide concentration occurred in 5-1, 5-2, and 7-1 brines 57 days after the final nutrient injection. This time, however, the sulfide concentration of 7-1 brine attained a maximum of 195 mg/l while values for 5-1 and 5-2 were 251 mg/l and 184 mg/l, respectively (Table 1). Sulfide levels also increased in the brine from wells in the 1A tract although the levels were substantially lower (Table 1). It should be emphasized that although large increases in sulfide production were noted, these increases were transient and did not affect the long-term sulfide concentrations in the reservoir which had returned to a baseline value of 44 mg/l by March, 1992.

The numbers of sulfate-reducing bacteria in the coproduced brines from 5-1 and 5-2 also were observed to increase with nutrient injection and sulfide

Table 1
Sulfide results of coproduced brines

Date	Day	5-1	5-2	7-1	1A-1	1A-9
Feb. 15, 1990					6.60	14.23
Aug. 29, 1991	0	35.59	64.82	59.79	16.96	30.56
Sep. 27, 1991	29	48.93	50.37	33.93	22.49	23.25
Oct. 14, 1991	46	26.19	21.10	23.20	ND	37.96
Oct. 22, 1991	54	52.82	46.10	73.16	50.04	5.94
Nov. 18, 1991	81	68.17	63.12	86.02	58.43	33.42
Nov. 22, 1991	85	153.95	131.88	429.44	124.45	92.17
Dec. 06, 1991	100	203.03	203.03	334.49	99.84	22.53
Dec. 10, 1991	104	52.23	60.75	65.88	47.28	28.94
Jan. 03, 1992	128	62.35	ND	64.12	29.65	27.39
Jan. 17, 1992	142	144.69	157.30	170.95	132.73	93.08
Feb. 14, 1992	170	251.16	184.34	195.13	87.62	112.97
Mar. 20, 1992	201	60.19	60.19	64.48	25.09	37.70
May 20, 1992	262	67.51	ND	49.90	22.89	35.80

concentration although the changes in bacterial counts were relatively small (Table 2). The number of sulfate-reducing bacteria in the 7-1 brine samples also appeared to peak although the increase occurred 25 days after that of both 5-1 and 5-2. The numbers of sulfate-reducing bacteria in the coproduced brine from the 1A tract remained relatively stable throughout the entire trial (Table 2).

Table 2
Numbers of sulfate-reducing bacteria in the coproduced brines (cells/ml)

Date	5-1	5-2	7-1	1A-1	1A-9
Aug. 29, 1991	12.40×10^2	9.33×10^1	4.27×10^1	9.30×10^1	4.27×10^1
Nov. 05, 1991	9.33×10^2	4.27×10^2	4.77×10^1	4.27×10^2	9.33×10^1
Dec. 06, 1991	4.27×10^2	2.40×10^2	2.30×10^1	2.40×10^2	9.33×10^1
Jan. 17, 1992	2.40×10^2	9.30×10^1	4.62×10^2	2.40×10^2	9.33×10^1
Feb. 11, 1992	9.30×10^2	4.27×10^2	4.27×10^1	2.05×10^2	9.33×10^1
Mar. 03, 1992	2.31×10^1	2.31×10^1	9.20×10^0	4.27×10^1	9.20×10^0
Apr. 22, 1992	9.33×10^1	ND	9.33×10^1	2.40×10^2	9.33×10^1

Table 3
Molasses-nitrate utilizing bacteria in the coproduced brines (cells/ml)

Date	5-1	5-2	7-1	1A-1	1A-9
Aug. 29, 1991	$1.10x10^3$	$4.62x10^2$	$4.27x10^1$	$2.40x10^2$	$2.34x10^5$
Nov. 05, 1991	$2.31x10^1$	$2.31x10^1$	$2.31x10^1$	$2.40x10^2$	ND
Dec. 06, 1991	$2.31x10^1$	$2.31x10^1$	$2.31x10^1$	$2.31x10^1$	$2.31x10^1$
Jan. 17, 1992	$2.31x10^1$	$2.31x10^1$	$2.31x10^1$	ND	ND
Feb. 11, 1992	$2.31x10^1$	$2.31x10^1$	$2.31x10^1$	ND	ND
Apr. 22, 1992	$9.33x10^2$	ND	$9.33x10^2$	$2.40x10^3$	$4.27x10^2$

The numbers of molasses-nitrate utilizing bacteria in 5-1 and 5-2 brines decreased 10-fold over the first 70 days of the trial and then remained constant at 23 cells per ml. These bacteria in the 1A-9 brine decreased by 10^4 during the same period while those in the 1A-1 brine remained relatively constant throughout the trial (Table 3). The low counts of the molasses-nitrate utilizing bacteria after the injection of molasses and ammonium nitrate during this trial may be the result of sulfide toxicity due to the high concentrations attained.

During the latter part of 1991, analyses of the alkalinities of coproduced brine were started. Alkalinity increases of almost 50 mg/l $CaCO_3$ for 5-1 and 100 mg/l for 5-2 and 7-1, respectively, were observed from April 1991 baseline concentrations. Regular analyses thereafter showed an increasing trend until concentrations peaked on January 17 (day 142) for 5-2, and 7-1, and on February 14 for 5-1 at 375, 275, and 275 mg/l $CaCO_3$ respectively. Similar profiles were noted for the coproduced 1A tract brines. Increases of about 100 mg/l $CaCO_3$ were observed in the 1A-1 and 1A-9 brines in January 1992 over a baseline value of 150 to 175 mg/l $CaCO_3$ determined between July 1989 and February 1990. By February 1992, these values had decreased and were close to baseline values. These increases in alkalinity are believed to result from microbial production of CO_2 due to metabolism of molasses in the reservoir. As none of the expected metabolic products, such as nitrite or volatile fatty acids, were detected in the brine waters from the production wells during the pilot study, it would appear that the rate-limiting step in the complete mineralization of the molasses was the initial metabolism of the carbohydrate.

4.3. Preferential permeability reduction

A permeability reduction factor (PRF), defined as the ratio of the permeability between wells in the reservoir after the microbial process (or any other process) to the initial interwell permeability, was determined as outlined previously [15] using pressure interference tests. The results of these tests, conducted before the trial and during the second incubation after the final nutrient injection in December 1991, indicate that there had been reduction in permeability in the pilot area. The initial permeability between wells 7-2 and 5-2 was three-fold higher than that between wells 7-2 and 7-1 (Table 4). The largest reduction in permeability was observed between wells 7-2 and 5-1 and between 7-2 and 5-2 which were also the regions with the highest initial permeabilities (Table 4). The results also indicate that permeability distribution throughout the pilot area became more uniform after the molasses and ammonium nitrate treatments.

Table 4
Results of interference pressure test

Well/Test	Date	Permeability (md)	PRF
5-1	1991	154	
	1992	56	0.37
5-2	1991	181	
	1992	49	0.27
7-1	1991	60	
	1992	43	0.72

The results of a third tracer study started on February 18, 1992 also indicated that a substantial alteration had occurred in the flow channel from well 7-2 to well 1A-9 following nutrient treatment. The two prior tracer studies had breakthrough times for the tracer of 16-18 days in the 1A tract. By the time of writing, no breakthrough had been observed in any of the sampled production wells.

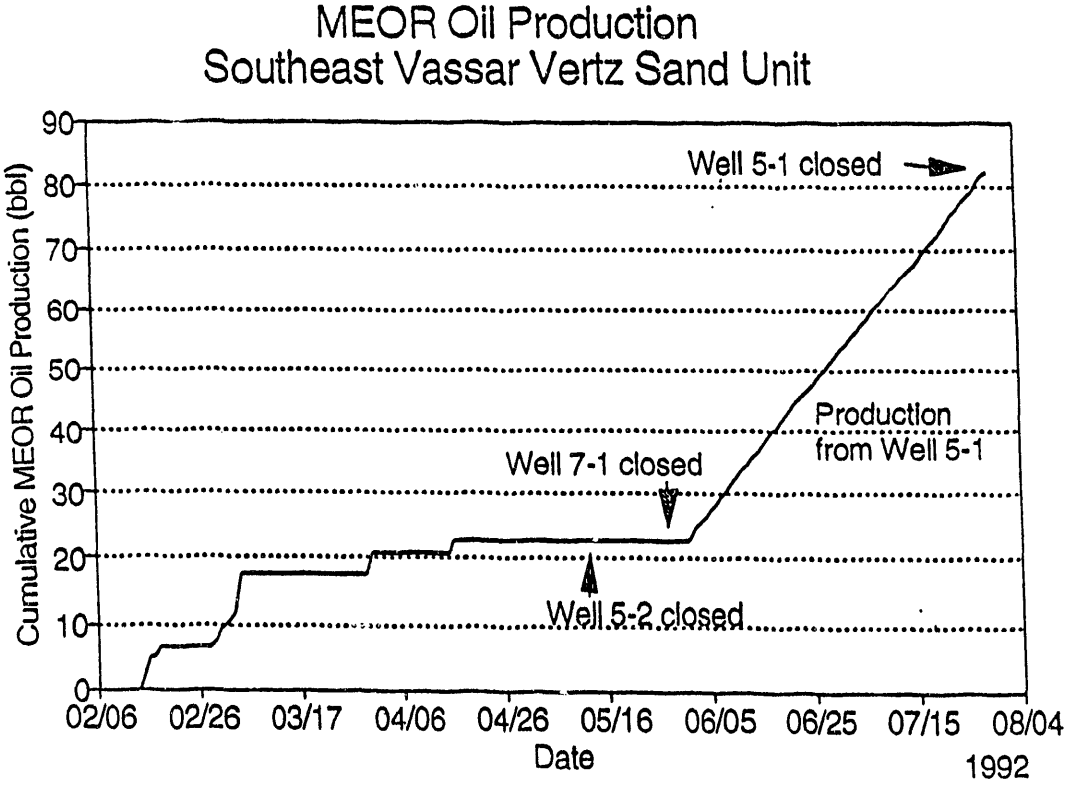


Figure 2. Cumulative tertiary oil production.

4.4. Tertiary oil production

Production of 22.5 barrels of tertiary oil was observed during the first half of 1992 before the shut down of the pilot wells 5-2 and 7-1 in April 1992. An additional 60 barrels of oil had been produced by well 5-1 prior to its shut down on June 30, 1992 (Figure 2). No oil was produced from the pilot wells during two months of waterflooding of the pilot area prior to the injection of nutrients, suggesting that the oil produced was a result of microbial stimulation in the reservoir pilot area. Significant tertiary oil recovery was not expected because of the near theoretical maximum recovery of oil (60% of original oil in place) from the swept portion of the field [16].

5. CONCLUSIONS

The original goal of the project was achieved, that is to preferentially plug zones of high permeability in the reservoir resulting in a more homogeneous distribution of permeability throughout the pilot area. Interference and tracer studies indicate that the permeability in the pilot area was preferentially reduced and the existing flow channel to the 1A tract was partially plugged as a result of the injection of 56.2 tonnes of molasses and 18.8 tonnes of ammonium nitrate into well 7-2. The alkalinity changes, resulting from CO_2 production in the reservoir, imply that the observed modifications of permeability were probably due to microbial metabolism of the molasses and ammonium nitrate by indigenous bacteria.

The initial injection of ammonium nitrate resulted in a significant decrease (about 50%) in the sulfide levels in the coproduced brines from the 1A tract as well as from the pilot area. However, insufficient nitrate was injected with the molasses to inhibit transient increases in sulfide production due to carbohydrate metabolism.

Finally, it should be emphasized that the overall biological effects of the stimulation of in-situ microbial populations in an oil reservoir were transitory and that 4 months after the last injection of nutrients both sulfide and alkalinity values had returned to pre-test levels with no permanent alteration in the coproduced brines being noted.

6. ACKNOWLEDGEMENTS

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Microbial Hydraulic Acid Fracturing

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Abstract

Compared with conventional hydrochloric acid treatments, microbial acid fracturing in carbonate reservoirs offers the technical benefits of a greatly increased fracture length coupled with the employment of non-corrosive, non-hazardous and environmentally friendly feedstocks. Following a discussion of the concepts underlying the technique, this paper describes in some detail the procedures, the results and the analyses of a first field trial performed in a carbonate reservoir in southern England in September 1991.

1. INTRODUCTION

Hydraulic fracturing of reservoirs is a common technique for improving the drainage of fluids into wellbores [1]. In sandstones, the inclusion of proppants in the fracture fluid prevents the fractures closing after injection. Proppants also are injected into carbonates but their cost, and the tendency of proppant particles to embed in often relatively soft rock, prompt the alternative use of acid etching. The use of mineral acid, however, suffers from several disadvantages:

- the severe limitations of rapid spending due to the enormous surface area of rock contacted and generation of worm holes;
- the corrosion of metal equipment and potential hazards to personnel.

Although retardant systems are able to delay spending, their efficacy is limited in long fractures; claims for retarded systems are common but evidence from the field suggests very limited success. Smooth etching can be overcome by the prior injection of a viscous pad into the fracture through which the acid penetrates by viscous fingering; however, the benefits are hard to quantify. Inhibitors may help to reduce the corrosion risk while proper safety precautions minimize danger to personnel. Nevertheless, inhibitors are highly toxic and their use results in very high disposal costs, up to £1,000 per ton of spent fluid. Collectively, these considerations encourage a search for less expensive alternatives.

As noted originally by ZoBell [2], *in situ* microbial systems can generate acid; this clearly offers an alternative approach to the conventional treatment of carbonate rock using mineral acid. The anaerobic conversion of carbohydrates and other feedstocks to a range of organic acids is a well-known feature of certain types of bacterial metabolism. The calcium and magnesium salts of those acids are sufficiently soluble in water to allow significant dissolution of a carbonate matrix and a consequential increase in permeability in the areas

contacted. The kinetics of microbial acid production make it particularly attractive for acid fracturing.

2. MICROBIAL ACID PRODUCTION

2.1. General considerations

The characteristic exponential pattern of bacterial growth (and hence also of acid production in the case of anaerobic acid producers) ensures that most growth occurs and most acid is generated late in the growth period. Thus, the injected fluid in microbial acidizing is itself barely acidic; in marked contrast to the direct injection of hydrochloric acid, microbial acid production is a progressive and, initially, a comparatively slow process. There is accordingly time for a large volume of the microbial acid-producing system to be injected into a well, either as an integral part of a fracturing operation or as a squeeze, with the fluid front being pushed far from the immediate neighborhood of the wellbore before the reaction is complete; most of the acid is, indeed, synthesized deep in the reservoir matrix, resulting in a correspondingly enlarged radius of action.

The injected fluid, based on produced or make-up water, contains both the bacterial inoculum and the nutrients appropriate to the strain being used. The volume of injection fluid is governed by reservoir considerations while the injection profile is determined with the aid of conventional fracture simulation programs. Mixing the bacteria and their nutrients some time before injection is commenced will ensure that during the injection the microbes are already making acid although most production will take place much later, as already noted. Individual microbial cells which may become impacted in pore throats will therefore tend to free themselves by etching the surrounding rock.

The downhole biological reaction is allowed to run for a period ranging from several hours to about three days (see below for factors influencing this period). The well is then flowed back and production restarted. The produced water contains the microbes and their metabolic products (primarily calcium and magnesium salts of organic acids) together with any unused nutrients. All these materials are environmentally safe and present minimal problems of disposal.

The benefits of microbial acid fracturing are the creation of an etched fracture face together with a high permeability zone adjacent to it. The extent and effectiveness of microbial etching of the fracture faces will depend on:

- the time during which the fracture is kept open by the injection pressure and hence during which microbial acid has access to the faces;
- the rate and the magnitude of microbial acid production in the injected fluid; and
- the reservoir rock composition and degree of heterogeneity.

The extent and effectiveness of the permeability increase in the adjacent matrix will be influenced by:

- the depth of leak off into the matrix;
- the depth of bacterial penetration into the matrix;
- the amount of metabolic acid generated in the leak off zone; and
- the geology of the matrix, i.e. the extent to which interconnected porosity can be increased.

The applicability of these factors to individual reservoirs must be evaluated on a case-by-case basis.

2.2. Technical, operational and cost factors

2.2.1. Temperature and pressure

These factors limit microbial acidizing opportunities just as they affect all *in situ* microbial procedures. Bacterial species able to produce acid at temperatures up to 65-70°C (149-158°F) are well known; extending the range upwards will become progressively more difficult. As noted in the field test described below, an injection pressure above 2,900 psig had no observed effects on subsequent microbial activity downhole.

2.2.2. Ionic composition of reservoir waters

All microbes are sensitive to some level of salinity. However, in many downhole microbial technologies, including acidization procedures, the ambient microbial environment will be provided largely by the injection fluid. Thus the salinity of the injection fluid may be chosen to suit microbial requirements as long as it remains compatible with injection into the reservoir. A demand for high salinity injection fluids will require correspondingly tolerant microbial systems. High concentrations of heavy metals in the reservoir waters, particularly of barium and strontium, are probably best avoided in all cases in which significant mixing is likely with the injection water.

2.2.3. Permeability and porosity

During injection there are no limitations to bacterial penetration into the generated fracture although plugging may occur in the neighboring formation. Both acid fracturing and matrix acidizing (i.e. injection below fracture pressure) will require a minimum permeability to permit significant bacterial penetration.

The form of the matrix porosity also may be important - it appears probable that the greatest enhancement of matrix permeability will result from increasing the interconnections between poorly connected or isolated pore spaces rather than marginally enlarging the channel diameters of a matrix with good communication. The geology of the formation will thus be of significance in evaluating susceptibility to bacterial acidizing.

2.2.4. Hydrogen sulfide generation

The introduction of organic materials into any reservoir environment will increase the risk of H_2S generation, particularly in fields already sour. Total exclusion of sulfate-reducing bacteria from oil reservoirs is virtually impossible, and the metabolites produced during acidizing constitute a source of organic nutrients for them. Bacterial H_2S production can be suppressed with non-biocide chemicals which specifically inhibit the reduction of sulfate to sulfide. However, sulfide ions already present in reservoir waters may be expelled from solution as H_2S gas by the microbial acids generated to promote carbonate dissolution - operational protocols must take this possibility into account. Nevertheless, it should also be noted that since a limited quantity of organic feedstock is injected, any H_2S generated will be correspondingly restricted and provision may be made at the wellhead to scrub it out of the vented gases.

2.2.5. Operational flexibility

The following parameters are variable:

- the volume of fluid injected can be tailored to the demands of the system;
- the aggregate amount of carbonate rock dissolved is proportional to the total quantity of feedstock supplied rather than to either the volume of the injected fluid or to the number of microbes injected; however, the quantity of carbonate dissolved *in any particular location*, and hence the

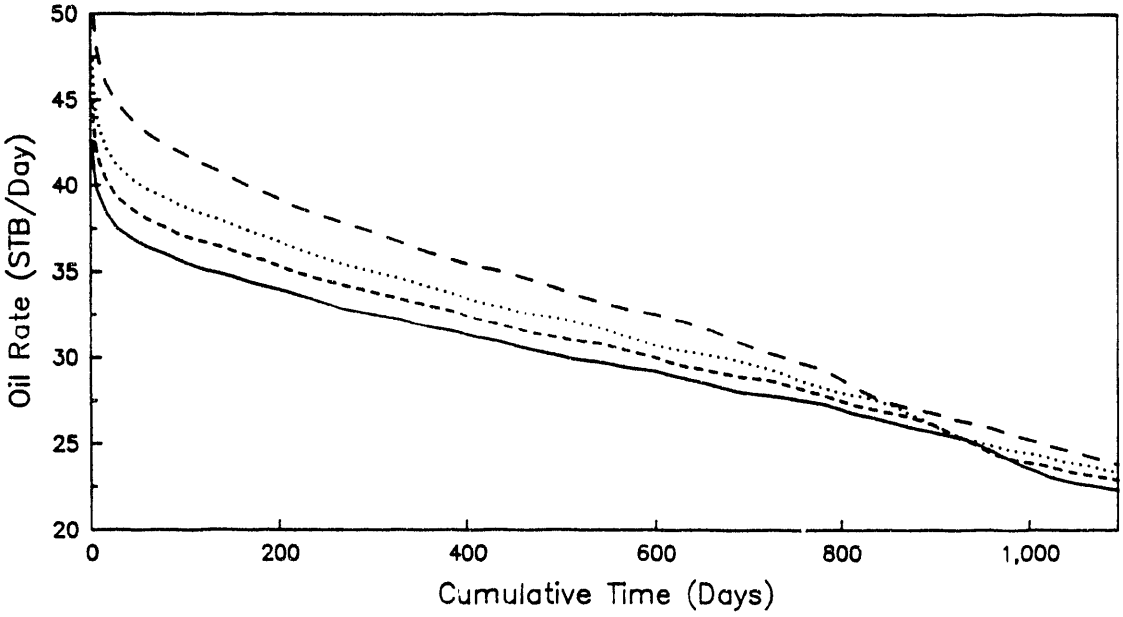


Figure 1. Effect of wing permeability on oil production rate with 20 acre spacing. Wing permeabilities: ——— base case; --- 20 mD; ••• 30 mD; --- 50 mD.

effect on local permeability, is largely dependent on the *concentration* of feedstock and the amount of acid generated at that site. With a predetermined volume of injected fluid, the concentration (and hence the total quantity) of feedstock chemicals can be varied over at least a ten-

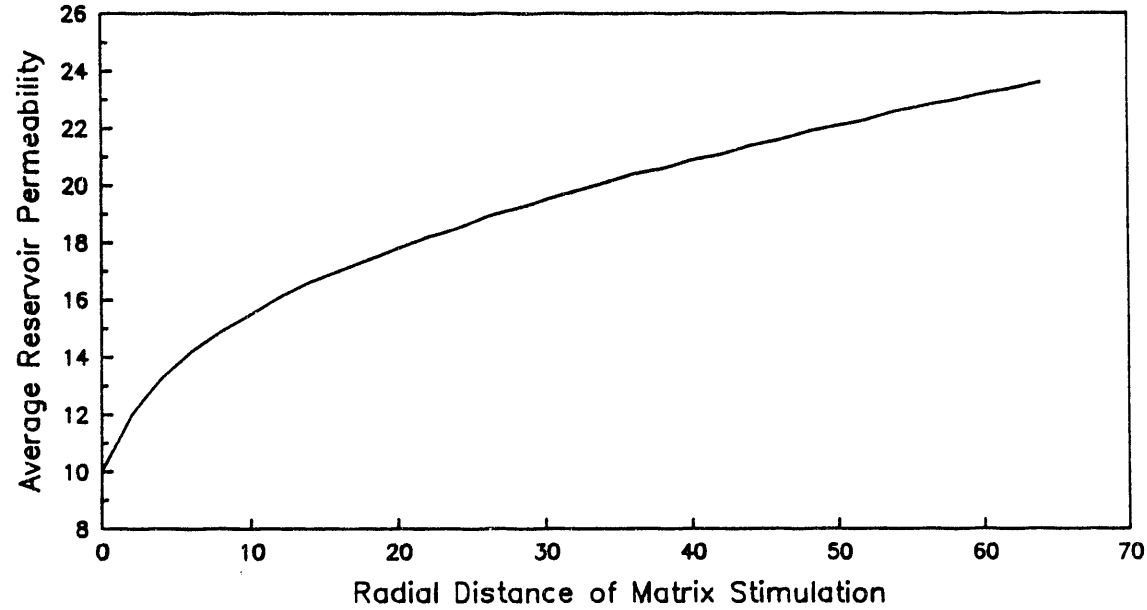


Figure 2. Increase in average reservoir permeability as a function of radial matrix stimulation. Assumptions: Reservoir permeability = 10 mD; simulated matrix permeability = 50 mD.

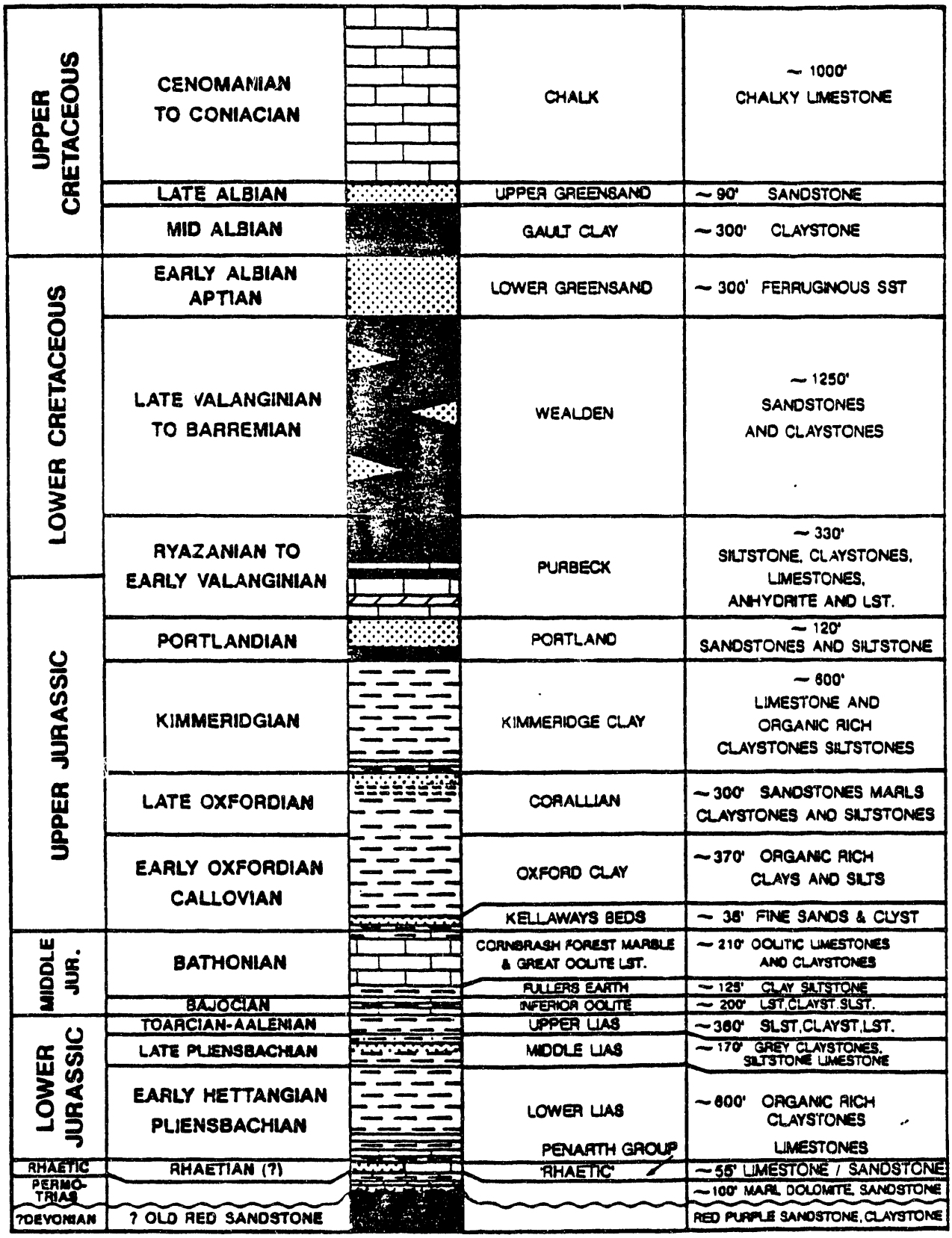


Figure 3. Stratigraphic sequences with thickness ranges.

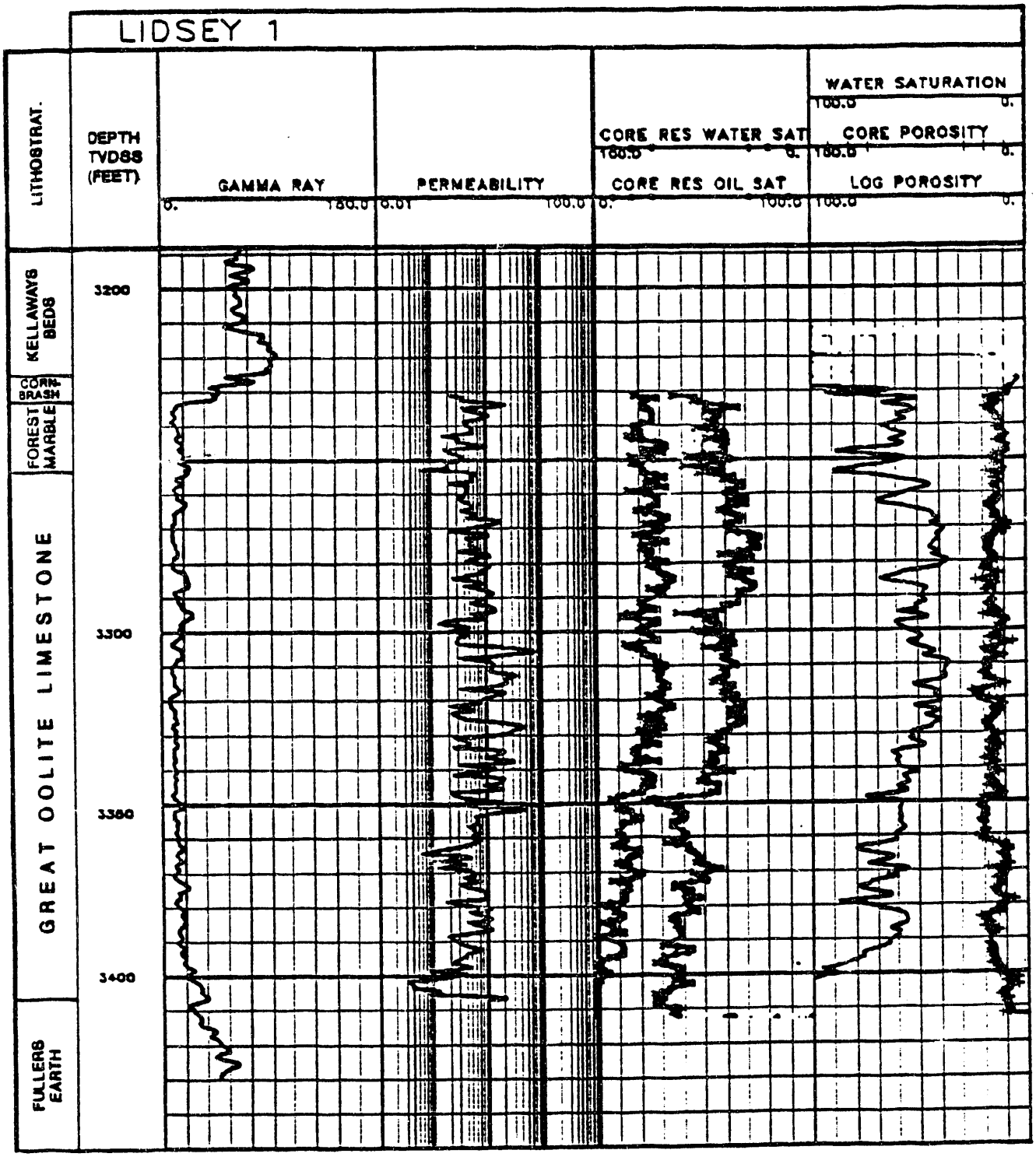


Figure 4. Great Oolite reservoir —— typical well section.

fold range. This has financial implications. Both the pore volume to be contacted and the quantity of rock to be dissolved are operating decisions to be taken in the light of reservoir engineering and cost considerations;

- the time for the reaction to complete varies from a few hours to several days depending on the downhole temperature and on the concentrations of feedstock and microbes in the injectate: the higher the temperature (within limits, see above), and the greater the number of bacteria introduced, the shorter the reaction time to completion; the higher the feedstock concentration, the longer is that time.

2.2.6. Technical and cost advantages

Compared with mineral acid, several clear benefits appear to follow from bacterial acidizing in carbonate reservoirs:

- an ability to dissolve rock at a considerably greater distance from the wellbore than is practical with mineral acid;
- a non-acidic, non-corrosive and non-hazardous inert injection fluid. The costs of equipment protection and personnel safety precautions are thereby reduced;
- equipment costs are lower because of the non-corrosive nature of the injected fluid;
- both the microbes used for injection and the products of their activity are entirely safe to the environment.

3. FRACTURE AND MATRIX ACIDIZING

Bacterial acid fracturing implies the introduction of the bacterial acid-generating fluid into an oil or gas reservoir above fracture pressure. The acid-generating fluid flows or "leaks off" into the rock matrix next to the generated fracture and creates a high permeability wing by dissolution of the carbonate matrix. This wing is designed to be 1-2 ft thick. Finite element simulation studies have shown the high permeability wing to behave similarly to a conventional propped fracture. Figure 1 illustrates the results of creating a horizontal wing 65 ft in diameter and 1 ft thick around a producing well in 20 acres. The reservoir permeability is 10 mD and the reservoir pressure is 500 psi. Finite element simulation reveals a 16% increase in cumulative oil production after creating a wing having a fivefold increase in permeability compared with the reservoir generally.

While acid squeezes are a common type of stimulation for carbonate wells, they are generally used mainly for cleaning up near-wellbore damage. Darcy's Law shows a decreasing benefit from a radial matrix stimulation treatment (Figure 2). A large benefit is observed close (<5 ft) to the wellbore; however, as the radius of stimulation is increased beyond 10 ft, the incremental percentage increase in average reservoir permeability is small. While 2,000 bbl of fluid may create a fracture about 1,000 ft long, a similar volume of fluid injected below the fracture pressure and distributed radially will penetrate no more than 10-16 ft in a reservoir 80 ft thick with a porosity of 20%. Bacterial acidizing is better suited to fracturing than to matrix treatments although matrix acidizing may be of value in injection wells in which it might be possible to produce acid continuously by batch feeding.

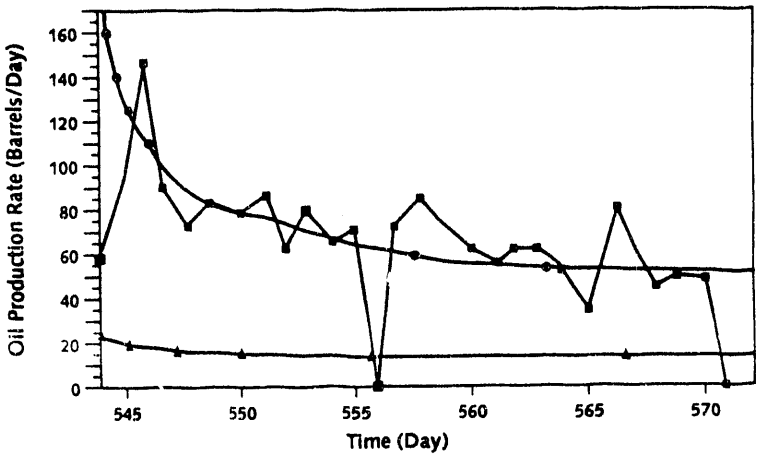


Figure 5. Production history match.

4. FIELD TEST OF BACTERIAL ACID FRACTURING

An *in situ* test of bacterial hydraulic acid fracturing was conducted in September 1991 in the Lidsey field, southern England.

4.1. Reservoir characteristics

The reservoir, within the Great Oolite Group (Middle Jurassic) of the Wealden Basin, reflects a water-wet shallow marine limestone facies characterized by clean, sparsely fossiliferous, cross-bedded bar sands, corraliferous units, and muddy bioturbated skeletal oolite (Figure 3). Seven cores taken from depths of 3,346-3,377 ft showed moderate to good porosity (16.5-19.8%) although this is virtually unconnected and the Klingenberg permeabilities are very low (0.62-4.38 mD) (Figure 4). The reservoir is drained by a single well.

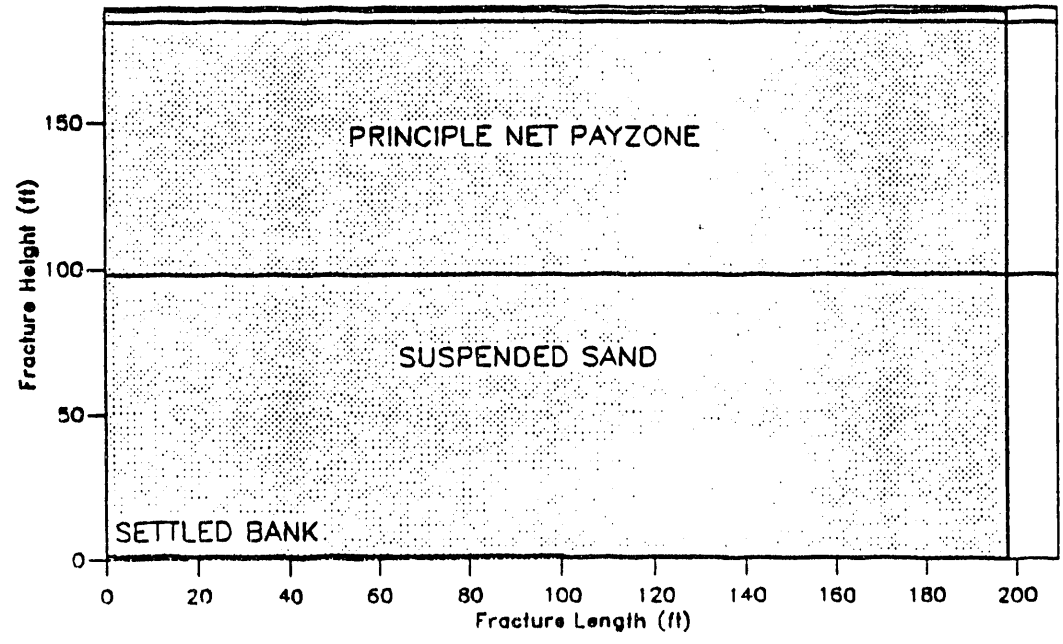


Figure 6. Simulated fracture treatment.

The well was spudded in March 1987 and drilled to a plugged back total depth of 3,850 ft. After coring, brief "barefoot" drill stem tests were performed using a packer set just inside the shoe of the 7 in casing. Minimal oil was recovered in these tests. A full suite of electric logs was run across the reservoir section allowing definition of a low- permeability oil-bearing interval from 3,270-3,450 ft measured depth to the rotary Kelly bushing, with an oil saturation of less than 30%. The remainder of the interval showed oil saturations declining from a peak of 70% at the top to 30% at the base and seemed to be divided at 3,368 ft into two zones of higher and lower oil saturation.

A Horner plot was used to calculate the initial reservoir pressure at 1,450 psig at a gauge depth of 3,205 ft. The reservoir temperature was 122°F (50°C).

4.2. Reservoir history

Before beginning production in May 1987, an acid wash and short flow test were performed; the results were poor. A hydraulic sand fracture treatment was therefore performed using 27,000 lbs of 20/40 Ottawa sand proppant in 9,000 gals of viscosified gel. Because the oil production rate from swabbing was much improved, the well was completed with 2-7/8 in tubing hanging packerless to facilitate gas separation during rod pumping. Perforations extended from 3,300-3,320 ft. A nipple below the rod pump allowed for memory pressure gauges to be hung during all operations.

An extended production test began in January 1989, the well being rod-pumped for 31 days followed by a 22-day build-up period. Production was disappointing: in those 31 days, the well produced 1,666 bbl of oil and 976 bbl of water. This production rate was not economical and the well was shut in until the present bacterial acid fracture test.

4.3. Reservoir modelling and production history matching

Using a two-phase "black oil" reservoir simulation model, the post-fracture production history was matched. General reservoir characteristics were taken from open-hole logs, PVT data, pressure build-up, core analyses and the report of the propped fracture treatment.

Figure 5 shows that a good history match (± 10%) is obtained. The treatment report and reservoir simulation model indicated good proppant placement (Figure 6). Modelling suggested that while increased sand volumes (even up to 100 tons) and fracture lengths beyond those actually used would have resulted in no more than small incremental production gains, the quantity of sand indeed employed was actually too small and that 30 tons would have been optimal.

In preparation for the microbial acidizing test, the production decline on reopening the well was simulated with the proppant in place (Figure 7); production was predicted to decline within 50 days to 40 bbl of oil per day. However, simulation showed that without the original fracture in place, production within 50 days would be less than 15 bbl of oil per day, the production rate to be expected if a microbial acid fracture removed sand from the previous fracture but failed to dissolve any matrix oolite. Any production above this level would be evidence for effective microbial action.

4.4. Design of a microbial acid fracture

Combining the rheological properties of the microbial injectate with the history-matched Lidsey model, a series of microbial fracture treatments was simulated using a range of pumping rates and fluid volumes to evaluate sensitivity and attainable fracture length. While showing some variability, the results suggested that fluid volumes of more than 750 bbl at pumping rates in

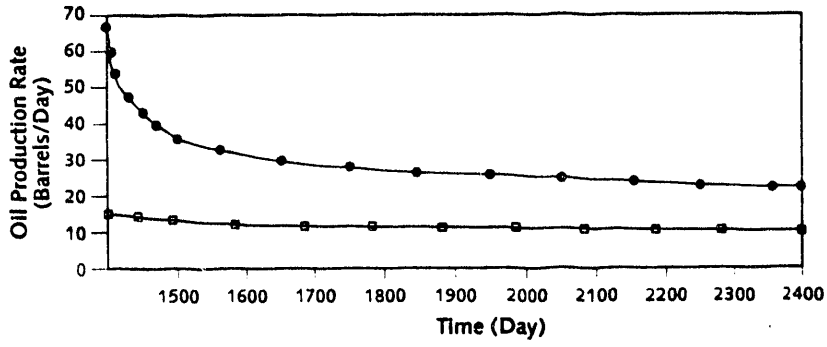


Figure 7. Anticipated production over 1,000 days upon reopening.

excess of 10 bbl/min would give half fracture lengths greater than 330 ft, theoretically exceeding the 200 ft long propped fracture already in place. Ar apparent skin value of about -4.0 derived from analysis of the 1989 build-up was compatible with an undamaged propped and fractured reservoir. A treatment to etch and extend the existing fracture, and generate a high permeability streak adjacent to it, was judged the only method likely to increase oil production from this well; a matrix type of treatment would show no benefit. This conclusion notwithstanding, because of the very tight formation neither the well nor the field could be regarded as good candidates for simulation.

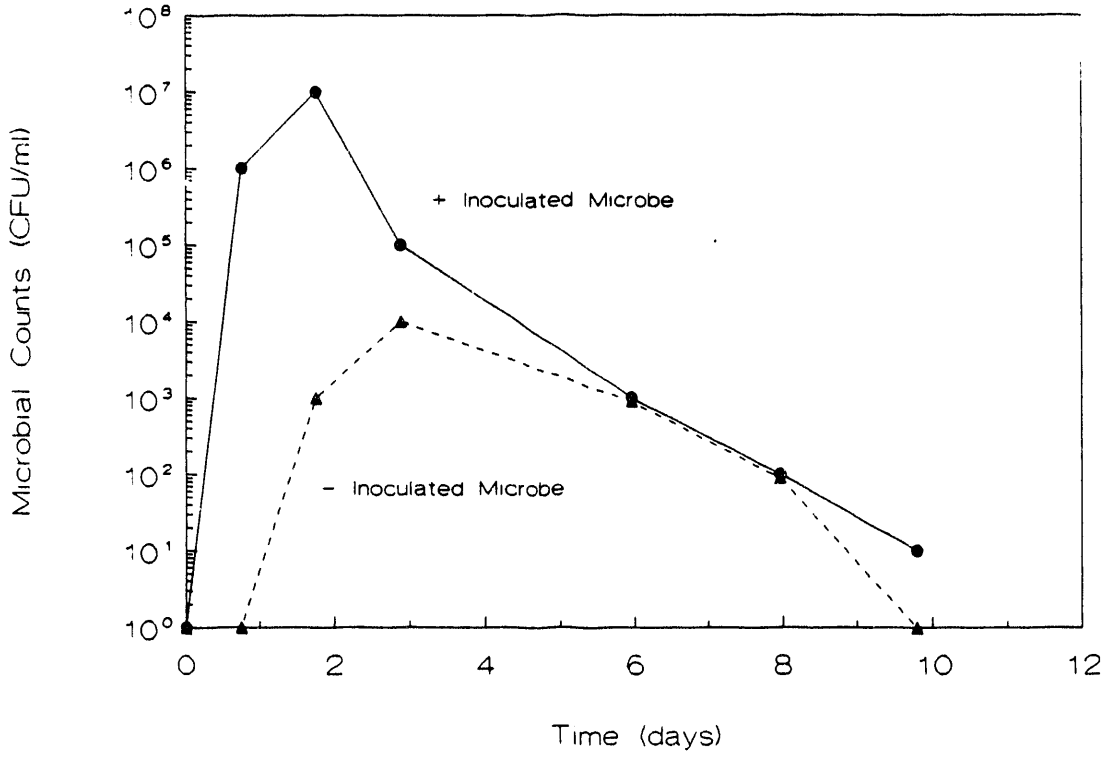


Figure 8. Reservoir model system —— microbial numbers.

In the light of this analysis and various operational constraints, it was recommended that more than 1,000 bbl of microbial injectate should be introduced. An injectivity test was to be carried out before the main injection and the treatment fluid would be left in place for at least two days before flowing the well back.

4.5. The microbial system

The system was based on a naturally-occurring anaerobic bacterial strain capable of generating organic acid from suitable carbohydrate sources. The strain was designated ACC004; no attempt was made to improve its performance with recombinant DNA techniques. The medium and operating procedures were optimized for acid production within certain cost constraints.

Laboratory culture of the microorganisms was routinely carried out in glass Wheaton bottles (160 ml volume) containing medium. It was important both to simulate the reservoir environment in which the bacteria would be producing acid in nearly stationary fluid physically very close to the carbonate rock, and to be able to measure total calcium dissolved rather than acid production *per se*. The Wheaton bottles were therefore packed with about 80 g of crushed oolite (particle diameters in the range 0.75-2.0 mm). Although neither normal autoclaving nor dry heat were entirely effective at sterilizing the rock, this was acceptable because the downhole matrix probably already carries an indigenous microflora which the microbial inoculum designed for introduction in the acid fracturing was configured to overwhelm. After sterilization, each Wheaton bottle was filled completely with inoculated medium and capped with a gas-impermeable butyl-rubber stopper. In each batch, some bottles were left uninoculated as controls.

The bottles, attached to a slowly revolving drum, were incubated at 49°C. At intervals, and without releasing the gas pressure which had developed, samples were removed to determine bacterial numbers (colonies on solid medium), pH, oolite dissolution (concentration of Ca^{2+}) and metabolic acid production. Within 48 hours microbial numbers rose to 10^7 colony-forming units/ml while remaining below 10^4/ml in the controls (Figure 8). The pH fell from 6.2 to 5.8 in the inoculated bottles, while soluble Ca^{2+} reached 6 mg/ml in 1-2 days, exceeding 7.5 mg/ml (equivalent to 18.8 g/l of calcium carbonate) after 8 days; the concentration of organic acid rose in parallel. In the controls, the pH stayed close to neutrality and the soluble calcium concentration never exceeded 1 mg/l, with a correspondingly low level of organic acid (Figure 9).

4.5.1. Preparation of the inoculum for the field test

A lyophilized preparation of ACC004 was prepared using commercial fermentation facilities: the fine white powder readily dispersed in aqueous medium. Inoculum viability was confirmed.

4.6. Core tests

4.6.1. Bacterial penetration through a Lidsey core

Core flood measurements on reservoir core samples were carried out with an EPS Coresystems CFA 100 Coreflood Apparatus. Sterilization of the system to permit subsequent determination of microbial numbers was achieved by flushing with disinfectant, followed by washing with distilled water. No microorganisms were detected in core effluent water after disinfection.

The core selected was recovered from a depth of 3,349 ft. Its properties are shown in Table 1.

Injection of sterile 3% (w/v) KCl solution through the core at 2 ml/min required a differential injection pressure of 8.82 bar, which had to be raised

Table 1
Characteristics of core for bacterial penetration study

core length	2.5 cm
core diameter	2.5 cm
core weight	26.1 g
bulk volume	12.3 ml
bulk density	2.1 g/ml
pore volume	2.4 ml
porosity	19.8%
permeability:	
gas	5.5 mD
Klingenberg	3.4 mD

to 10.97 bar when the fluid also contained ACC004 at a concentration of 10^6 cells/ml. Bacteria were detected in the effluent after 17 pore volumes showing limited penetration through this very tight formation, a factor to be borne in mind when evaluating the field data presented below.

While restricted microbial penetration through the matrix was one of the main reasons why the field was regarded as a poor one for microbial testing, availability and accessibility were overriding factors in its selection.

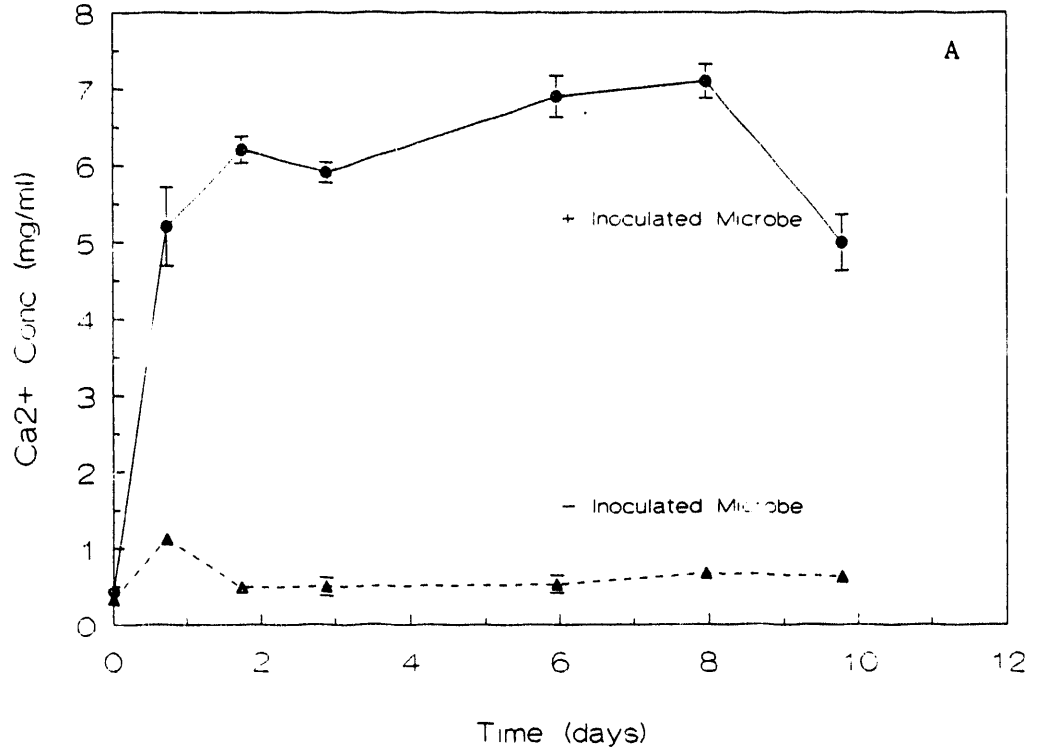
4.6.2. Effect of microbial action on the permeability of a Lidsey core

A second core was used to evaluate the ability of the microbial system to increase matrix permeability. Before inoculation, permeability with 3% KCl was measured as 6.5 mD. Nutrient solution inoculated with ACC004 (40 pore volumes) was passed through the core; the Hassler cell enclosing the core under a confining pressure of 140 bar was incubated for 88 hours at 49°C. After incubation the permeability was 20.0 mD.

4.7. Protocols for the field test

The Department of Energy as the regulatory agency issued a license allowing 84 days of production on an experimental basis. The field trial was therefore organized in the following sequence:

1. a re-entry program for installing downhole pressure gauges and a rod pump into the well which had been shut-in for two years;
2. a production period of 42 days before stimulation so as to provide a pre-treatment baseline for the subsequent production rate and pressure behavior;
3. a 27-day shut-in period, with pressure gauges in place to measure pressure build-up, to restore the well to the *status quo ante*;
4. the microbial fracture treatment comprising:
   (a) on-site mixing of about 1,350 bbl of stimulation fluid containing water, nutrients, and the microbial inoculum. The nutrients were to be mixed with 250 bbl of fresh water by recirculation through holding tanks, the concentrate subsequently being diluted with fresh water on the fly before discharge to the fracture pumpers;
   (b) injection ahead of the stimulation fluid of a 50 bbl water pad to serve as a test of injectivity, fill the well and break down the formation;



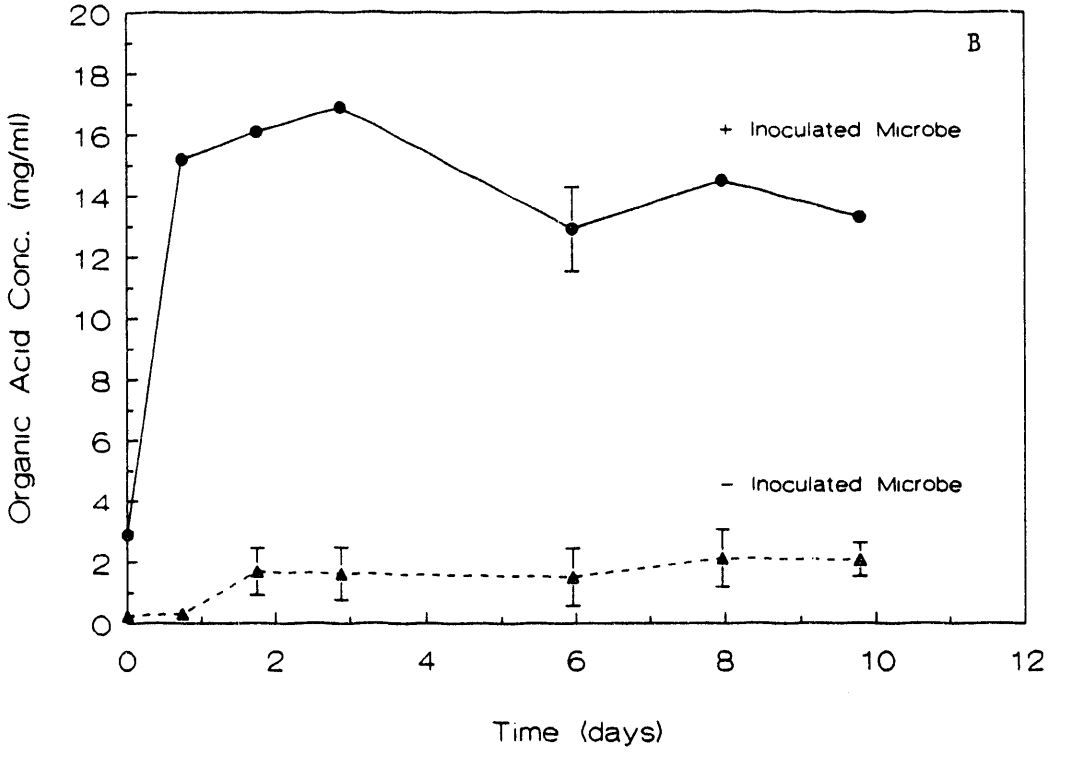


Figure 9. Reservoir model system —— concentrations of (a) dissolved calcium and (b) organic acid.

Table 2
Characteristics of the formation water

pH	6.06 ± 0.03
color (extinction at 680 nm)	0.02 ± 0.01
carbohydrate concn.	0.02 ± 0.00 mg/ml
soluble calcium	3.13 ± 0.06 mg/ml
organic acid	0.03 ± 0.00 mg/ml
microbial numbers (on nutrient agar at 49°C)	0.0 colony forming units/ml.

(c) injection of the stimulation fluid over a 3-hour period as described below. The lyophilized, powdered microbial inoculum was to be added by hand at a predetermined rate on the fly via the condor tub. This method of introducing the microorganisms was adopted because of uncertainty about the ability of the bacterial cells to withstand the prolonged vigorous agitation of the mixing process. In practice there were no deleterious effects; in future operations the inoculum will be mixed with the other components before the injection begins. With a surface injection pressure of 200 bar, the pumping rates were planned for 1,345 bbl of fluid to be injected over about three hours. Arrangements were made to sample the fluid at 30 min intervals to analyze the various components and a further sample was to be taken at the wellhead immediately after injection was complete. These analyses later showed that mixing of the concentrated nutrient solution with the bulk of the fresh water used for injection was not entirely uniform, with a fluctuation of roughly ± 20%;

(d) flushing the tubulars with 12 bbl of clean water, representing a 7 bbl underflush.

5. shutting the well in for 7 days to allow a generous time margin for the fermentation to complete; laboratory tests had already shown that microbial growth and acid production were confined essentially to the first 48-72 hours after inoculation;
6. a 30-day production period following the stimulation during which an extensive monitoring program was to be undertaken;
7. scrubbing the gases vented from the stock tank through a zinc oxide scrubber because the well was known to be sour.

5. FIELD TEST DATA

5.1. Pre-stimulation analyses

5.1.1. Well water analysis

The chemical composition of the formation water showed little variation throughout the pre-treatment production period. Table 2 shows important characteristics of the formation water.

5.1.2. Oil and water recoveries during baseline production

Daily production declined fairly rapidly on opening the well and stabilized at about 40 bbl oil and 25 bbl water (i.e. 38% water cut) (Figure 10). The rates were similar to those obtained during the production test in 1989.

5.1.3. Pre-stimulation build-up period

Although the main criterion of success in a fracture process is an increase in oil and/or total fluid production, changes in fracture length, fracture conductivity, apparent reservoir permeability and wellbore skin can all serve as indicators of stimulatory effects.

During well shut-in following the baseline production period, the bottom hole pressure reached 75.2 bar compared with an initial value of 102 bar. Horner analysis was used to estimate reservoir permeability and skin via a log δpressure vs. δtime plot to determine the end of wellbore storage. This plot was also used together with one of the pressure derivative and pressure vs. superposition time to verify the permeability and skin factor values derived from the Horner plot.

The effects of sand and microbial simulation fractures were modelled on the basis of type curves from Heber Cinco et al.[3], using Weltest software by Intera ECL.

The results of the pressure build-up, not presented here in detail, paralleled those of 1989. The salient conclusions were:

- the calculated values for permeability and skin were in the ranges 0.29-0.45 mD and -3.88 to -4.38, respectively;
- the curve shape indicated the presence of a possible boundary or fault;
- the fracture half-length was shown to be 20.4 m, matching the calculated value from the previous build-up, but less than the original design length of 51 m.

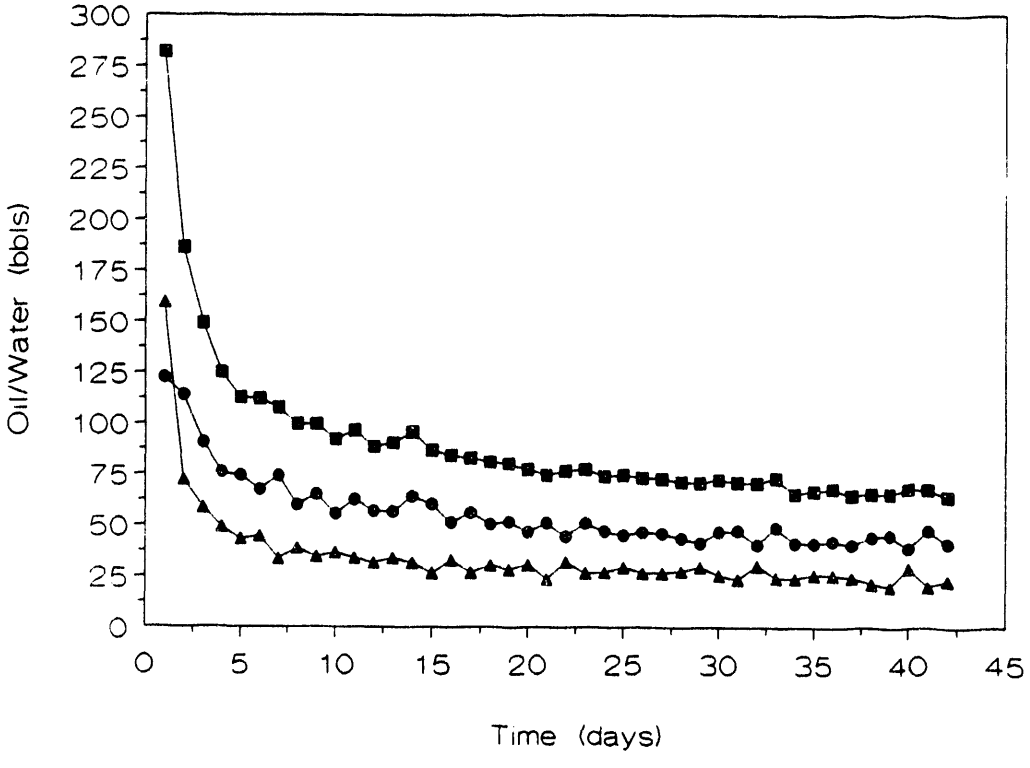


Figure 10. Pre-stimulation production —— oil and water.
—●— oil; —▲— water; —■— total.

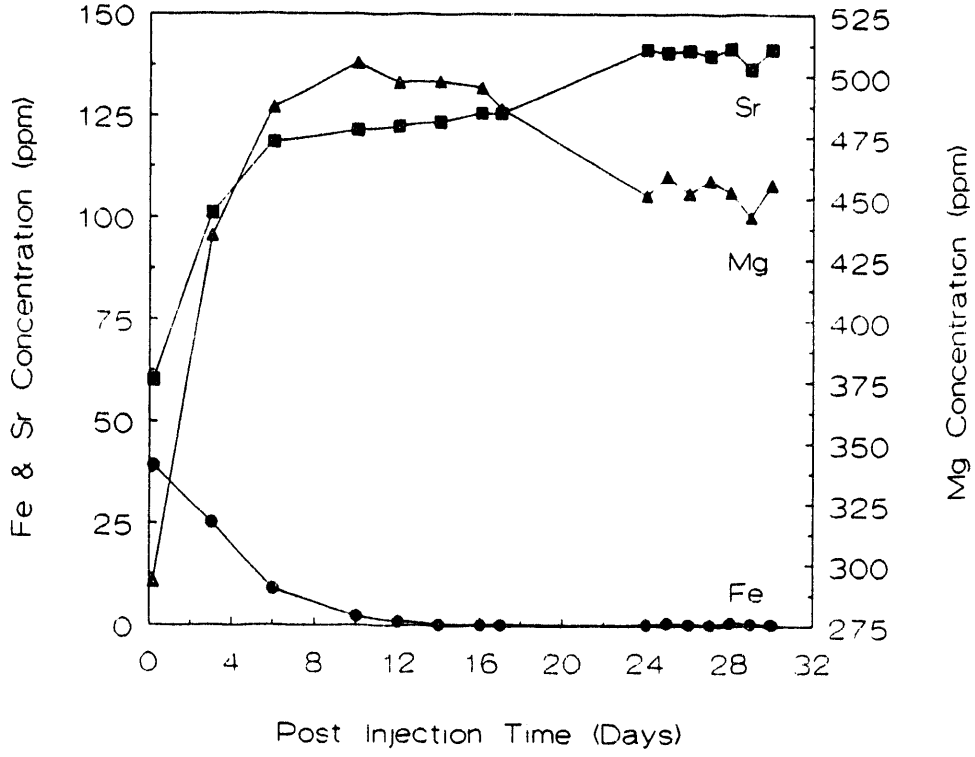


Figure 11. Post-stimulation water analysis —— changes in elemental composition.

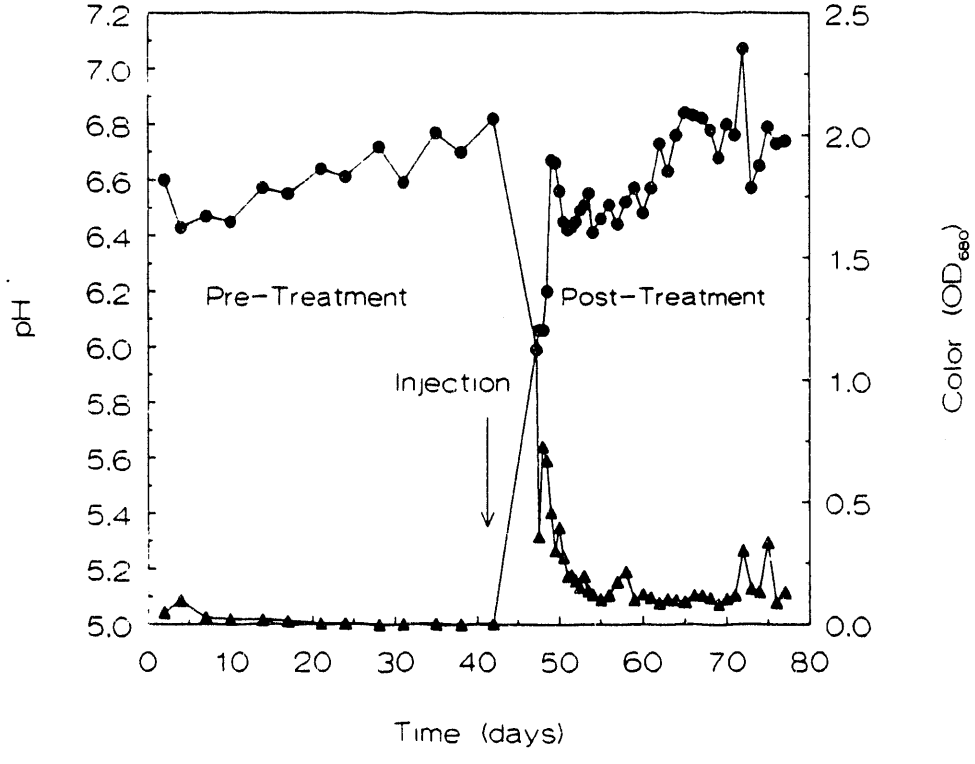


Figure 12. Post-stimulation water analysis —— pH and color.
—●— oil; —▲— water; —■— total.

Table 3
Ionic composition of the injection and formation waters

Element	Concentration (mg/l) Injection fluid	Formation water
Ag	0.25	0.26
Al	0.02	0.00
B	0.17	16.7
Ba	0.0	0.39
Be	0.15	0.15
Cd	0.19	0.18
Co	0.13	0.03
Cr	0.12	0.16
Cu	0.03	0.02
Fe	5.16	0.27
Mg	34.0	507.6
Mn	0.41	0.18
Mo	0.33	0.24
Ni	0.22	0.19
Pb	0.10	0.10
Sr	0.45	124.3
V	0.0	0.92
Zn	0.91	0.17

5.2. Post-stimulation results

5.2.1. Recovery of injection fluid

The different elemental compositions of the injection and formation waters as measured with an inductively coupled plasma spectrophotometer enabled the recovery of injection water to be determined: Table 3 shows the results.

Figure 11 presents the concentration changes for Fe, Mg, and Sr; it demonstrates that initially the produced water originated mainly from the injection, but after about 10 days essentially all was from the formation. While not entirely consistent, the data show that about 75% of the injection fluid had been recovered within that period.

5.2.2. pH and color of the produced water

Figure 12 reports (a) that despite the buffering capacity of the rock, the pH fell after treatment, recovering gradually to the pre-treatment value, and (b) that water samples taken soon after treatment were turbid and dark brown in color, the extinction values rapidly diminishing with time.

5.2.3. Carbohydrate content of the produced water

Before injection, carbohydrate was barely detectable in the produced water. After injection the concentration rose rapidly and equally rapidly fell to a slowly declining level (Figure 13). The data indicate that at least 80% of the injected carbohydrate was removed, presumably by fermentation.

5.2.4. Microbial population in the produced water

While the produced water before the microbial injection was sterile, the bacteria recovered after treatment were all identified as ACC004; numbers rose to 10^4-10^5 colony-forming units/ml, where they remained for several days (Figure 14). Although these data clearly demonstrate the growth of ACC004 *in situ*, they cannot be taken as quantitative evidence for growth because the extent

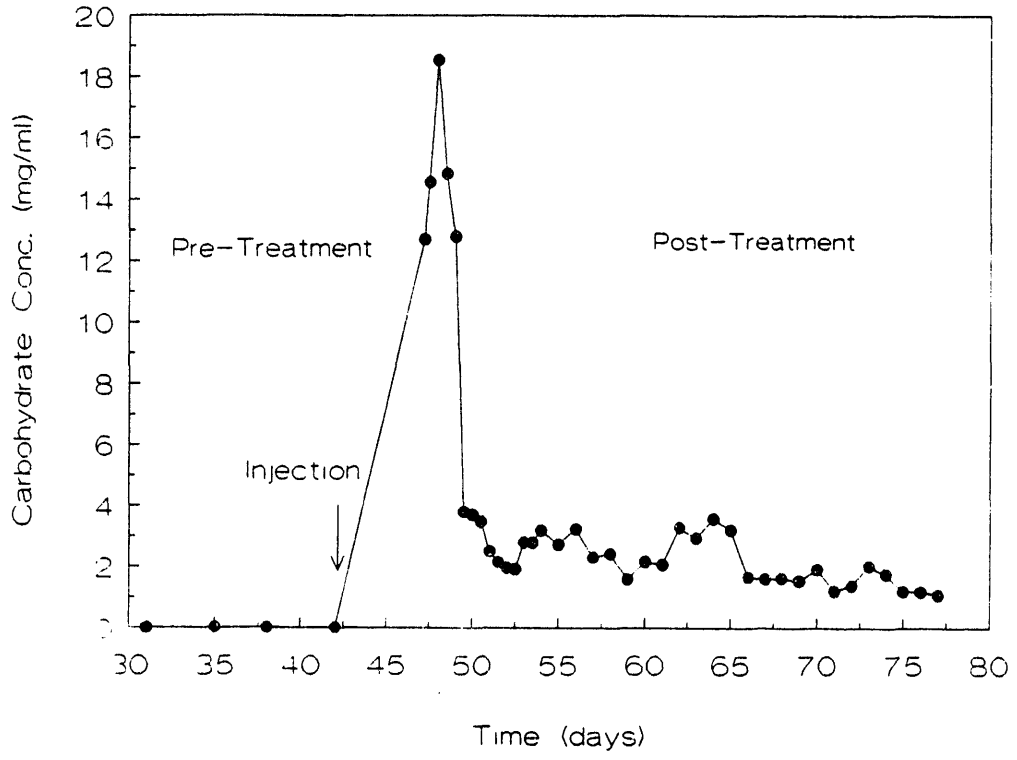


Figure 13. Post-stimulation water analysis —— carbohydrate concentration.

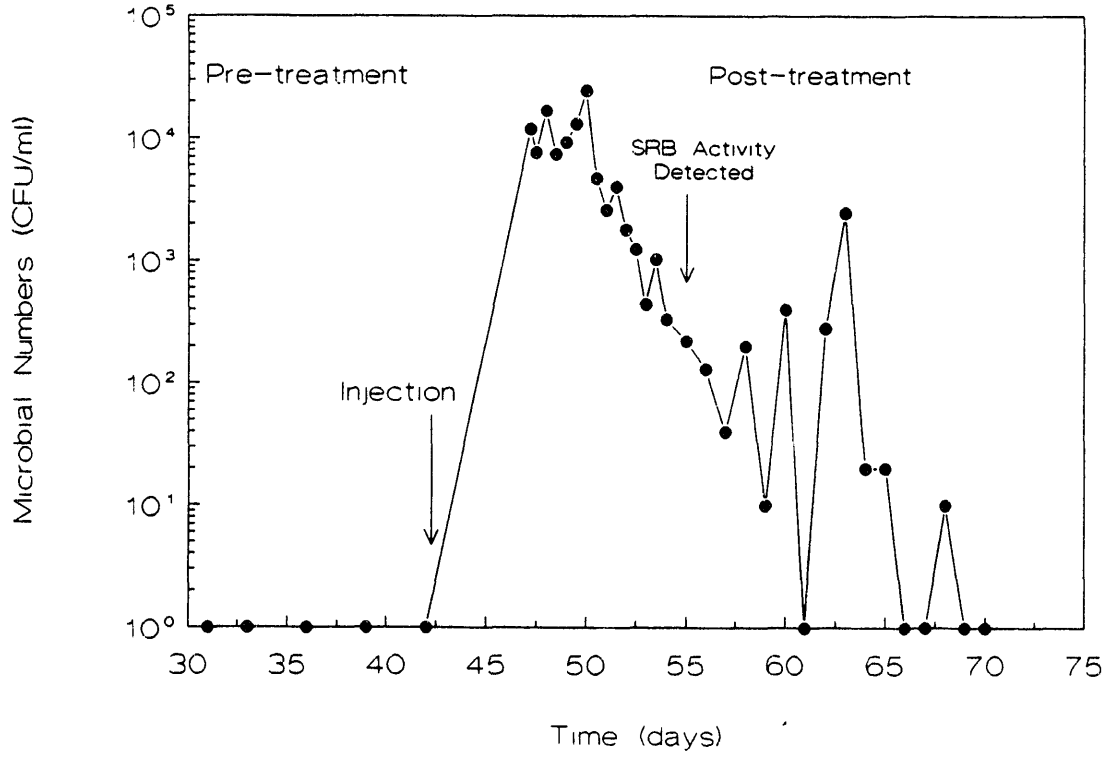


Figure 14. Post-stimulation water analysis —— microbial numbers.

of bacterial absorption onto rock and hence the efficiency of recovery is unknown. Judging by the quantity of carbohydrate consumed it is likely that no more than a small proportion of the bacteria were recovered in the produced water. Some hydrogen sulfide was produced but as the well was already sour before the treatment, it is not clear what proportion resulted from the *de novo* bacterial generation of H_2S following the injection and how much by the fall in pH releasing sulfide ions already in solution.

5.2.5. *In situ* acid generation and oolite dissolution

Figure 15 gives the concentrations of Ca^{2+} and organic acid in the produced water. The results closely resemble those obtained under similar conditions in the laboratory and suggest that the microbial treatment dissolved 15 g $CaCO_3$/l of injected fluid. While this is less than the quantities dissolved by conventional treatment with 15% hydrochloric acid, the advantage of the microbial acidization lies not in the total quantity of carbonate dissolved but in activity located beyond the range of mineral acid and in the non-corrosive nature of the injection fluid.

5.2.6. Oil and water production

Figure 16 shows the production of oil and water following treatment. In the first two days nearly 700 bbl were produced, almost all of it water (93% water cut). The water cut declined with time but so did the total volume of produced fluid; production rates then fell slowly through 50-60 bbl/day for oil and 40-50 bbl/day for water (45% water cut).

Figures 17 and 18 compare before-and-after data for total fluids and for oil production, respectively. Using as a baseline the rates before the treatment, there is weak evidence in both cases for increased production rates after the bacterial treatment. A more interesting comparison, however, is one based on the simulated rates of production assuming that the injection of the bacterial system above fracture pressure caused the proppant to fall out and the fracture to seal. On that basis the bacterial system had a major beneficial effect, increasing oil production from 10-15 to 40-50 bbl/day. The crucial question is: did the proppant really drop out?

Uncertainty about the proppant and the baseline would have been avoided had resources allowed the trial to include a stage before the microbial treatment in which water alone was injected and the subsequent production of oil and water measured. In the event this was not possible measurements of a post-stimulation build-up were recorded which provided additional information.

5.2.7. Post-stimulation build-up

The downhole gauges recording pressure build-up following the period of post-stimulation production were recovered 41 days later; the data they provided were analyzed in a manner similar to those described earlier. Several model runs were made with slightly different input parameters, giving correspondingly slightly different results (Table 4).

While the skin showed no significant differences, the permeability values were higher than for the pre-stimulation results. Furthermore, there was evidence that the fluid flow was less fracture-dominated, i.e. there was a lesser presence of fracture after the bacterial treatment than before. The calculated post-stimulation fracture half-length was shorter, and the dimensionless fracture conductivity much higher, perhaps due to negation of the earlier sand fracture and suggesting some positive improvement within the fracture itself arising from the stimulation.

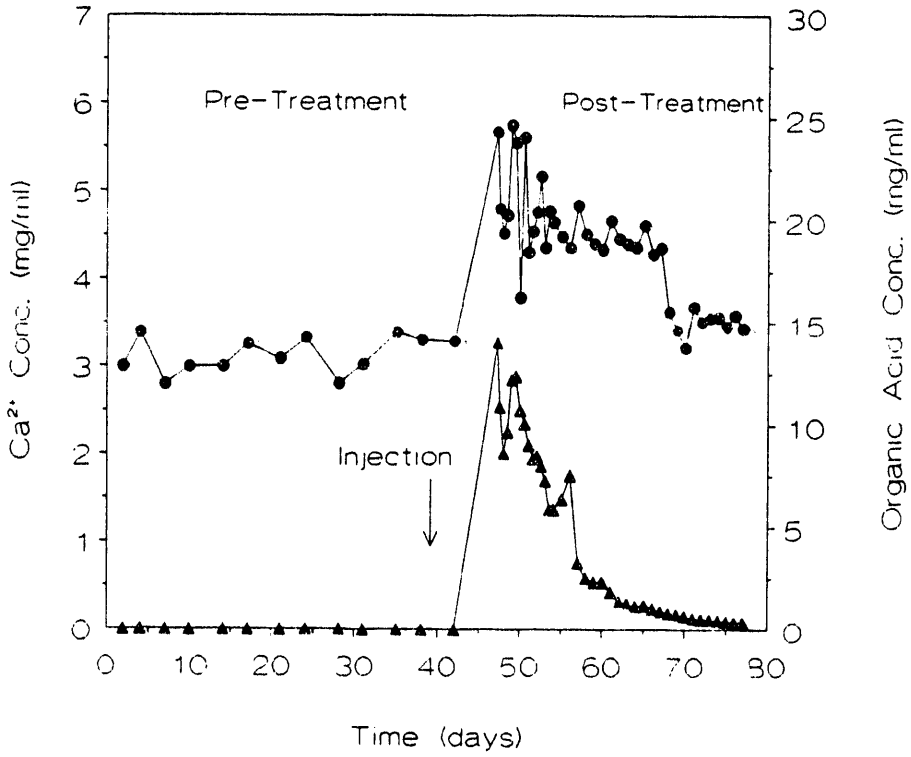


Figure 15. Post-stimulation water analysis —— Ca^{2+} and organic acid concentrations. —●— Ca^{2+}; —▲— acid.

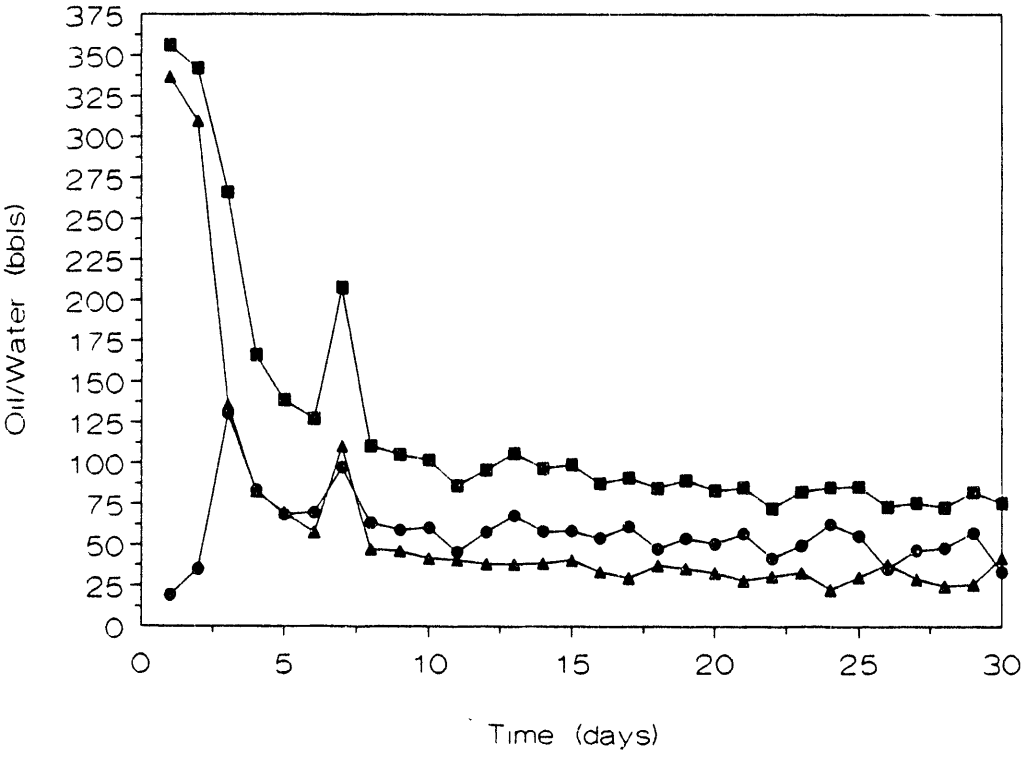


Figure 16. Post-microbial treatment —— oil and water production data. —●— oil; —▲— water; —■— total.

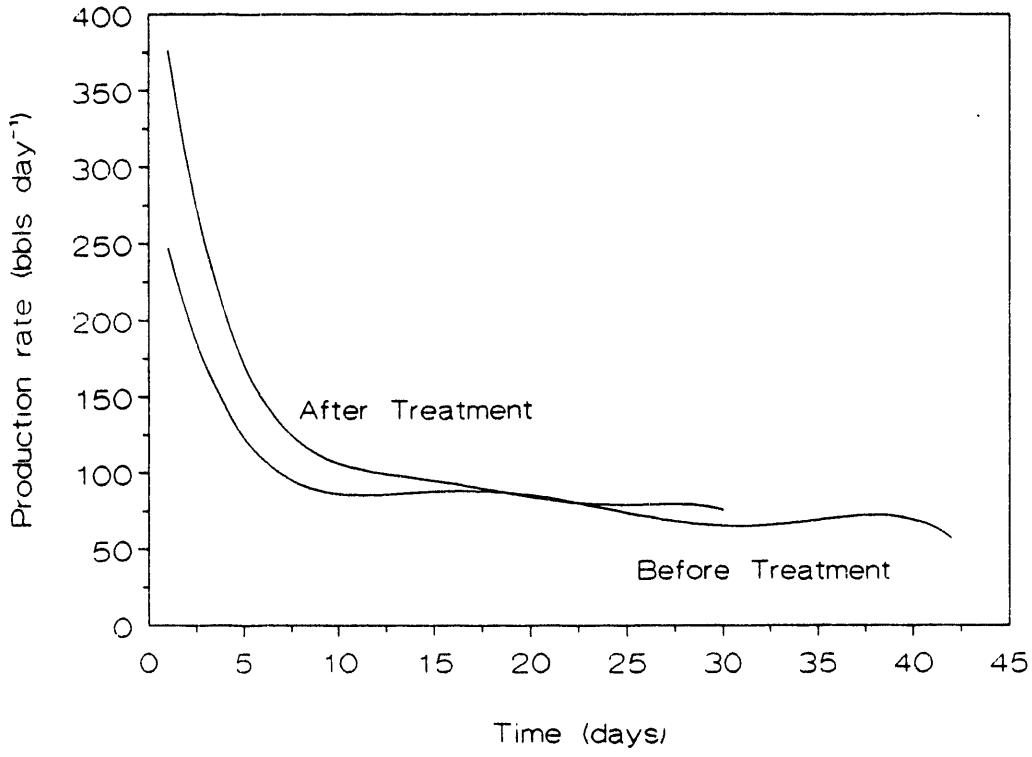


Figure 17. Total fluid production before and after microbial treatment.

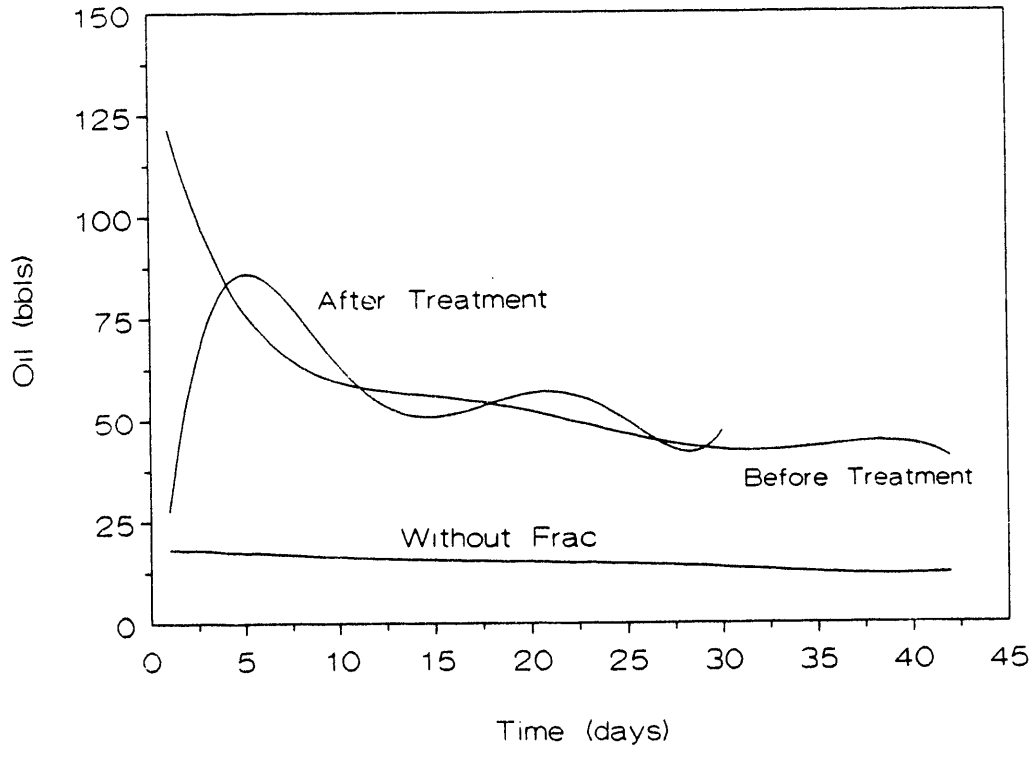


Figure 18. Oil production before and after microbial treatment.

Table 4
Post-stimulation build-up data

	Pre-stimulation	Post-stimulation
reservoir permeability	0.29 mD	0.48 mD
skin	-4.38	-3.98
fracture half-length	67.0 ft	43.0 ft
dimensionless fracture conductivity	49.0	834.0

The simulation model and all the analyses performed with it are based upon numerically generated solutions with very well-defined assumptions, many of them not strictly applicable to the reservoir which was actually used for the trial. The analyses, therefore, should be used only as indicators of relative changes in reservoir properties. No dramatic increases occurred in the production either of oil or of water and from the transient analysis there was no significant change in the reservoir system. The permeability of this reservoir appears to be too low to allow the leak-off into the matrix which was essential for the successful achievement of all the trial objectives.

6. CONCLUSIONS

The development of a new technology in the laboratory and its transfer into the field is rarely smooth. As well as throwing up a few technical modifications to be effected in further trials, this test led to several conclusions significant for the future design of bacterial acid fracturing procedures:

- operational logistics and equipment requirements for the microbial acid fracture treatment differed little from conventional acid fracturing methods and no special equipment was necessary;
- none of the components of the injection fluid is corrosive or hazardous, either at the point of injection or when later produced back;
- no difficulties were encountered in transferring the bacterial methodology from the laboratory to the field and the performance of the bacteria *in situ* was as expected. The trial showed the successful production downhole of large quantities of metabolic acid and the solution of about 1.1 tonnes of rock;
- the micro-effects of bacterial etching on various carbonate rocks need to be better understood with a view to maximizing the potential benefits of the bacterial treatment;
- no well damage resulted from the test except for some enhancement of H_2S production. Subsequent work in our laboratories has shown how this may be controlled by including suitable low-cost chemicals in the injection fluid.

The work reported in this paper was funded in part by the Offshore Supplies Office, U.K. Department of Energy (now incorporated into the Department of Trade and Industry).

7. REFERENCES

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2. C.E. ZoBell. U.S. Patent 2 413 278 (1946).
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A Pilot Test of EOR by In-Situ Microorganism Fermentation in the Daqing Oilfield

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Abstract

Using microorganisms for in situ fermentation to enhance oil recovery is a new technology in the petroleum industry. This paper discusses the screening and evaluation of bacterial strains both in the laboratory and in a pilot test in the Daqing oilfield.

The bacteria used are *Pseuodomonas aeruginosa*, *Xanthomonas campestris*, *Bacillus licheniformis*, and 5GA, which is like Bacteroides. Oil recovery in the laboratory tests were increased by 34.3%, and the residual oil recovery was 69.8%. A huff-and-puff pilot test on two wells, Dong 6-J22 and Dong 5-J18, which are located in the heavy oil pay zones in the oil-water transition zone of eastern Daqing was run in June-July, 1990. After the two wells were shut off, for 40 and 64 days, respectively, the pressure in the tubing and casing of Well Dong 6-J22 had increased by 1.6MPa and 1.9MPa, respectively. Also daily liquid production was increased by 15-20 t/d, water cut decreased from 94% to 84%, and oil production increased from 3 t/d-5.6 t/d and lasted about 8 months. After Well Dong 5-J18 was shut off for 64 days, liquid production increased, oil production increased from 7.6 t/d to 10-11 t/d, and gas production increased from 234 m^1/d to 547 m^1/d and lasted about 18 months.

1. INTRODUCTION

Today, a new technological revolution, bioengineering, and its applications, is developing all over the world to exploit energy resources. One of the most promising fields in bioengineering, EOR, has greatly progressed since the first petroleum crisis in 1973. Along with this development, microbial enhanced oil recovery of most studies has made enormous strides.

In the 1950s, the target of most studies focused only on bacterial oil recovery. With the appearance of sophisticated equipment and analytical approaches, the development of the basic theory of microbial techniques, especially molecular genetics, and new microbial reactors, has progressively entered a new stage; some promising single-well tests have been made [1-9]. In the last two or three years, more tangible results have been obtained. The NIPER group, U.S.A., made a pilot test in 1988 [10], and field tests were also made in Western Germany and Australia; all these results were satisfactory, and the techniques show excellent prospects for further development and application.

However, this is the first time that China has injected bacteria into reservoirs to improve oil recovery: this paper presents the results of our study.

2. LABORATORY EXPERIMENTS

2.1. Bacterial strains and working principles

The bacteria used were aerobic and facultative bacteria obtained from sugar refineries, from water produced from oil wells in the Daqing oilfield, and from

soil samples damaged by oil. They were *Xanthemonas compestris*, *Bacillus licheniformis*, and *Pseuodomonas aeruginosa*, which were designated as SU_{2-1}, SU_{1-2-3}, and SU_{4-1-2}, respectively, and another strain 5GA, similar to Bacteroides, designated SU_{5-2}.

The working principles of SU_{2-1}, SU_{1-2-3}, SU_{5-2}, and SU_{4-1-2}, are: (1) to degrade a heavy crude oil into a light one and improve its mobility; (2) to generate organic acid and reduce the oil-water interfacial tension; (3) to generate CO_2 by oxidation and degradation: the produced CO_2 enhances fluid mobility. Strain 5GA generates much CO_2, with a maximum of 15–20 ml CO_2 for every ml pore volume, while, at the same time, oxidizing organisms generate organic solvents, so improving the flow of crude oil and enhancing oil recovery.

The culture medium for SU bacteria was:

KH_2PO_4 10g NaH_2PO_4 5g
$(NH_4)_2SO_4$ 2g $MgSO_4 \cdot 7H_2O$ 200 mg
$CaCl_2 \cdot 7H_2O$ 1 mg $FeSO_4 \cdot 7H_2O$ 1 mg
Crude oil 50g (or Hexadecane 25 ml)
Water 1000 ml with a pH 8.7–7.4

The culture medium for 5GA bacteria was:

Molasses 5%
Residue of sugar 4% (replaced by 4% filter mud afterward)
Crude oil 5%

2.2. Evaluation of the bacterial strains

Two evaluations must be made for the fermentive bacteria strain: first, to evaluate the effect of changing the fermentive oil, and second, to evaluate what changes in biological chemistry have taken place to the fermentive fluid. It is difficult to establish methods for evaluation of these properties in a short period.

Using analytical instrumentation, five methods of evaluation were set up by our research team:

2.2.1. To determine changes in the components of crude oil before and after fermentation, three methods were used

a. The Engler distillation method was used to check differences in the volume of distillate under the same distillation conditions.

b. Chromatography was used to compare total hydrocarbons, and to analyze changes in the saturated hydrocarbons before and after fermentation, and to determine the biodegradation of crude oil based on the changes in the ratio of pristane/C_{17} (Pristane–2.6.10.14–tetramethyl pentane (isononadecane)) and phytane/C_{18} (Phytane–2.6.10.14–tetramethyl hexane (isoeicosane)).

The ratio of pristane/C_{17}: phytane/C_{18} is taken as an index of the biodegradation of crude oil; the larger the value, the more n–alkane is transformed into cycloalkanes, the flow of the crude oil becomes better, and the solidification point is decreased. This value is easily determined in the chromatograph.

c. Chromatography of the composition of oil was used to analyze the percentage of saturated hydrocarbons, aromatic hydrocarbons, non–hydrocarbons, and asphaltenes in the crude oil before and after fermentation. From the changes seen, the parts of the crude oil that were mainly affected by the microbes can be analyzed.

Table 1
The results of Engler distillation

Temperature (°C)	Crude Oil (%)	Fermentation Oil %			
		SU_{2-1}	SU_{1-2-3}	SU_{5-2}	SU_{4-1-2}
<100	1.2	43.8	40.56	6.22	6.92
100–150	2.2			2.46	8.22
150–200	4.0			1.12	
200–250	5.8			3.98	6.58
250–300	8.2			9.68	48.54
>300	78.0	56.0	55.82	75.72	28.28
Total Recovery:	99.40	99.8	96.36	99.18	98.54

Table 2
Effect of bacteria on the properties of crude oil

	Pristane /C^{17}	Phytane /C^{18}	Flow
Crude Oil	0.184	0.142	
SU_{2-1}	1.0	0.667	Good
SU_{2-1}	2.230	1.571	Good
SU_{1-2-3}	3.166	1.578	Good
SU_{1-2-3}	0.795	0.633	Better
SU_{4-1-2}	2.36	1.18	
SU_{4-1-2}	0.83	0.73	
SU_{5-2}	0.29	0.20	
SU_{5-2}	0.2	0.16	

2.2.2. Determining the change in the properties of the fermented fluid

a. Interfacial tension.

An important factor in evaluating the bacterial strains is to measure whether the interfacial tension of fermented fluid changes, to determine if any active material is generated or not and the amount generated.

b. Content of organic acid in the fermented fluid.

2.2.3. Evaluation of the simulation

a. Evaluation under atmospheric pressure; some equipment has been improved.

b. Evaluation in a high pressure model.

2.3. Results of evaluation of the bacterial strains

2.3.1. Evaluation of SU_{2-1}, SU_{1-2-3}, SU_{5-2}, and SU_{4-1-2}

a. Crude oil is strongly degraded by SU_{2-1}, SU_{1-2-3}, SU_{5-2}, generating the lighter components. From chromatography of 87–54, 87–41, 87–51, and 87–11, the curves generally appear to be shorter, and the amounts of the components lower than C_{17} increase. For example, the chromatographic curve of 87–41 is much

shorter than the control chromatograph of crude oil, and the peak value becomes longer, showing these bacteria play an important part in the fermentation of crude oil. Figure 1 shows that the SU_{5-2} bacteria degrade the heavy components of crude oil, generating many light components, much lighter than C_{15}. The results of the Engler distillation further validate this finding (Table 1). For example, SU_{2-1} and SU_{1-2-3}, fermented oil, when distillation was carried out at 100°C, yields a 43.8% and 40.56% cut at atmospheric pressure, respectively, while under the identical experimental conditions the original crude oil cut is only 1.2%, and up to 300°C, the total distillation cut of crude oil is only 20.8%. In Table 1, the increment of the light components in the fermentation oil from the SU_{4-1-2} and SU_{5-2} bacterial strains is not as large as that from SU_{4-1-2} fermented oil. When distillation is carried out at 0-200°C, the distillation cut of crude oil is only 7.4%, while the distillation cut of SU_{4-1-2} fermented oil reaches 15.15%, about double the above value while the light component of SU_{5-2} increases slightly. This method improves the production of oil.

b. The SU_{2-1}, SU_{1-2-3}, and SU_{5-2} bacteria increased the flow of the crude oil. The chromatographs of n-alkanes show that the curves not only are shorter, with more isopeaks, but the peak values of C_{17} and C_{18} are much shorter than the peak values of pristane and phytane, and the ratio of pristane/C_{17}, phytane/C_{18} increases markedly. The ratios of crude oil increase from 0.1846 and 0.1429 to 2.0-3.0 and 1.2-1.6, respectively, which is an important indicator showing that the flow of the crude oil has improved (Table 2).

c. Producing organic acid and active material.

The pH of the fermented liquid decreases significantly (generally, the pH falls from 7.4 to less than 6.0), and the acid value of the crude oil increases significantly. SU_{5-2} can cause an increase in the acid value of the fermented crude oil from 0.01 mg/g(KOH) to 0.11 mg/g (KOH). These changes in pH reflect the production of organic acids by oxidation during bacterial growth. The organic acid content of the fermented liquid increases to varying degrees (Table 3), with a maximum value of 226.3 mg/l. Because the organic acid content and fermentation increase, the interfacial tension decreases to varying degrees (Table 4). Usually, the interfacial tension of the fermented liquid falls by 10 mN/m. For example, SU_{2-1}, which gives the best results, causes the interfacial tension of the fermented liquid to decrease from 36 dyne/cm to 35 mN/m. SU_{5-2} causes the interfacial tension of the fermented liquid to decrease to 8.32 mN/m and 6.01 mN/m.

In laboratory tests, we found that SU_{2-1}, SU_{1-2-3}, SU_{4-1-2}, and SU_{5-2} could degrade the gelatine asphalt in the crude oil.

Summing up our findings, the screened bacterial strains, SU_{2-1}, SU_{1-2-3}, SU_{5-2}, and SU_{4-1-2} can degrade crude oil and its heavy components, generating lighter components, organic acids, and surface active materials. These products are all favorable to EOR.

d. Simulation using the LAZAR device under normal pressure.

The four kinds of bacteria and the field water were mixed together, and a few nutrients were added. Using conventional methods, the model was saturated by water and oil, and then displaced by water. When the water cut reached 98%, the model was displaced continuously by water for more than two hours, then water displacement was stopped. At this time, 2PV of fluid containing bacteria was injected into the model, and it was placed in a box at a temperature of 45°C for cultivation and observation.

The withdrawal in the LAZAR model, which was cultured for 113 days, increased as the culture time increased; i.e., over 30 days, the discharged water was 189 ml, the discharged oil 8 ml, and the discharged gas 250 ml. After 30 days, the discharge of gas stopped and the discharged gas in the culture increased

Table 3
Organic acid content of fermentation liquid

	Fatty Acid Content mg/l	Remarks
Blank	0	
SU_{2-1}	21.36	28/2 bench
SU_{2-1}	48.07	19/3 bench
SU_{1-2-3}	122.84	14/5 bench
SU_{1-2-3}	69.43	18/7 tank
SU_{4-1-2}	110.42	11/7 tank
SU_{4-1-2}	117.15	18/8 tank
SU_{4-1-2}	226.36	28/7 tank
SU_{5-25}	110.42	10/10 bed
SU_{5-26}	98.86	10/10 bed

Table 4
Interfacial tension of fermented liquid

	Interfacial tension mN/m	Remarks
Distilled water	35.69	Interfacial tension to simulated crude oil
Culture fluid	34.3	Interfacial tension to simulated crude oil
SU_{2-1}	24.2	Interfacial tension to simulated crude oil
SU_{2-1}	3.5	Interfacial tension to simulated crude oil
SU_{1-2-3}	28.76	Fermentation jar
SU_{5-2}	8.32	Bed 10/10
SU_{5-2}	6.01	Bed 23/10
SU_{4-1-2}	24.50	Jar 7.18
SU_{4-1-2}	24.76	Bench

significiantly. By the 58th day, the produced gas was up to 1800 ml, and the maximum production rate was 250 ml per day. The O_2 content had fallen to zero by day 90, while the methane content continued to increase. Thus, initially, the methane content was 16.59%, but on the 58th day it was 51.78%, and by day 90, it was up to 82.53%, and contained a little ethane, propane, and butane. The content of N_2 gas decreased from 63.43% to 33.22% by day 58, and by the 90th day, it had decreased to 16.33%. N_2 and O_2 were used up by microbial growth. The methane was produced by microbial consumption of hydrocarbons and other organic materials. The ultimate increment in oil was 41 ml, representing 8.3% of the original oil and 27.80% of the residual oil.

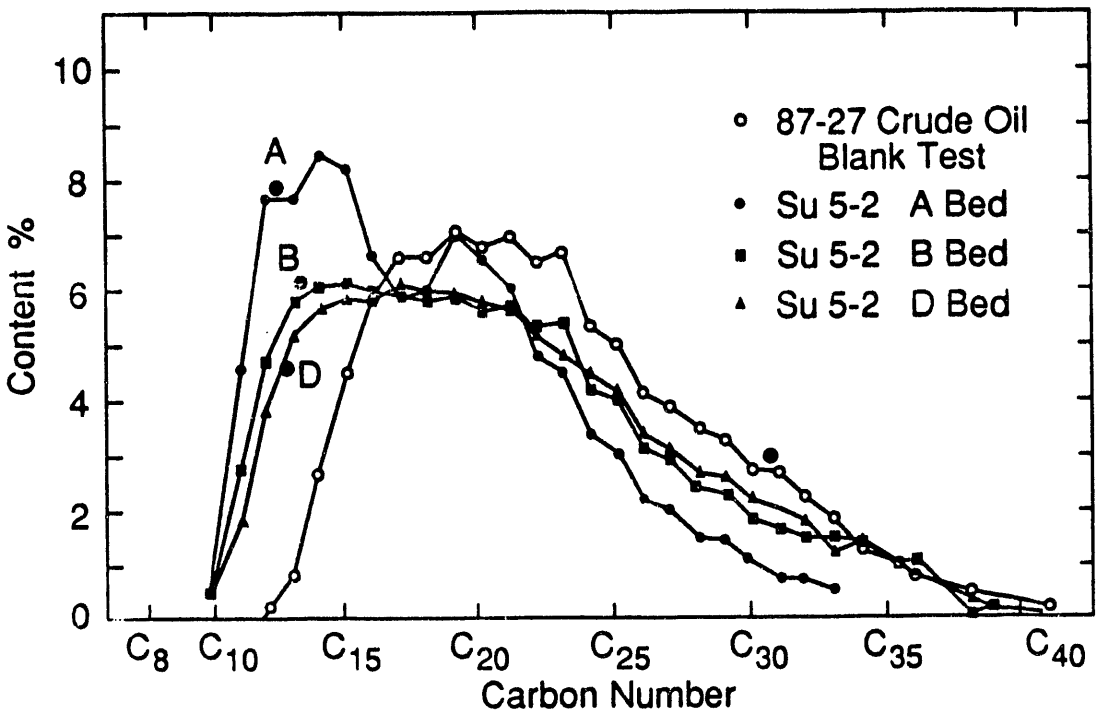


Figure 1. Curves of SU_{5-2} degrading crude oil.

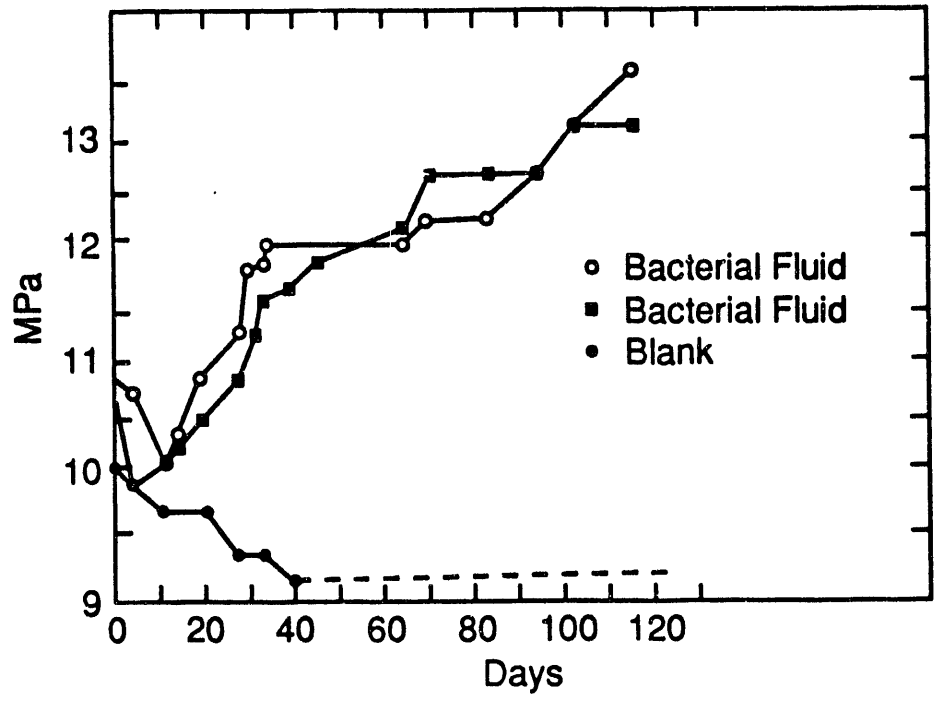


Figure 2. Gas pressure variation curves of high pressure model injected by bacteria.

The LAZAR test was repeated using the same methods. Increments of more than 2000 ml of gas and 27 ml of oil were produced, equivalent to 5.5% of the original oil and 17.4% of the residual oil.

e. High pressure simulation.

A high pressure simulation was conducted to determine whether the above results can be repeated under conditions of temperature and pressure that occur in the reservoir. We used bacterial strains SU_{2-1}, SU_{1-2-3}, and SU_{5-2}, adding the specified amount of the active field water and nutrients required by these bacteria. Permeability and porosity were measured, then the model was saturated by oil and displaced by water until 98% of the water cut was reached and maintained for 2 hours. Oil displacement was stopped and 2 PV of the bacterial fluid was injected. After the pressure inside the model became lower than the pressure outside the model, the entry and discharge valves were shut (maintaining a pressure of 10.0 MPa) so that the culture was held under high pressure.

On the 18th day, the pressure inside the model started to increase, and by day 60, the pressure had increased by 2.0 MPa, i.e., from 10.0 MPa to 12.0 MPa. After 113 days, the pressure inside model-1 had increased to 13.6MPa (an incremental of 3.6 MPa), and the pressure inside model-2 increased to 13.0MPa, an increment of 3.0MPa (Figure 2).

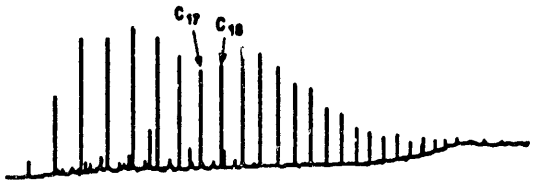
After culturing for 113 days, the process of oil recovery was carried out. The procedures were as follows: first, the elastic drive was started and a small amount of gas appeared. Then, the elastic drive was changed into the water drive, with the pressure being maintained at 10.4-11.6MPa, and a small amount of oil was produced. The amount of oil produced for both models was about 0.5 ml each. The amount of water produced was 788 ml and 819 ml for model-1 and model-2, respectively. The amount of gas produced was 603 ml and 618 ml, respectively. By comparison, there was little gas in a blank test. Later, the water drive was changed into the pressure depletion drive (the pressure was dropped from 10.5 to 0.5 MPa). For model-1, the gas produced was 632 ml, the oil was 1.5 ml, and the water was 2563 ml. For model-2, the gas produced was 1165 ml, the oil was 0.8 ml, and the water was 2570 ml. The interfacial tension of the fermented liquid fell, from 40 mN/m to 27.8 mN/m and 35.1 mN/m, respectively. A chromatographic analysis of normal paraffinic hydrocarbon was conducted for the produced oil (chromatographs 88-471 and 88-472, and Figure 3). We found that the produced oil contained more lighter components than the original oil. The lighter components of less than C_{17} increased by 30%. The gas produced was also analyzed; 92.47% of the gas from model-2 was methane, and for model-1, the value was 79.43%, i.e., 981 ml methane was produced from the model-1, and 1077 ml from model-2. These increases were significant.

According to our calculations, the oil produced from model-1 was 16.2% of the original oil and 41% of the residual oil, and from the model-2, the values were 13.21% of the original oil and 34% of the residual oil.

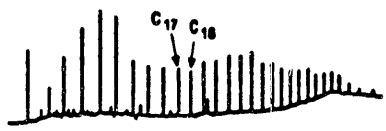
2.3.2. Evaluation of bacterium 5GA

a. Analysis of the properties of single bacterial strains and mixed bacterial strains; screening of six mixed aerobic strains (6A, 10A, 26A, 5GA, 22B, and 20A).

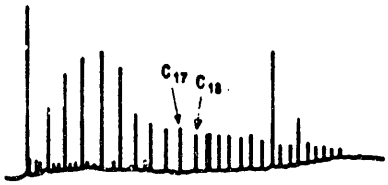
Based on work at the Beijing Microbial Institute, Chinese Academy of Sciences, the scope of the tests was extended. We know that the rates of gas production by 6A, 5GA and 26A were higher than those produced by other bacteria, so we gave priority to these three strains in fermentation tests. The results indicated that both the single bacterial and the mixed bacterial strains strongly degraded crude oil. The heavy hydrocarbon components were markedly degraded. The degradation of crude oil by the mixed bacterial (6A+26A+5GA) culture was obvious.



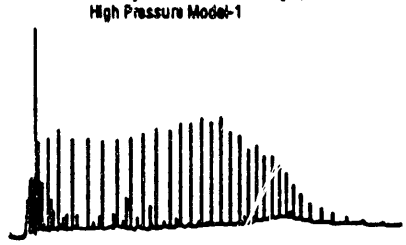
87-11 Su_{5-2}A Bed Total Hydrocarbon Analysis



88-471 Total Hydrocarbon Chromatograph Chart of High Pressure Model-1



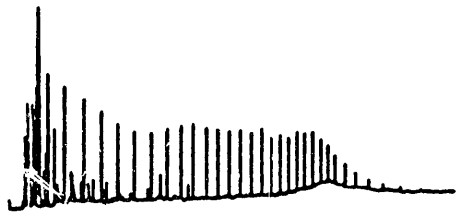
88-472 Crude Oil Total Hydrocarbon Chromatograph Chart of High Pressure Model-2



Crude Oil Total Hydrocarbon Chromatograph Chart of Well Dong 6 D22 (90-39) Before Bacterial Injection



Crude Oil Total Hydrocarbon Chromatograph Chart of Well Dong 6-D22 (90-88) After Bacterial Injection



Crude Oil Total Hydrocarbon Chromatograph Chart of Well Dong 6 D22 (90-11) After Bacterial Injection



Total Hydrocarbon Chromatograph Chart of Well Dong 5-D18 (90-38) After Bacterial Injection



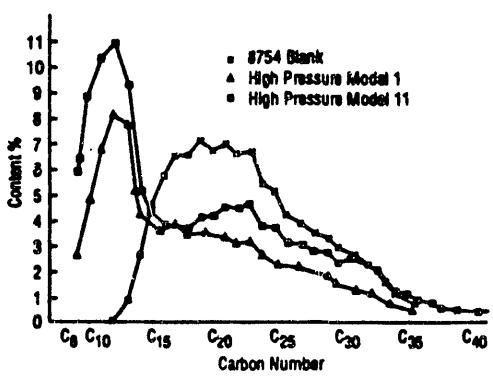
Total Hydrocarbon Chromatograph Chart of Well Dong 5-D18 (90-39) After Bacterial Injection



Crude Oil Total Hydrocarbon Chromatograph Chart of Well Dong 5-D18 (90-40) After Bacterial Injection



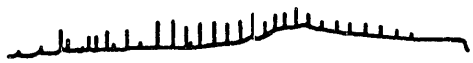
Total Hydrocarbon Chromatograph Chart of Well Dong 5-D18 (90-41) After Bacterial Injection



Crude Oil Component Variation Curve of High Presure Model



Blank Test Oil Sample Total Hydrocarbon Analysis



Fermented Oil Sample Su2-1 Total Hydrocarbon Chromatograph Chart



Fermented Oil Sample Su1-2-3 Total Hydrocarbon Analysis

Figure 3. Hydrocarbon Analyses: Chromatograms.

Both 5GA+Daqing bacteria, and 26A+Daqing bacteria degraded the heavy components in the crude oil, producing a large amount of the lighter components.

During fermentation, we found that all these bacteria could metabolize molasses and generate organic acid. The pH of the fermented liquid decreased from 7.4 to less than 6.0. The single bacterial strains generated a little organic acid, while the mixed bacterial strains generated a larger amount, the difference being about one order of magnitude. When the bacterial cultures were aerated, the amount of the organic acid increased by a factor of 3-4 (the maximum reached was 7621 mg/l). At the same time, the surface tension of the fermentation liquid decreased from 71 mN/m to less than 50 mN/m and the interfacial tension of the fermentation liquid decreased from 36.69 mN/m to less than 24 mN/m. This result showed that some active materials were generated in the fermentation process.

b. LAZAR simulation of a single bacterial strain.

In the light of above results, these bacteria were thought to be of benefit to EOR, because they can cause a decrease in the interfacial tension. In the crude oil test, three models (6A,5GA,26A) generated a large amount of gas. Within 10 days, 26A produced 1540 ml of gas, 6A produced 2000 ml of gas, and 5GA 1750 ml of gas. Gas production did not continue for long after a large amount of gas had been produced. The gases produced were mainly H_2, CO_2, and N_2. However, when a large amount of gas had been produced, methane appeared. The methane content rose gradually as the total amount of gas increased, to a maximum of 76%. At this time, the amounts of CO_2 and H_2 approached to zero. As gas was produced, oil also was produced. After four months, 26A had produced 12040 ml of gas, and 5GA had produced 6500 ml. The incremental oil produced by 6A was 29 ml, equivalent to 7.20% of the original oil and 21% of the residual oil; the incremental oil produced by 5GA was 50.5 ml, equivalent to 11.12% of the original oil and 34.6% of the residual oil (Table 5). Finally, when water was injected, there was little residual oil left, and the oil sand was almost white.

c. LAZAR simulation of mixed bacterial strains.

From these data, we can see that SU_{2-1}, SU_{1-2-3}, and SU_{5-2} have a stronger capability of degrading crude oil and simultaneously producing more cycloparaffinic components and improving the mobility of the oil. However, these bacterial strains do not produce a large amount of oil. Strains 6A, 26A, and 5GA have a lesser capability of degrading the crude oil, and there is no cycloparaffin petroleum in the oil produced. Hence, the mobility of the fermented oil is relatively poor, although the rate of gas production in molasses and oil is higher which helps to enhance the oil recovery. To make these properties of the different strains of bacteria complementary, and useful in EOR, these bacterial strains were mixed in a simulation test. The following three combinations were used.

$6A + SU_{1-2-3} + SU_{2-1} + SU_{5-2}$ (Model 6)
$26A + SU_{1-2-3} + SU_{2-1} + SU_{5-2}$ (Model 7)
$5GA + SU_{1-2-3} + SU_{2-1} + SU_{5-2}$ (Model 8)

Results from these three models are given in Table 5. Model 6, 6A+SU, generated 1250 ml of gas within 10 days and produced 44.5 ml of oil within 37 days, giving an oil recovery factor of 8.3%, equivalent to a residual oil recovery factor of 30%. After four months, an additional 10.4 ml of oil was produced by water drive, giving an oil recovery factor of 12.5%, equivalent to a residual oil recovery factor of 38.2%. Model 7, 26A+SU, generated 4750 ml of gas within 10 days and produced 48 ml of oil within 37 days, giving an oil recovery factor of 8.3%, equivalent to a residual oil recovery factor of 28.9%.

Table 5
Results of bacteria in LAZAR simulation

No	Oil Rate (ml)	Oil recovery factor(%)	Oil Rate (ml)	Oil recovery factor(%)	Oil Rate (ml)	Oil recovery recovery factor(%)	Residual oil recovery factor(%)	gas generated (ml)	Notes
1	27	5.5	296.5	60.3	41	8.3	27.8	2400	SU_{2-2}+SU_{1-2-3}
2	241	49	80	16.3	27	5.5	17.4	2650	SU_{5-2}+WH_1
3	245.5	55.80	42.5	9.6	32	7.3	21	12040	26A
4	188.7	46.59	75	18.5	29	7.2	21	4750	6A
5	243.5	52.37	72	15.4	50.5	11.12	34.6	6500	5GA
6	181	34.2	200	37.7	66.4	12.5	38.2	1250	6A+SU
7	290	51.8	108.1	19.3	104.4	18.6	55.9	4750	26A+SU
8	115	24	153	31.9	165	34.4	69.8	1250	5GA+SU

Notes:
6. 6A+SU_{2-1}+SU_{1-2-3}+$SU_{2-4}Wh_1$
7. 26A+SU_{2-1}+SU_{1-2-3}+$SU_{5-2}Wh_1$
8. 5GA+SU_{2-1}+SU_{1-2-3}+SU_{5-2}+Wh_1

Wh_1. Active mire water

After four months, an additional 10.4 ml of oil was produced by water drive, giving an oil recovery factor of 18.6% equivalent to a residual oil recovery factor of 55.9%. Model 8, 5GA+SU, generated 1250 ml of gas within 10 days and 30 ml of oil, giving an oil recovery factor of 16.7%, equivalent to a residual oil recovery factor of 44.2%. After four months, another 165 ml of oil was produced, giving an oil recovery factor of 34.4%, equivalent to a residual oil recovery factor of 69.8%.

On the basis of above results, we recommended using the bacterial combination in model 8 for field testing.

3. FIELD TEST

3.1. Test plan for microbial injection

Based on the laboratory results, "the microbial huff and puff EOR test" for two producers was conducted. The main points of the test plan were as follows:

1. According to the ability of the chosen bacteria to degrade crude oil and generate CO_2, a test well was chosen in the eastern part of the central area, which is in the oil-water transition zone or the viscous oil area; here, the viscosity of the oil is higher and the remaining oil saturation is also higher. Well Dong 6-D22 is located in the 4th zone and Well Dong 5-D18 is located in the 3rd zone.
2. Both wells were tested by a "microbial huff and puff test."
3. For convenience and for comparison of the results, the test wells were not reworked, the pumps were not pulled out, and the injection was made through the casing. Thus, the working system was consistent before and after the test.
4. The bacterial fluid was prepared in the fermentation plant. Then, the fluid for injection was formulated in the well sites and was injected by high-pressure piston pumps.
5. After microbial injection, the wells were shut for no less than 40 days to allow the microbes to ferment in situ.
6. The field test results were evaluated by using data on the production performance and by analyses of the physical and chemical properties of the oil, gas, and water before and after the test.

Boiler trucks were stationed at well sites. All facilities and pipelines were sterilized, and care was taken to ensure that all personnel were protected. The flow system was sealed to prevent outflow of microbes to the environment.

3.2. RESULTS

After the microbial injection, Well Dong 6-D22 was shut for 40 days and opened up on August 15th; Well Dong 5-D18 was shut for 64 days and opened up on September 20th. The following results were obtained.

3.2.1. In situ bacterial fermentation during the shut-in period

a. After the well was shut, wellhead pressure went up. For the first nine days, the wellhead pressure in Well Dong 6-D22 fell from 11.0 MPa to 7.4 MPa (casing pressure) and 7.2 MPa (tubing pressure). From the 10th day, the wellhead pressure gradually rose and continued to increase for about 20 days. The casing pressure was 9.3 MPa, and the tubing pressure was 8.8 MPa, increasing by 1.9 and 1.6 MPa, respectively (Figure 4). Similar phenomena were seen in Well Dong 5-D18. After the well had been shut for 16 days, the tubing pressure started to increase, but the magnitude of the pressure increase was smaller than in Well

Dong 6-D22 (Figure 5). A preliminary analysis indicated that the pressure increase was due to the growth of the injected microbes. The metabolic activity in the reservoir produced a large amount of gas, increasing the pressure. During this period, the working condition of other producers and injectors around this well were not changed.

b. Gas sample analysis during the shut-in period.

During the shut-in period, gas samples were taken and analyzed. The CO_2 content of Well Dong 6-D22 increased from about 1% before the test to 11.7%; the CO_2 content in Well Dong 5-D18 increased to 55.8%. These results are in agreement with the results of laboratory tests.

3.2.2. Variation in the properties of oil, gas, and water after opening up the wells

a. There was a large increase in the numbers of live bacteria in the produced water. On the day when Well Dong 6-D22 was opened up, the number of live bacteria in the water was up to 1.6×10^7/ml. At present (February 1991), the numbers in the water are still at a high level of 6.0×10^4/ml. On the day when Well Dong 5-D18 was opened up, the number of live bacteria in the water was 9.5×10^3/ml; now, the number is 1.3×10^2/ml.

All the strains of bacteria injected were detected in the produced water. Our results show that the bacteria injected into the wells can live and grow in the reservoir for a long time, signifying that production could continue over an extended period.

b. Increase of organic acid in the water produced from oil wells.

The organic acid content in the water produced from wells increased by a factor of more than eleven. The oil-water interfacial tension decreased slightly. These changes are useful in helping to displace the oil.

c. After opening up, the CO_2 content in the gas produced from Well Dong 6-D22 had increased to about 7%, nearly ten times the content before the test. At present (February 1991), the CO_2 content has decreased to about 1%, which is the normal value. The CO_2 content in Well Dong 5-D18 was always higher and has been maintained in a range of 6-10%. CO_2 provides not only the energy to displace the oil but also can improve the properties of the oil.

d. The components with values below C_{15} increased in the crude oil by 3-5% in Well Dong 6-D22, and by 3-9% in Well Dong 5-D18. The initial distillation point of oil from Well Dong 5-D18 decreased by 10°C. These changes are shown in the chromatographs (see chromatographic profiles and curves).

3.2.3. Increase of rate of oil and water production

a. After microbes were injected into Well Dong 6-D22, the water cut decreased by about 5% and oil production increased from 3.5 t/d before the test to 5.5 t/d. The incremental oil production lasted for 8 months. The cumulative incremental oil recovery was 480 tons.

b. After microbes were injected into Well Dong 5-D18, the oil production rate increased. The average production rate was 7.6 t/d before injection, and increased to 10-11 t/d after injection, with a maximum rate up to 17-25 t/d. By the end of December 1991, this continuous increase in oil production had lasted for 18 months. The net increment was 988 t. Even now, there is still a trend of increase in the rate of oil production. The rate of gas production increased from 234 m^3/d before the test to 457 m^3/d (average gas production rate after 14 months).

From these results, we conclude that Well Dong 5-D18 with the higher oil saturation gives better results than Well Dong 6-D22 with low oil saturation.

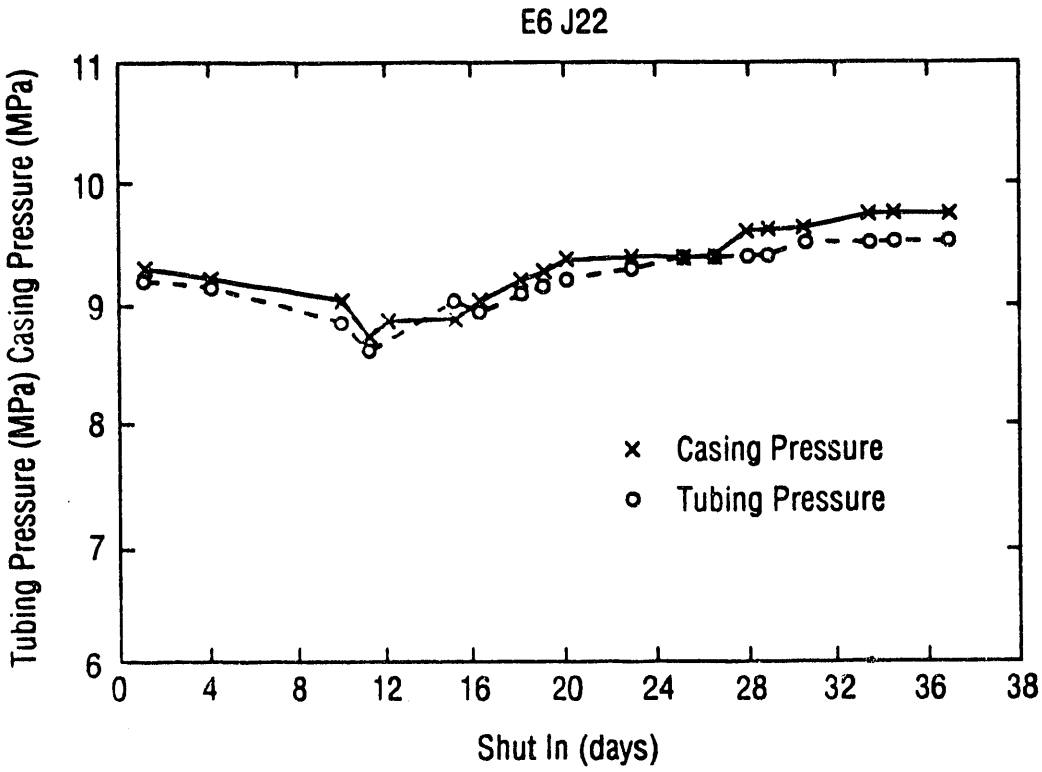


Figure 4. Wellhead pressure build-up curves of Well Dong 6-D22 after shut in.

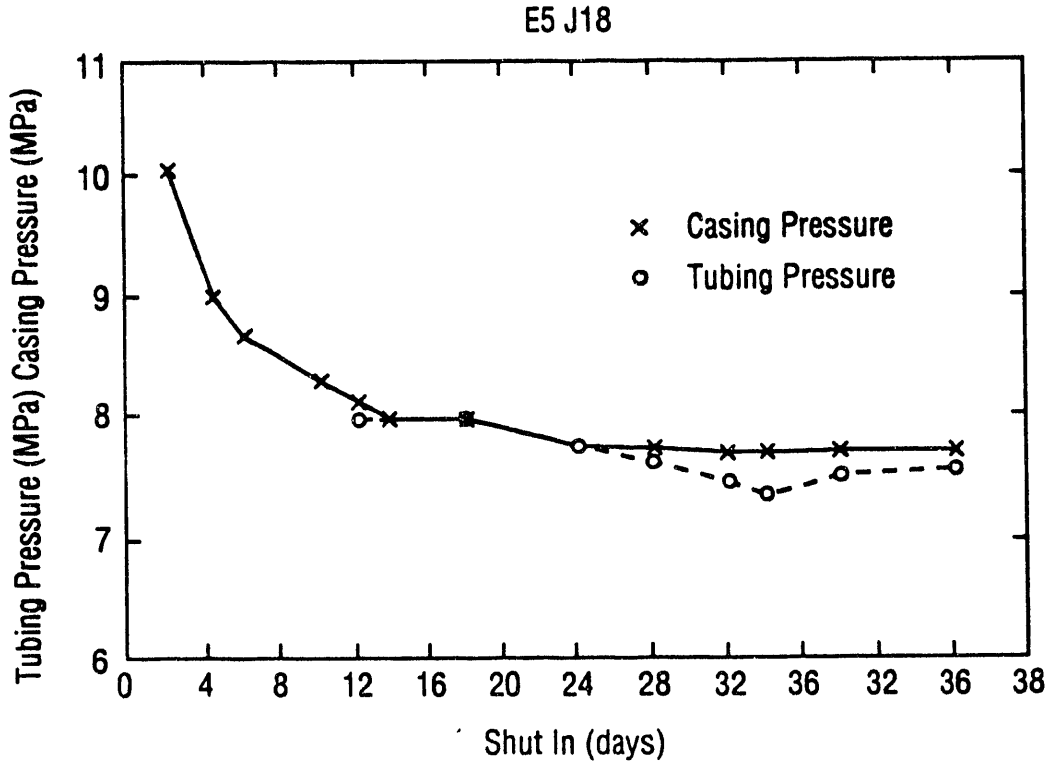


Figure 5. Wellhead pressure build-up curves of Well Dong 5-D18 after shut in.

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The Application of Microbial Enhanced Oil Recovery to Trinidadian Oil Wells

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Abstract

The Trinidad and Tobago Oil Company Limited (TRINTOC) possesses approximately 1300 active oil wells, of which 75% produce less than fifteen (15) barrels per day. The decline in natural production was 15-18% per annum over the last five years. Efforts are underway to examine ways to enhance the oil recovery from existing reservoirs. Since Trinidad and Tobago produces sugar, it was anticipated that MEOR using sugar by-products is a technique by which stripper oil wells may economically produce incremental oil.

Of the various options available for using microbes to improve well productivity, it was felt that single well stimulation would provide the most attractive opportunity for TRINTOC to evaluate the potential of this technique. The indigenous stimulation of bacteria to produce gases, surfactants, and acids using nutrients available from the sugar industry was chosen as the first approach. The Caribbean Industrial Research Institute (CARIRI) was contracted to investigate the effects of various nutrients in selected TRINTOC well salines.

A set of screening criteria for the use of this technique was developed and applied to all of TRINTOC's oil wells. The screening process identified those wells producing oils of gravities greater than 25° and oil production ranging from 5-12 bopd and some water; this represented approximately 10% of the active wells, most of which were located within four reservoirs/fields. Two of these reservoirs, the Catshill CO 30 sand and the Guayaguayare Lower Gros Morne sand, were further identified as good prospects for the techniques, if they were successful in field tests.

To start the laboratory work, waters from wells GY 212 and CO 34 of these two reservoirs were treated to stimulate the indigenous bacteria to determine the production of gases, surfactants, and acids. A preliminary investigation was carried out of the microorganisms present in well salines GY 212 and CO 34. The waters were also chemically analyzed. The production of gases was considered the most important recovery factor of single-well stimulations, and therefore, nutrient mixture ratios were varied to optimize the production of gases. The nutrient mixture used had varying amounts of molasses, ammonium nitrate, and Tanner's solution.

The production of biosurfactants by microorganisms in the well salines of selected wells was determined by quantifying the changes in surface tension of the experimental solution before and after incubation; these data showed changes of a surface tension of 16mN/m. It also was found that the change of pH ranged from a maximum of 2.5 units (pH 7.3-4.8) in well saline GY 212, to 0.6 units (pH 6.4-5.8) in CO 34.

The experimental work carried out at CARIRI establishes a protocol for selecting reservoirs for single well stimulation. Optimizations also will be conducted on other reservoirs. The results indicate that the technique has significant potential for application. Future work in this area includes further

identification of the microorganisms present, further optimization using locally available nutrients, and execution of a field test program.

1. INTRODUCTION

Following a request from the Caribbean Industrial Research Institute (CARIRI), the United Nations Industrial Development Organisation (UNIDO) funded the visits of two consultants, R. M. Knapp and M. McInerney to Trinidad & Tobago in December 1990. They held discussions with technical personnel from the Trinidad and Tobago Oil Company Limited (TRINTOC), the Trinidad and Tobago Petroleum Company Limited (TRINTOPEC), and TRINMAR Limited about the potential for the application of Microbial Enhanced Oil Recovery (MEOR) technology in Trinidad and Tobago. After R. M. Knapp's site visits to TRINTOC and TRINTOPEC field operations, a Workshop was held on January 7 and 8, 1991 at which a proposal for an initial MEOR Research program for Trinidad was outlined. It was decided to focus mainly on single-well microbial treatments because this approach appeared to have the most immediate potential for increasing oil productivity from Trinidadian reservoirs. Such treatments could be carried out inexpensively and the analytical resources were available at TRINTOC and CARIRI.

A joint project was subsequently developed by TRINTOC and CARIRI in which TRINTOC screened all its land-based wells, CARIRI undertook benchscale investigations of the effects of various nutrients on the indigenous microorganisms in saline samples obtained from selected TRINTOC wells, and TRINTOC agreed to provide CARIRI with the background technical information required to carry out the project.

2. SELECTION OF CANDIDATE WELLS FOR MICROBIAL STIMULATION

Most Trinidadian onshore wells produce less than 20 stb/day, and they suffer from a continuous decline in productivity. TRINTOC considered that individual well treatments to remove local barriers to flow and to increase production rates would be an attractive use of microbial techniques since the anaerobic production of gases, acids, biosurfactants, and solvents from carbohydrate substrates by microorganisms had been reported to improve well productivity in other locations [1-4]. Such production could be accomplished by stimulating indigenous microbial populations in the water phase of the reservoir. Initial targets for MEOR application, therefore, had to be wells with a substantial water phase present in the reservoir. Chosen wells should also span a variety of states of permeability and oil gravity, as recommended by R. M. Knapp and M. J. McInerney [5] in their report compiled after the UNIDO-funded Workshop in January 1991. TRINTOC wells were screened according to the criteria listed in Table 1. The wells were screened in three phases.

Phase I of the screening process eliminated all wells which were subject to CO_2 or Steam Injected Projects.

Phase II of the screening listed all land-based wells producing approximately 10 bopd, and with water production. Three hundred and forty-seven wells were listed after this phase.

Phase III listed all wells with oil gravities greater than 25° API which produced approximately 10 bopd with water production. Eighty-five candidates were identified.

Table 1
Selection of candidates for microbial stimulation using single-well treatment technique

Phase I screening	Eliminate all wells in CO_2 or Steam Injection Projects
Phase 2 screening	List land-based producing wells up to 10 bopd with water production
Phase 3 screening	Eliminate wells with API gravity <25 degrees

List of wells within TRINTOC Leases with potential for application of microbial stimulation

Candidate Selection

Exclude wells with suspect wellbore mechanical integrity

Exclude wells with wax deposition problems

Rank wells favoring those not producing emulsions

Rank wells in batches in order of potential for expansion (Result: Listing of good candidates for testing for potential microbial stimulation).

Select representative wells for follow-up studies on chemistry and microbiology.

TRINTOC recommended that initial investigations should be concentrated in the Guayaguayare, Barrackpore, Oropouche, Catshill, and Trinity/Inniss areas.

Table 2 lists the average recovery parameters in these areas. Further screening of possible candidates for MEOR treatment resulted in the selection of twelve wells for chemical and microbial analyses.

Areas		Wells Selected for Analyses
Guayaguayare	33	GY212, GY 315, GY 341
Barrackpore/Wilson	13	BP 336, BP 450, BP 504
Trinity/Inniss	10	IN 4, IN 26
Oropouche/Siparia	10	CO 70, CO 118, CO 34, AO 39
Ortoire (Catshill/Balata)	8	
Forest Reserve	3	
Point Fortin	3	
Penal	3	
Brighton	1	
Palo Seco	1	

Table 2
Recovery Parameters by Fields

Field and Code Number	Producing Horizons and Code Number	Oil Gravity *API	Oil Visc. 100*F/cp	ϕ*	Sw*	Klinkenberg Permability to Air, md Range	Klinkenberg Permability to Air, md Average	Comments
Barrackpore 10	Shallow Herrera 23	33	5.8	0.24	0.27	31-1801	389	(2) 2 samples only
Guayaguayare 23								
a) Goudron	Goudron Sands 10	38	4.5	0.27	0.20	38-982	459	(1) Well GY-168
	Cruse Sands 12			0.27	0.33	-		
b) Navette	Lower Gross Morne 40	35	3.3	0.24	0.25	5-634	187	
c) Marcelle Valley	Lower Gross Morne 40	35	3.3	0.22	0.39	3-529	92	
d) Beach	Upper Gross Morne 11	30(1)	6.2	0.23	0.30	-	-	(1) Range 17-36° API
	Middle Gross Morne 39	26(2)		-	-	-	-	(2) Well GY-602
Oropouche 15	Retrench 30	32	5.0	0.29	0.31	1-192	64	
	La Fortune 31			0.26	0.32			
Trincity 16	Shallow Herrera 23	33	3.1	0.19	0.35	0-299	45	
Catshill 32	Catshill Formations	40	0.8	0.31	0.30			CO-38 Fault Block

*Core Values

All the listed wells have good well bore integrity, no wax or emulsion problems, and reservoir temperatures of less than 70°C (158°F).

Wells CO 34 and GY 212 were selected for a preliminary investigation of well salines at CARIRI and the development of a methodology which would subsequently be applied to all twelve wells.

Figure 1 shows the location of Trinidadian onshore and offshore oil and gas fields including Catshill (Well CO 34) and Beach Marcelle Valley (Well GY 212).

3. THE CO 30 SAND WATERFLOOD

CO 34 is an offtake well in the CO 30 Sand Waterflood (Figure 2). Figure 3 shows the production/injection profile of the Catshill CO 30 sand waterflood project from January 1986 to May 1992. The project produced 665 mbbls of

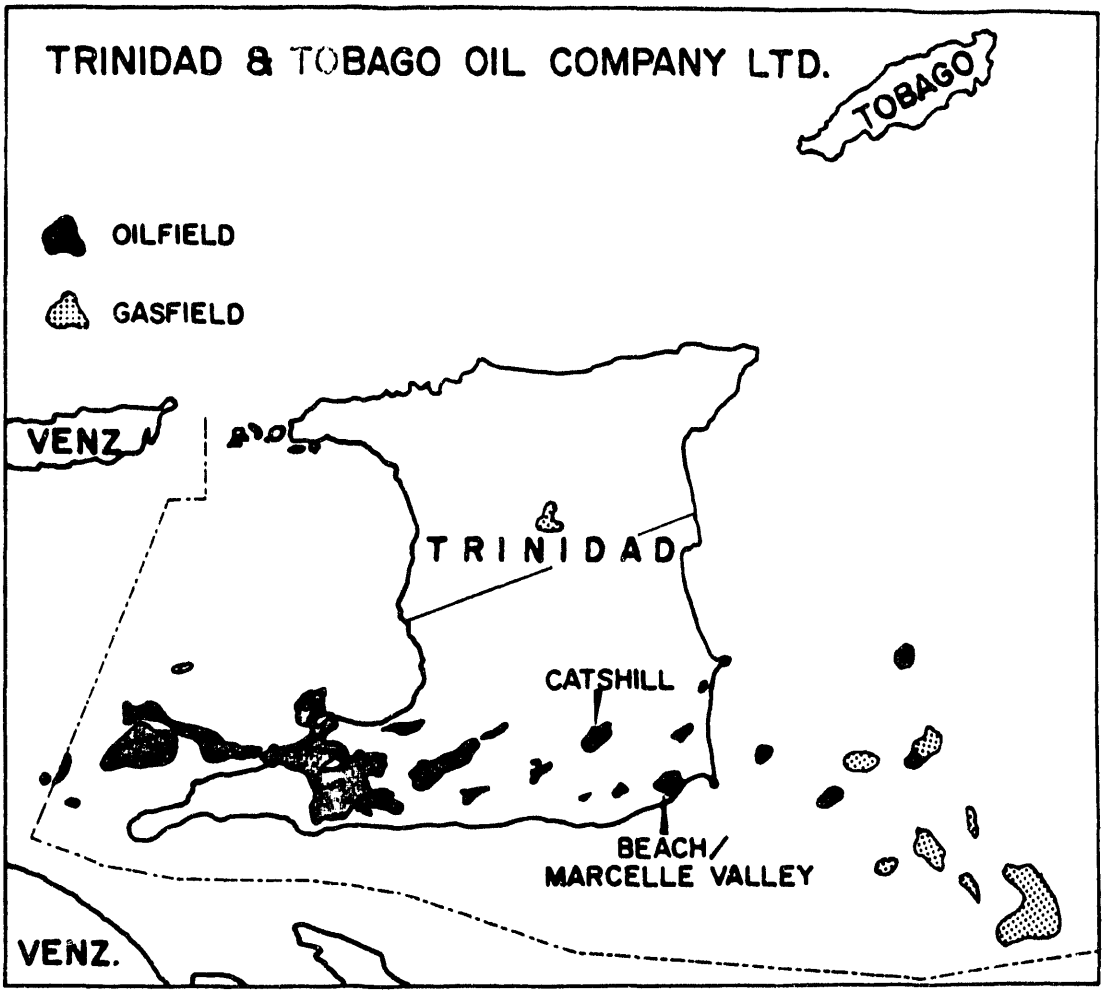


Figure 1. The location of Trinidadian onshore and offshore oil and gas fields including (Well CO-34) and Beach Marcelle Valley (Well GY-212).

secondary oil to May 31, 1992 for a cumulative injection of 7,409 mbbls of water. In May 1992, secondary oil was being produced at 86 bopd and the average water injection rate was 1153 bwpd. Table 3 summarizes the injection/production cumulatives to May 31, 1992. Table 4 summarizes the reservoir and rock fluid properties of the CO 30 sands, CO 24, and CO 38 blocks.

CO 34 is part of pattern Nos. 5 and 6 and its production/GOR from January 1987 to May 1992 is shown in Figure 4. It consists of injection wells CO 65 and CO 67. Injection well CO 65 is the center well in an inverted five-spot injection pattern. Offtake wells are CO 30, CO 34, CO 38, and CO 48. To date, CO 34 well saline has shown very promising results to nutrient stimulation on a laboratory scale and has produced a significant amount of biogas and biosurfactants. In addition, the bottom hole pressure is very low (≈240 psi), making it a suitable candidate for a single-well microbial stimulation.

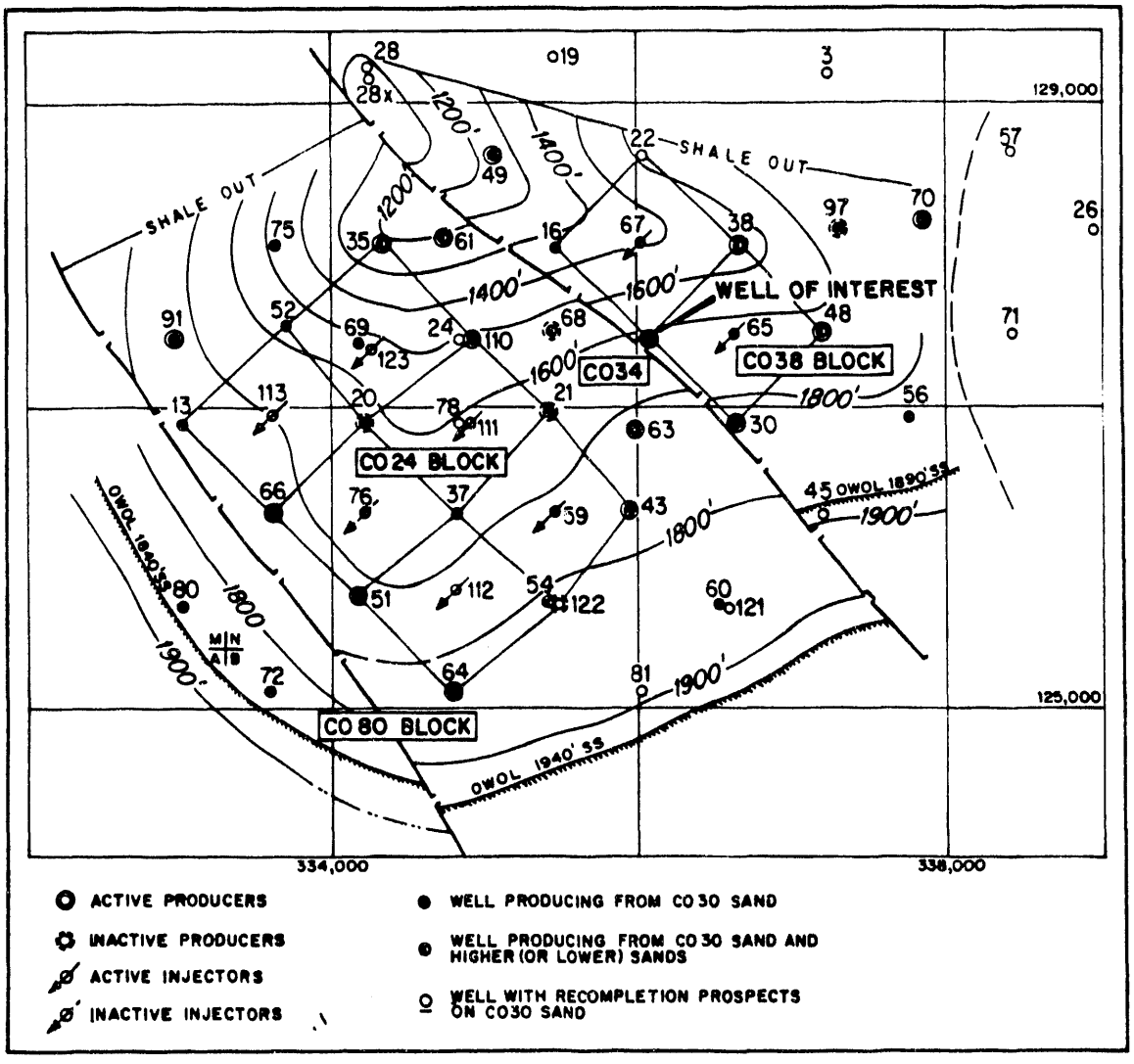


Figure 2. Contour map on top of CO 30 Sands Catshill central field.

Table 3
CO 30 Sand waterflood: injection/production cumulatives from March 1967 to May 31, 1992

Cumulative Water Injected	7409 mbw
Cumulative Total Oil Produced	1154 mbo*
Cumulative Secondary Oil Produced	665 mbo
Cumulative Water Produced	232 mbw*
Cumulative Gas Produced	800 mmcf*
Estimated Fill-up Volume for CO 30 Sand	10,700 mbw
Estimated Fill-up	70%
Cumulative GOR	693 scf/bbl
Cumulative WOR	0.17 bw/bo
Cumulative Injection/Production Ratio	6.4 bw/bo

*From project inception, March 1967.

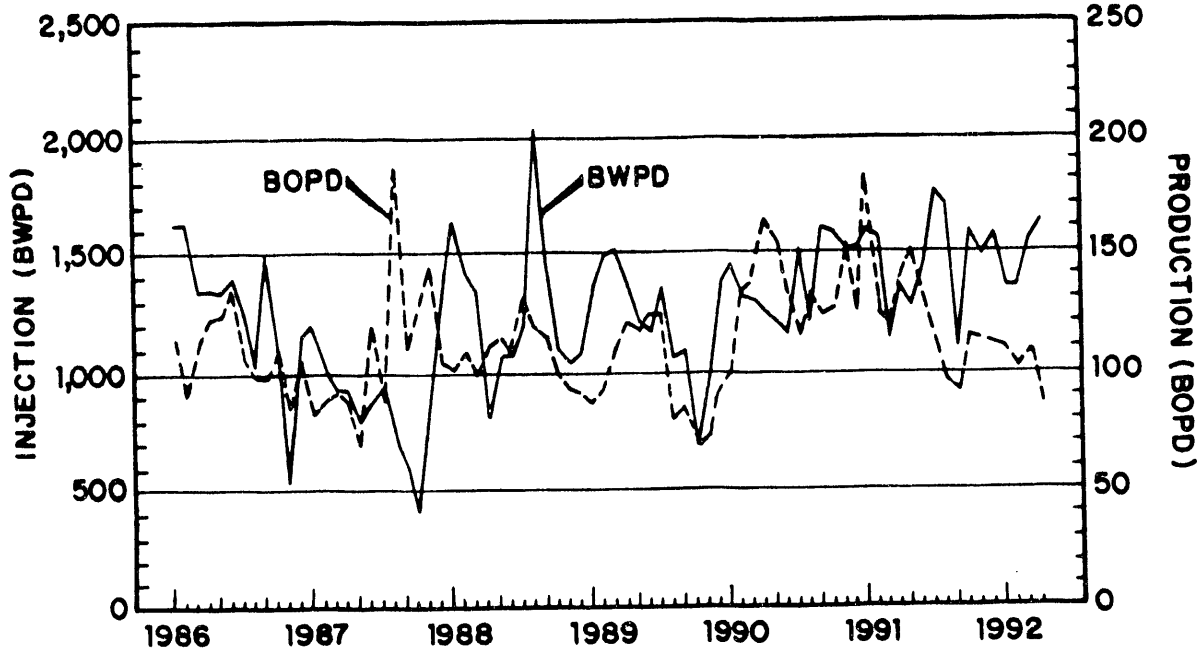


Figure 3. The production/injection profile of the Catshill CO 30 Sand waterflood project, January 1986-May 1992.

Table 4
Reservoir rock and fluid properties of the Catshill CO 30 Sands

	CO 24 BLOCK	CO 38 BLOCK
Porosity, ϕ	0.31	0.31
Initial Water Sat, S_{wi}	0.30	0.30
Present Water Sat, S_w	0.43	0.43
Initial Oil F.V.F. β_{oi}	1.15	1.15
Present Oil F.V.F.* β_o	1.04	1.04
Average Permeability, K_{so} (md)	5.0	4.2
Dykstra Parson Coefficient (Vdp)	0.47	0.64
Oil Gravity, °API	40	40
Average Oil Saturation, S_o	0.46	0.46
Average Residual Oil Sat, S_{or}	0.20	0.20
Oil Viscosity, cps.	0.84	0.84
Water Viscosity, cps. at 110°F	0.7	0.7

*Formation Volume Factor

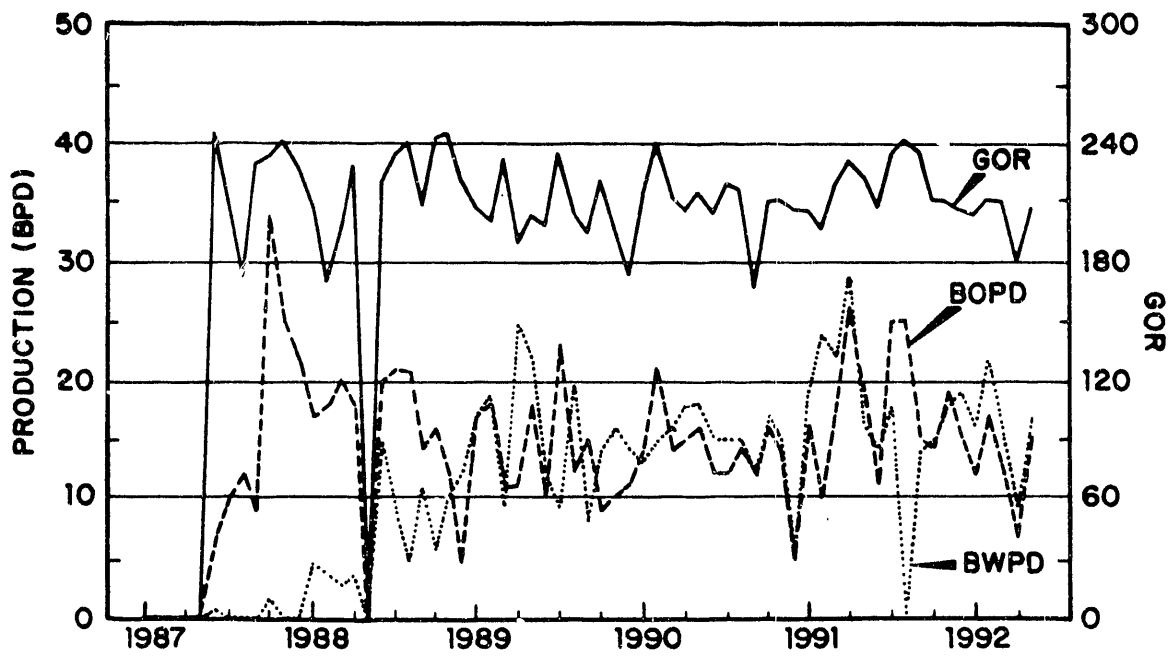


Figure 4. CO 34 production/GOR, January 1987-May 1992.

Pattern No. 5		Pattern No. 6	
Injector:	CO 65	Injector:	CO 67
Producers:	CO 30, CO 34, CO 38 and CO 48	Procedures:	CO 34, CO 38
		Offwells:	CO 16 and CO 22

This pattern shows some response to injection in CO 65, as evident by the trend of the injection/production profile given in Figure 5. CO 65 injected a cumulative total of 682 mbw at an average rate of 266 bwpd.

CO 34 is also part of Pattern No. 6, which consists of injection well CO-67 and offtake wells CO 34, CO 38, CO 16 and CO 22.

CO 67 injected a cumulative total of 466 mbw from inception. CO 67 had an erratic injection pattern, as shown in Figure 6.

4. MARCELLE VALLEY

GY 212 well is completed in the Marcelle Valley, Gros Morne, as shown in Figure 7, Zone 6. The WSW-ENE trending Gross Morne Anticline and the Marcelle Valley fault system are the dominant structural features which influence hydrocarbon accumulation in the Beach/Marcelle Valley area, and together give rise to the Beach and Marcelle Valley fields, located on the North South Flank of the anticline, respectively.

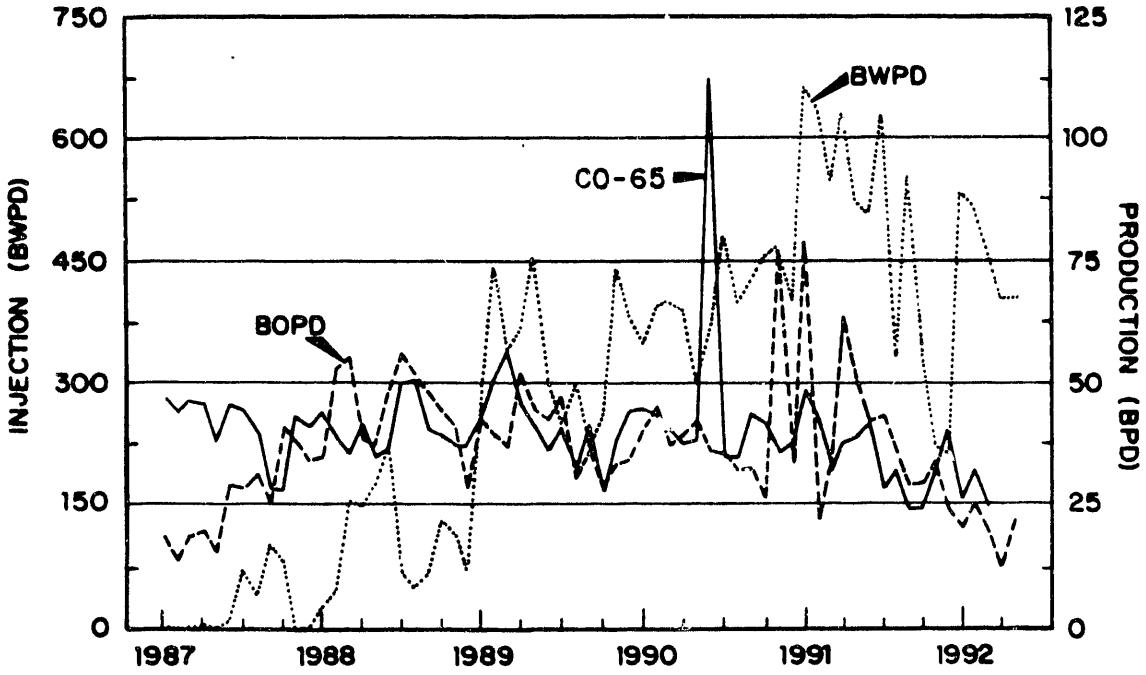


Figure 5. Production/injection profiles of production wells CO 34, CO38, CO 48, CO 30 and injection well CO-65, January 1987-May 1992.

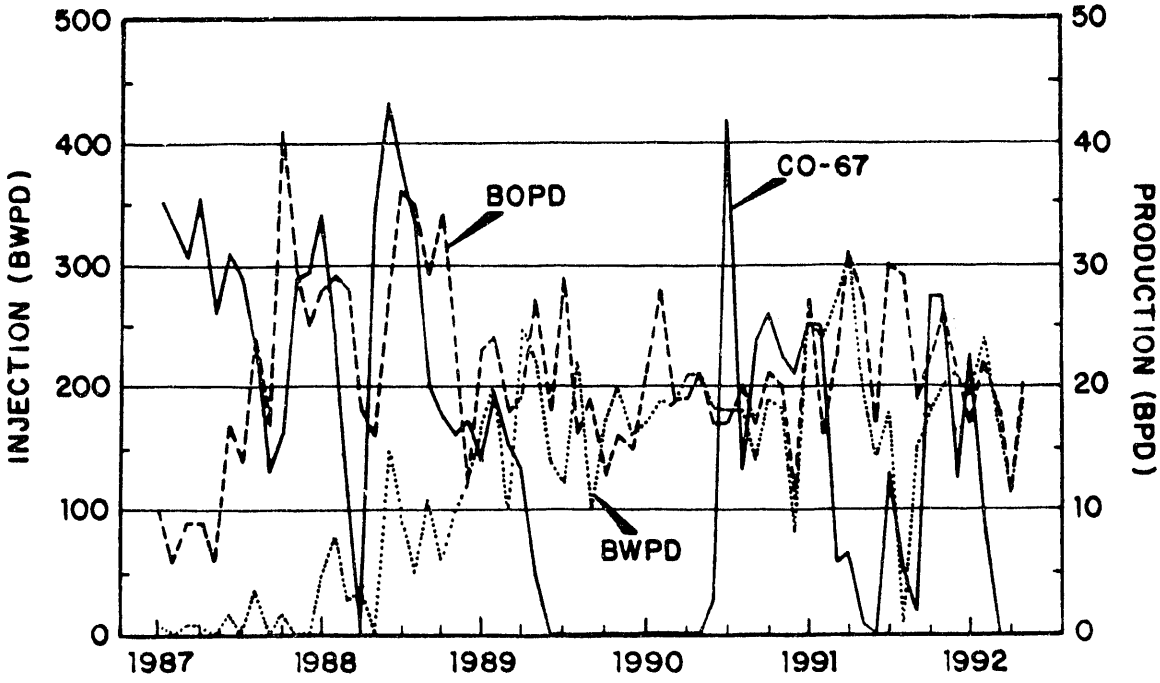


Figure 6. Injection/production profile of production wells CO 16, CO 22, CO 38, CO34, and injection well CO-67, January 1987-May 1992.

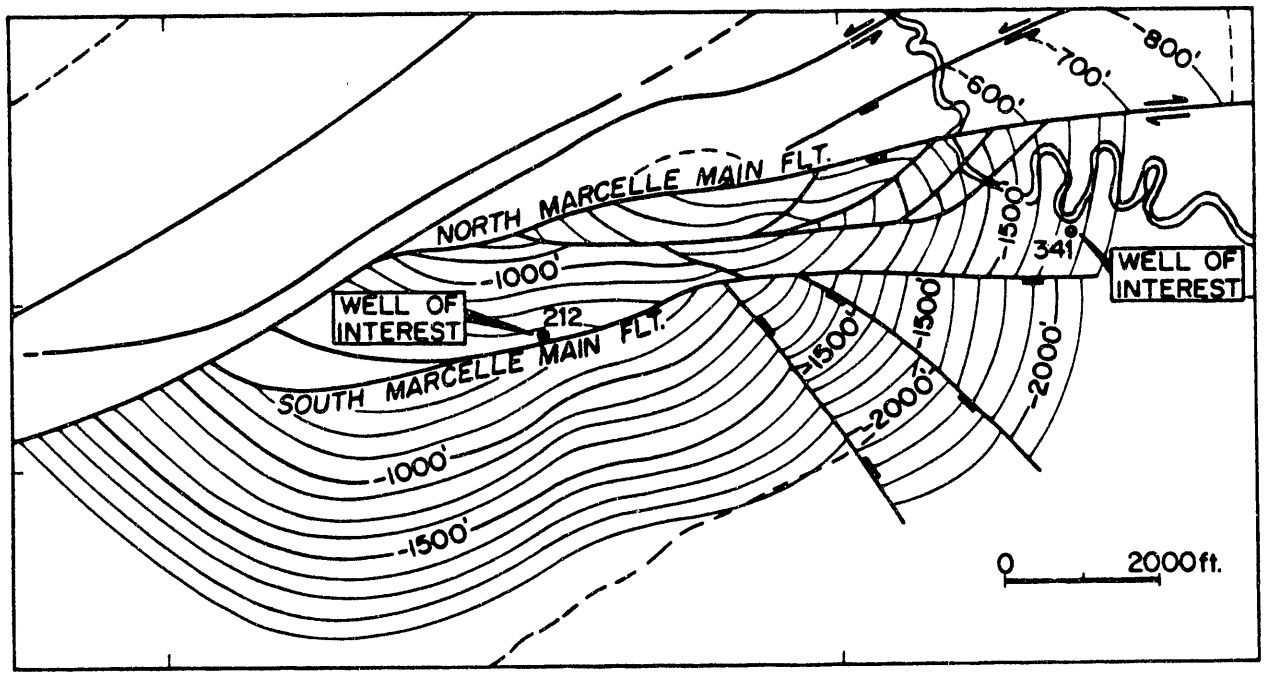


Figure 7. Marcelle Valley Field - structure on top of Zone 6 - Lower Gros Morne.

The deltaic, Pliocene Gros Morne Sands have been interpreted to be stacked coastal barrier bars and beach sands, and average five thousand feet in the study area. These sands, found between 500-7000 feet, are the primary producers, with cumulative production to date at about 29 mmbo (≈12 mmbbls of oil ex Marcelle Valley and ≈17 mmbo oil ex Beach).

The Marcelle Valley/Beach Field area is subnormally geo-pressured and this usually causes lost circulation problems while drilling. The Beach and Marcelle Valley Fields are at a mature stage of development and future primary production lies in workovers, development drilling to the NE and SE as identified, and enhanced recovery techniques, including Microbial Enhanced Oil Recovery.

The present productive area of the Beach and Marcelle Valley fields covers about 650 acres and contains 230 wells which have exploited the productive Pliocene Gros Morne sands. This area has produced 28.8 mmbo at an average of 125,000b/oil/well. The depth of the productive horizon varies from 500 feet to 5000 feet in the down flank position. The drive mechanism is solution gas drive.

The Lower Gros Morne sands (most prolific) have been interpreted as stacked coastal barrier bars and beach sands deposited in a near shore environment. These sand bodies show thick axes of NW-SE deposition providing the reservoir rocks which are fine-to medium-grained, fairly well-sorted sandstones in a clay matrix. Porosities vary from 15-35% with an average of 22%. Permeabilities vary from 10-520 md, with an average of 65 md. The average pay thickness is 250 feet and sand influx into the producing well bore is not a problem. Oil gravities range from 15°-40° API, with an average of 34° API.

The estimated recovery factor is 10%. Table 5 summarizes the reservoir and fluid parameters for Marcelle Valley Zone 6 where GY 212 is located. The production/GOR for January 1987 to May 1992 is shown in Figure 8. GY 212 is currently pumping 10 bopd/61 bwpd and has a cumulative oil production of 285 mbo. The current bottom hole pressure for GY 212 is 160 psi.

5. ANALYSIS OF TRINTOC WELL SALINES

5.1. Collection of samples

Before collecting the samples, each sample bottle (4 liter) was thoroughly cleansed and steam-sterilized. The bottles then were sealed and dispatched to TRINTOC where individual bottles were aseptically filled with brine from the twelve specific reservoirs and labelled. Samples then were transported to CARIRI's Fermentation Laboratory where they were stored at 4°C. Samples CO 34 and GY 212 were used in preliminary trials to develop the methodology which was subsequently used to evaluate all samples.

5.2. Experimental procedure

Preliminary experiments focused on stimulating indigenous microbial population in brines to produce biogas. Specific nutrients were added to brine samples and oxygen-free gas was introduced into each sample bottle to enhance production of the desired metabolite.

The basic carbohydrate source used in all experiments was sugar cane molasses, which was acquired from Caroni (1975) Limited. Mineral supplements were formulated with Analar grade chemicals.

Preliminary experiments on biogas production were carried out using a gassing station and an anaerobic gas mixture containing 10% H_2, 10% CO_2, and 80% N_2. Brine samples were aseptically dispersed into 30mL sample bottles with

Table 5
Reservoir parameters: Marcelle Valley - Gros Morne Zone 6

Area		300 Acres
Volume		105,000 Ac. ft.
Average Thickness		350 feet
Average Porosity		17%
Average Water Saturation		43%
Average Permeability		51 md
Formation Temperature		100°F
Oil Viscosity -	Initial	3.1 cp
	Current	4.0 cp
Oil Gravity (API°)		35°
Water Viscosity		0.8 cp
F.V.F.	Initial	1.08 bbl/stb*
	Current	1.03 bbl/stb
Gas Oil Ratio (est.)	Initial	130 SCF/stb
	Current	49 SCF/stb
Reservoir Pressure (est.) @ 1200' Datum		
	Initial	535 psi
	Current	160 psi
Oil in Place		73.1 mm stb
Reserves @ 10% Rec. Factor		7.31 mm stb
		70 stb/Ac-ft
Cumulative Production to December 1991		5.1 mm stb
Remaining Primary Reserves		2.21 mm stb

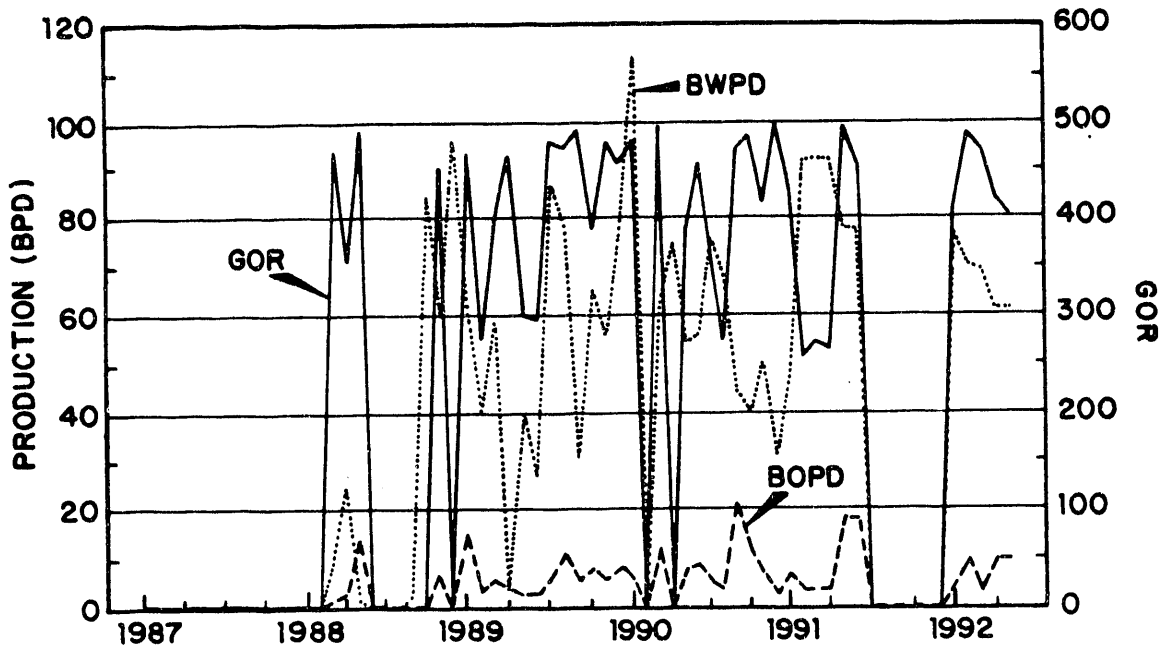


Figure 8. GY 212 production/GOR, January 1987-May 1992.

Table 6
Composition of Tanner's Mineral Solution

	Grams/Liter
NaCl	40
KCl	5
NH_4Cl	50
KH_2PO_4	5
$MgSO_4.7H_2O$	10
$CaCl_2.2H_2O$	1

septum-lined screw covers which allowed the introduction of a syringe needle. Sterilized sugar cane molasses and mineral supplements were added to each brine sample in varying concentrations to determine optimum concentration of nutrients for maximum gas production. Control samples were sterile saline solution supplemented with the same quantities of nutrients and minerals.

5.3. Results

Significant quantities of biogas were produced from CO-34 brine in the presence of molasses alone. In repeated experiments, the greatest increase in gas production (22-25%) occurred when the molasses (0.8%) was supplemented with 0.1% ammonium nitrate and 0.3mL Tanner's solution (Table 6). Table 7 shows the composition of sugar-cane molasses.

The effects of this nutrient mixture on the twelve brine salines is shown in Table 8, using the nutrient mixture and conditions optimized for CO 34 and GY 212. The biogas produced was analyzed by gas chromatography using a thermal conductivity detector, and it was found to consist mainly of CO^2. No other gases were identified.

5.4. Modification of methodology to determine biogas production

The necessity to frequently replace the septa in the serum sample bottles, to avoid leakages after repeated withdrawals of gas with gas syringes, led to the development of an alternative methodology to measure biogas production. The equipment used is shown in Figure 9. A layer of oil from the well under investigation was used to maintain an anaerobic environment in the treated saline in the flask. In principle, generation of biogas in the first flask (A) displaces an equivalent volume of water from the second flask (B) into the third flask (C). Therefore, the amount of biogas generated can be determined by monitoring the level of water that collects in the third flask.

5.4.1. Materials and methods

Preliminary experiments to demonstrate enhanced gas production were done on samples of brine from reservoirs CO-34 and GY 212. Control experiments were set up for each reservoir by using sterile saline (0.5%) in place of reservoir brine. The inoculum used was taken from a sealed serum bottle (30 mL) that was first incubated under anaerobic conditions for three days at 35°C. The nutrient mixture used was described in Section 5.3. Before commencing each experiment, an inoculum (10mL) from this three-day culture supplemented with additional reservoir brine 75mL and molasses solution (4% W/V) was aseptically introduced into a 125mL sample flask. To maintain anaerobic conditions in the flasks, a

Table 7
Composition of sugarcane molasses

Component	% (w/w)
Protein	1.85
Ash	6.73
Total Solids	80.98
Total Sugars as invert	67.90
Calcium	0.93
Magnesium	0.25
Sodium	0.19
Potassium	1.31
Phosphorous	0.14
Sulphur	0.52

Table 8
The effects of 0.1% ammonium nitrate, 0.3mL of Tanner's Solution, and 0.8% molasses on brine samples obtained from twelve TRINTOC wells

Well	Mean Gas Production, ml
CO 34	13.25
GY 315	13.0
GY 212	14.0
B 336	15.5
B 504	13.0
GY 341	11.0
IN 26	10.5
IN 4	11.5
BP 450	5.5
AO 39	4.5
CO 70	3.5
CO 118	4.5

small volume (5mL) of crude oil from the relevant reservoir was layered on top of sample solutions. The flask was then attached to the water-displacement apparatus and the entire system was placed in an incubator (Lab-Line) maintained at 35°C.

The molasses solution was pasteurized in a water bath (New Brunswick Scientific) at 68°C for forty-five minutes.

The flasks were incubated for nine days. The results of investigations carried out on salines obtained from wells GY 212 and CO-34 are shown in Tables 9 and 10.

5.4.2. Results

The results, shown in Tables 9 and 10, indicate that nine days incubation is required for full gas production on this scale. Saline GY 212 proved very promising with a production of 425 cc of biogas: this value was substantiated in repeated determinations.

Biogas production from CO 34 (375 cc), while lower than that from saline GY 212, was also significant and compared well with published values for similar investigations of well salines in Romania [4].

5.5. Analysis of well salines

Formation water associated with oil producing reservoirs is generally saline. Since the EOR technique involves the stimulation and growth of microorganisms, the downhole aqueous environment is an important consideration in predicting the species that would thrive best to produce the desired effects. The factors of importance are pH and total salinity, which dictate the strain of bacteria that would proliferate; the presence of nutrients, such as nitrogen, phosphorous, and potassium; the presence of heavy metals, which are essential for cell growth and reproduction, and the presence of heavy metals which are toxic to the microorganisms.

5.5.1. Materials and methods

Wellhead water samples were taken in glass bottles and analyzed within twenty-four hours. Sodium, potassium, calcium, magnesium, and iron were assayed by atomic absorption spectroscopy. Chloride was determined by $AgNO_3$ titration, bicarbonate/carbonate by acid base titration, sulfate by turbidimetric method, pH by an Orion Research meter, and total dissolved solids by calculation.

5.5.2. Results

Tables 11 and 12 show the results of these analyses for the CO 34 and GY 212 well waters. The range in the values reflects variations in the results taken over one year. There were no significant changes in the water quality of either well, with one exception; sulfate levels of GY 212 water varied from 7.5 to 50 mg/liter. The variations of the waters of the wells within the parent reservoirs

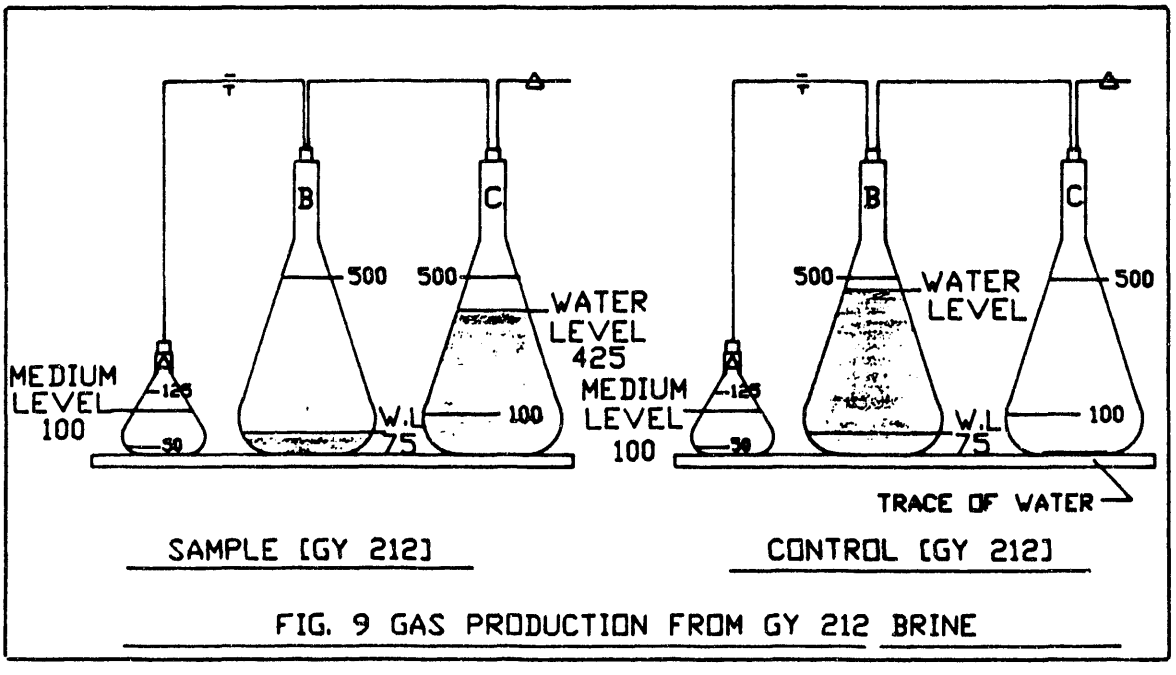


FIG. 9 GAS PRODUCTION FROM GY 212 BRINE

Figure 9. Gas production from GY 212 Brine.

Table 9
Production of biogas from reservoir GY 212

Hours	Time	Date	ML Biogas Produced
0	2pm	9/16/91	0
24	2pm	9/17/91	100
66	8am	9/19/91	250
162	8am	9/23/91	375
170	4pm	9/23/91	400
210	8am	9/25/91	425
234	8am	9/26/91	425

Table 10
Production of biogas from reservoir CO34

Hours	Time, am	Date	ML Biogas Produced
0	10	10/04/91	0
24	10	10/05/91	75
72	10	10/07/91	200
120	10	10/09/91	0
144	10	10/10/91	300
240	10	10/14/91	375
263	9	10/15/91	375

were determined and also are shown in the Tables. The variation of the chemical characteristics of the waters of the CO 30 sand were wide, and well CO 34 water was of average quality. The formation waters of the Gros Morne Sand showed some variation; however, GY 212 water was generally towards the lower levels of the analyzed ions, except for bicarbonate, where it was the highest. Analyses of the waters of the reservoir are important to indicate the tolerance expected of the microorganisms, if the technique is to be applied on a larger scale.

5.6. Determination of biosurfactant production

Selected well salines were investigated for biosurfactant production by:

1. Streaking the brine on blood agar plates to determine whether biosurfactant producers were present. The plates were incubated in an anaerobic jar at 35°C;

2. Measuring the changes in surface tension of anaerobic cultures grown in various concentrations of glucose;

3. Measuring the changes in surface tension of anaerobic cultures grown in various concentrations of sucrose.

Table 11
Analysis of CO 34 water in relation to waters characteristic of the CO 30 Sand

Analysis	Well CO 34[1]	CO 30 Sand[2]
Na	4,250-4,500	1,400-8,500
K	22-26	5-46
Ca	22-55	4-55
Mg	30-44	4-140
Fe	1.2-1.5	0.2-2.0
Cl	5,540-5,680	1,200-12,000
HCO_3	1,580-2,440	1,580-3,050
SO_4	3.0-55	3.0-7.0
CO_3	NIL	NIL
TDS*	11,480-12,720	4,400-24,000
pH[3]	7.3-7.5	7.0-8.3

*Total Dissolved Solids
1 Range of values for 3 samples over one year.
2 Range of values for wellhead samples of six wells.
3 All values except pH in mg/litre.

The blood agar plates turned completely green, which is characteristic of α haemolysis but does not give conclusive indication of biosurfactant production indicated by β haemolysis. Therefore, further tests were carried out by measuring the surface tension of brine cultures before and after incubation, using a Fischer Surface Tensiometer Model 20. The instrument was calibrated and the surface tension of distilled water was measured each time to check the accuracy of the calibration. Surface tension measurements were carried out at

Table 12
Analysis of GY 212 water in relation to waters characteristic of the Gros Morne Sand

Analysis	Well GY 212[1]	Gros Morne Sand[2]
Na	6,110-6,800	6,110-10,500
K	34-52	34-57
Ca	75-105	75-175
Mg	225-250	148-295
Fe	0.2-0.8	0.2-2.2
Cl	7,310-8,520	7,310-15,830
HCO_3	4,570-5,335	915-5,33550
SO_4	7.5-50	0.5-500
CO_3	NIL	NIL
TDS*	18,960-20,610	18,960-27,920
pH[3]	6.9-7.4	6.8-7.9

*Total Dissolved Solids.
1 Range of values for 4 samples over one year.
2 Range of values for wellhead samples of six wells.
3 All values except pH in mg/litre.

Table 13
Total aerobic and anaerobic counts

	Total Aerobic Count cfu (mL)	Total Anaerobic Count cfu (mL)
GY 212	1.2×10^5	1.2×10^2
CO 34	2.2×10^5	1.7×10^2

room temperature (24°C). Density measurements of the anaerobic cultures also were determined before and after each incubation.

Optimum concentrations of glucose and mineral supplements were determined in preliminary experiments on 30 mL samples of brine. The samples were incubated under sterile conditions at 35°C for six days. No surfactant production occurred in the presence of sucrose, and, therefore, glucose was used in all subsequent determinations of surface tension. GY 341 and CO 34 brines were most promising, with changes of surface tension of 16.10 and 15.80 mN/m respectively. GY 212, a promising gas producer, showed a negligible change in surface tension (<2mN/m).

6. IDENTIFICATION OF MICROORGANISMS IN CO 34 AND GY 212 WELL SALINES

6.1. Materials and methods

Microorganisms in GY 212 and CO 34 well salines were incubated on Calcium carbonate medium, Plate count agar, Molasses nitrate medium (1% NaCl), and investigated for the presence of both aerobic and anaerobic microorganisms. Plate count agar was used for total aerobic courts by the Pour Plate Method. Molasses nitrate medium was used for total anaerobic counts, using the Hungate technique.

6.2. Results

6.2.1. Aerobes

Gram-positive and gram-negative rods, and gram-positive and gram negative cocci were identified in GY 212 well saline. Gram-positive and gram-negative rods were identified in well saline CO 34.

6.2.2. Anaerobes

Gram-negative and gram-positive cocci were identified in both GY 212 and CO 34 salines incubated on molasses nitrate medium under anaerobic conditions.

6.2.3. Microbial counts

The results of total aerobic counts carried out by the Pour Plate Method on Plate Count Agar and total anaerobic counts using a modified Hungate technique on molasses nitrate medium are shown in Table 13.

7. SUMMARY AND CONCLUSIONS

The results presented in this report indicate that gas and biosurfactant production occurred when pasteurized molasses and mineral salts were added to

saline samples from selected TRINTOC wells. This production indicated indigenous microorganisms were present in the saline with a potential for Microbial Enhancement of Oil Recovery (MEOR). The identification of carbon dioxide (CO_2) as the major gas produced by the microorganisms in the well salines is promising in view of the known effects of CO_2 in increasing reservoir pressure, thereby enhancing oil recovery. Hydrogen sulfide (H_2S) was produced in certain well salines (AO 39, BP 450) used in preliminary investigations: these wells were not considered suitable candidates for MEOR because of the corrosive properties of H_2S.

Following the screening of the selected well salines for gas and biosurfactant production in relatively small-scale trials, two promising salines (GY 212 and CO 34) were further investigated for gas production by a methodology based on the work of Lazar et al. Lazar is a pioneer in the field of MEOR and he has been carrying out field trials in Romania since 1975 with increased oil production ranging from 16-200%. The results obtained during these scaled-up investigations substantiated results obtained in previous trials. They also compared very favorably with results obtained by Lazar et al. in similar benchscale investigations [4]. The methodology employed made gas quantification simple and it was also suitable for further scale-up to field trial scale.

Overall, the encouraging results obtained in these preliminary investigations indicate that the indigenous microorganisms present in selected TRINTOC wells have potential to enhance oil recovery. This potential should be further explored with a view to subsequent field trials should these investigations continue to produce promising results under actual reservoir conditions.

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MEOR, Recent Field Trials in Romania: Reservoir Selection, Type of Inoculum, Protocol for Well Treatment and Line Monitoring

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Abstract

From 1975 to 1980, the first generation of MEOR field trials were carried out in Romania, based on microbial flooding recovery technology and using the level of technology of that time, with its pioneering deficiencies. The results were promising, but after 1980, it became apparent that many aspects of the MEOR technology we used should be improved, because a miracle is not possible with very simple MEOR technologies. This was the reason why, between 1980-1986, we prepared a "second generation" of MEOR field trials which took place between 1987-1991. Compared with the first generation of field trials, in this second generation we tried to improve the methods in the following ways: 1. enhancing the performance of the bacterial inoculum; 2. testing the inoculum for the production of substances involved in oil release; 3. with mathematical models, simulating the release of residual oil; 4. formulating a protocol for injecting the wells; 5. ensuring that adequate quantities of inoculum were used; and, 6. controlling nutrients and the time of their administration.

In this paper we discuss the results obtained from 1987-1991 at the two reservoirs where cyclic microbial recovery and microbial flooding recovery trials were carried out. The possibilities of extending and improving these technologies at other reservoirs also are discussed.

1. INTRODUCTION

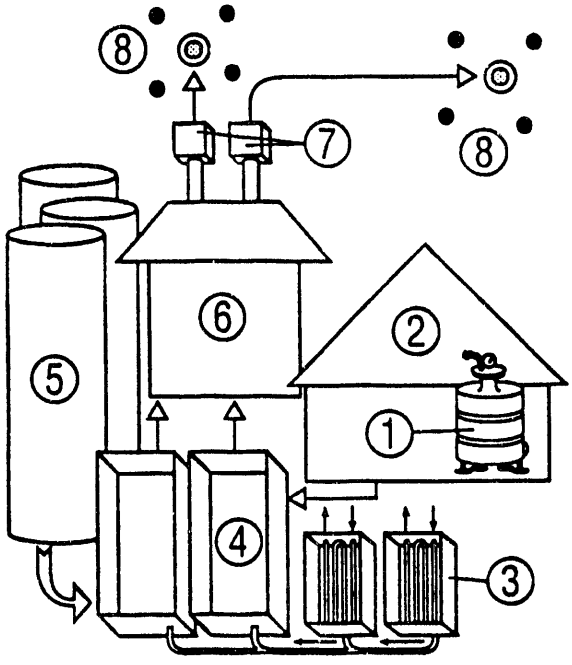
During the last ten years, interest in MEOR technologies has increased significantly. This fact is demonstrated not only by the MEOR international scientific meetings (sometimes two in a year), but also by the increasing number of field trials. The interest in MEOR technologies also is demonstrated by the publication of several important books and volumes of Proceedings.

During the pioneering phase of MEOR technology, between 1950-1960, countries such as the United States of America, Czechoslovakia, Hungary, Poland, and to some extent, the previous Soviet Union played an important role in developing this field. After 1970, other countries also participated, such as Romania, the previous East Germany, Canada, Australia, Great Britain, China, France, Norway, and Pakistan. In the last year, interest has increased to include some countries of the Caribbean and South America. Thus, at the International Conference on MEOR at Norman, OK, USA in May 1990, scientists and specialists from the petroleum industry of 15 countries participated. The 1990 MEOR International Conference underlined several very important conclusions, namely:

It is time now to go in the field and to prove, in practice, the importance and the future of the MEOR method;

There are at least five to six different technologies in this method which could be applied *in situ*. The economical efficiency of some of these has already been demonstrated;

The sinuous evolution of petroleum crises imposes the need to develop some technologies with high efficiency, which could mitigate the necessity of abandoning a large number of wells each year. Such wells could be reinstated and work again with reasonable efficiency. This is an important aspect, because, for instance, in the United States about 13,000-15,000 wells are abandoned each year, just because an efficient EOR method is not available to clean up the wellbore area. It is important to pay more attention to simplifying the EOR method to make it cost-efficient;



1. Recipient for bacterial inoculum
2. Thermostatic room for preparing inoculum
3. Tank with steam serpentine for molasses (30 m^3)
4. Tank for homogenizing nutrient support (brine + molasses 2%) and bacterial inoculum
5. Storage tank for brine
6. Homogenization pump (brine + molasses)
7. Distribution system for injection wells
8. ◎ Injection well
   • Reaction wells

Figure 1. Technical facilities for oil recovery either by cyclic or microbial flooding system (Bragadiru Reservoir).

Table 1
Requirements for oil reservoirs treated with MEOR technologies

Characteristics	The limits required by MEOR method
1. The oil	
Type of oil	preferable, in the following order: paraphinic type S, semiparaphinic type B, mixed without S, or with maximum 1% S
Density	0.83-0.95 Kg/dm^3 → preferable 0.83-0.88 Kg/dm^3
Viscosity	5-50 cp → preferable 5-30 cp
Rock saturation	40-70% → preferable 60-70%
2. The collector	
Mineralogical	preferable: calcarous collectors, calcarous marl, calcarous sandstone with a content of at least 20% $CaCO_3$, as well as sands with calcarous cement with minimum 10-20% $CaCO_3$. If needed, soda can be added. Structure with very good continuity of level (strata) between the injection and reaction wells
Porosity	minimum 20%
Permeability	more than 150 mD, and better, more than 300 mD
pH	5-8 → prefereable 6-8
Temperature	15-70°C → preferable 30-50°C
3. Brine	
Co-produced water	30-90% → preferable 60-70%
NaCl content	5-150 g/l → preferable 5-50 g/l
$CaCl_2$ content	10-70 g/l → preferable 10 g/l
SO_4^{2-} content	0.1-5 g/l → preferable under 1 g/l
Water mineralization	up to moderate
Type of mineralization	preferable, in the following order: carbonated, sulfatated, chlorinated
Content of toxic substances	preferable: under 0.1 g/l

The development of the MEOR method must be based on interdisciplinary participation, because this cooperation is characteristic of any high efficiency biotechnology. At present, many research groups over the world are involved both in basic and practical investigations in MEOR. The main focuses are on improving MEOR technologies already tested in some countries, and on activities in microbial oil recovery concentrating mainly on the ecology and microbiology of the reservoirs, in fracture and matrix acidizing in carbonate reservoirs, in biopolymer flooding as well as in combined flooding of biosurfactants and biopolymers, and on various kinds of selective plugging.

This paper will discuss the results of the Romanian field trials carried out after 1986; some of the preliminary data were presented in a previous paper [1]. The results were obtained from 1987 to the end of 1991 at two reservoirs where the wells were treated by MEOR methods using technologies like microbial flooding recovery and cyclic microbial recovery (for cleaning up the wellbore, for instance). These wells are still under observation, so, in a way, this paper and the results are still preliminary ones.

2. EXPERIMENTAL PROCEDURES

2.1. The characteristics of the reservoirs subjected to microbial treatment

In the previous papers of Lazar et al. [1,2], some data were given on the geological and physico-chemical characteristics of the reservoirs subjected to MEOR treatment. Two reservoirs, namely CALDARARU and BRAGADIRU, are both located in Sarmatian III. Another two reservoirs, BALACEANCA and GRADINARI located in the same area, are soon going to undergo MEOR treatment, and the bacterial inoculum has already been selected.

Table 1 gives data on the requirements for reservoirs that are to be treated by MEOR methods. Generally speaking, the reservoirs Caldararu and Bragadiru seem suitable for microbial treatment.

Table 2 summarizes the main geological and physico-chemical characteristics of the two reservoirs used in our work.

2.2. The type of bacterial inoculum

The bacterial inoculum used at the Caldararu and Bragadiru reservoirs was AMEC (Adapted Mixed Enrichment Culture) selected according to the stages presented in a previous paper [3]. The bacterial preinoculum was prepared in containers of 25 l (Figure 1), and then the quantities of inoculum for injecting into the wells was prepared in a special installation, as described in a previous paper [4]. The bacterial inoculum injected into the reservoirs was characterized according to the methodology presented by Lazar et al. [5]. The performance of the inoculum in the production of gases, acids, solvents, biosurfactants, and biopolymers in the Caldararu and Bragadiru reservoirs, as well as the ability of the inoculum to release residual oil from porous media have been presented in several recent papers [3-5]; the findings are synthesized in this paper (Tables 3 and 4).

At the same time, we determined the concentration of bacteria in cells/ml, and the main genera of bacteria, as well as the ability of this inoculum to use crude oil aerobically and anaerobically.

2.3. The nutrient support

Along with the bacterial inoculum (AMEC), brine was injected into each reservoir, fortified with 2-4% molasses, which has about 50% polarizable sugar.

No special minerals, such as nitrogen or phosphorous sources were added, and our laboratory experiments demonstrated that these supplements were not necessary.

2.4. The protocol for injection

A description has been given earlier of the treatment protocol for microbial flooding recovery, as well as the local technical facilities for carrying out this protocol at the Caldararu reservoir (Figures 2 and 4, [1]) and the Bragadiru reservoir (Appendix 17, [3]). The technical facilities are shown here in Figure 1.

For cyclic microbial recovery (single well-stimulation or wellbore clean up) at both the Caldararu and Bragadiru reservoirs, we used the injection protocol given in Appendix 5 of Lazar [3] and in Figure 5 of Lazar et al. [1].

Table 2
Some characteristics of the Caldararu and Bragadiru reservoirs

Characteristics	Caldararu	Bragadiru
Mineralogical structure	Calcarous sandstone or calcarous marl with thin intercalations of sand, with microfissures and microcaves	Sandstones + calcarous sand, limestone with microfissures
Temperature (°C)	47	36
Depth (m)/Pressure (atm)	750-800/90	780
Pay zone (m)/inclination	5 - 6/4°	6 - 7/2 - 4°
NaCl (g/l)	4 - 4.5	3 - 6
Permeability (mD)	245 - 248	150 - 300
Porosity (%)	25.5	36
Type of crude oil	A_3, nonparaffinic	A_3, nonparaffinic
Crude oil saturation (%)	75	60
Crude oil viscosity (cp)	26	9
Crude oil density (Kg/dm^3)	0.912	0.880
Coproduced water	95 - 99	92
pH/interstitial water saturation (%)	6.7 - 6.8/25	7.0 - 7.4/40
$CaCO_3$ content	45	60 - 80

Details of the local technical facilities for preparing the bacterial inoculum (AMEC) for some isolated wells in the oil field are presented in Figure 2.

2.5. Line monitoring of field trials at the Caldararu and Bragadiru reservoirs

Before starting the MEOR treatment for microbial flooding recovery or for cyclic microbial recovery, samples were collected of the fluids extracted from the wells that would be subjected to microbial treatment. Microbiological and physico-chemical analyses of these samples were made.

A graph of oil production was generated, to get an idea about the history of oil production in the reservoirs before microbial treatment was started [1]. These curves were illustrated in Lazar et al. [1], which include results up to April 1990; Figures 3 to 7 of this paper extend the results up to the end of 1991. Both reservoirs are still under observation, so that the curves will be completed at the end of each year, for at least a further two to three years, when general conclusions can be drawn.

After starting microbial treatment, periodical samples (at least, monthly ones) of extracted fluids were analyzed microbiologically and physico-chemically. Also, measurements of the fluids extracted were taken daily by the field specialists responsible for the two reservoirs.

The first results of these analyses were presented by Lazar et al. [1,2]; further analyses covering the last 1½-2 years are presented in this paper (Tables 3 to 8).

Table 3
Performance of the bacterial inoculum used at the Caldararu reservoir for some MEOR technologies

Number of cells/ml inoculum	2.2×10^7 - 4.5×10^{11}
Principal genera of bacteria	*Clostridium, Bacillus, Pseudomonas, Arthrobacter, Mycobacterium, Micrococcus, Enterobacteriaceae*
Substances produced in the first 5 days (from 10 ml medium)	Gases (110-160 ml), acids (pH: initial 7.0-7.5; final 6.8-4.8), solvents (+ to ++), biosurfactants (++++), biopolymers (++)
Efficiency of residual oil recovery (%)	Cyclic microbial recovery (33.0-34.1) microbial flooding recovery (43)
Field results	
cyclic microbial recovery	Very good
microbial flooding recovery	Satisfactory

3. RESULTS AND DISCUSSION

3.1. Reservoirs subjected to MEOR methods of microbial flooding recovery and microbial cyclic recovery (mainly to clean up the wellbore)

As discussed in the Introduction, from 1975 to 1980 the "first generation" of MEOR field trials took place in Romania while the "second generation" covered the period 1987 to 1991. These data are summarized in Table 9. All the reservoirs meet the requirements for MEOR set out in Table 1.

Table 4
Performance of the bacterial inoculum used at the Bragadiru reservoir for some MEOR technologies

Number of cells/ml inoculum	1.5×10^9 - 4.0×10^{11}
Principal general of bacteria	*Clostridium, Bacillus, Pseudomonas, Arthrobacter, Enterobacteriaceae, Mycobacterium, Micrococcus*
Substances produced in the first 5 days (from 10 ml medium)	Gases (140 - 145 ml), acids (pH: initial 7.2, final 5.5), solvents (+ to ++), biosurfactants (++++), biopolymers (++)
Efficiency of residual oil recovery (%)	Cyclic microbial recovery (35.8) Microbial flooding recovery (41.3)
Field results	
cyclic microbial recovery	Very good
microbial flooding recovery	Satisfactory

The encouraging results of the field trials of the "first generation" seem to be proved by the data in Table 9; details are given in some previous papers, mainly that of Lazar and Constantinescu [6].

Within the period 1987 to 1991, the "second generation" of field trials was carried out at the reservoir Bragadiru (one well treated by wellbore clean up), and at Caldararu (four wells treated by wellbore clean up and a group of wells treated by microbial flooding recovery). The results are shown in Figures 5 to 9, and in Tables 10 and 11.

Keeping in mind the requirements listed in Table 1, these reservoirs seem suitable to MEOR applications; meanwhile, petroleum engineers have found some deficiencies which could have been avoided from the beginning. Because MEOR will soon be applied to other reservoirs, the petroleum engineers and geologists will be able to efficiently use the experience they have gained in choosing suitable ones for treatment.

3.2. The performance of the bacterial inoculum injected into the reservoirs

During the last 15 to 18 years, a rich experience was accumulated in Romania about the isolation and selection of AMEC for MEOR [1,2]. These experiences are synthesized in Lazar et al.'s paper [2]. The methodology was arrived at after many years of research because, initially, we were working mainly with selected pure cultures of bacteria or mixed cultures adapted to the conditions in the reservoirs.

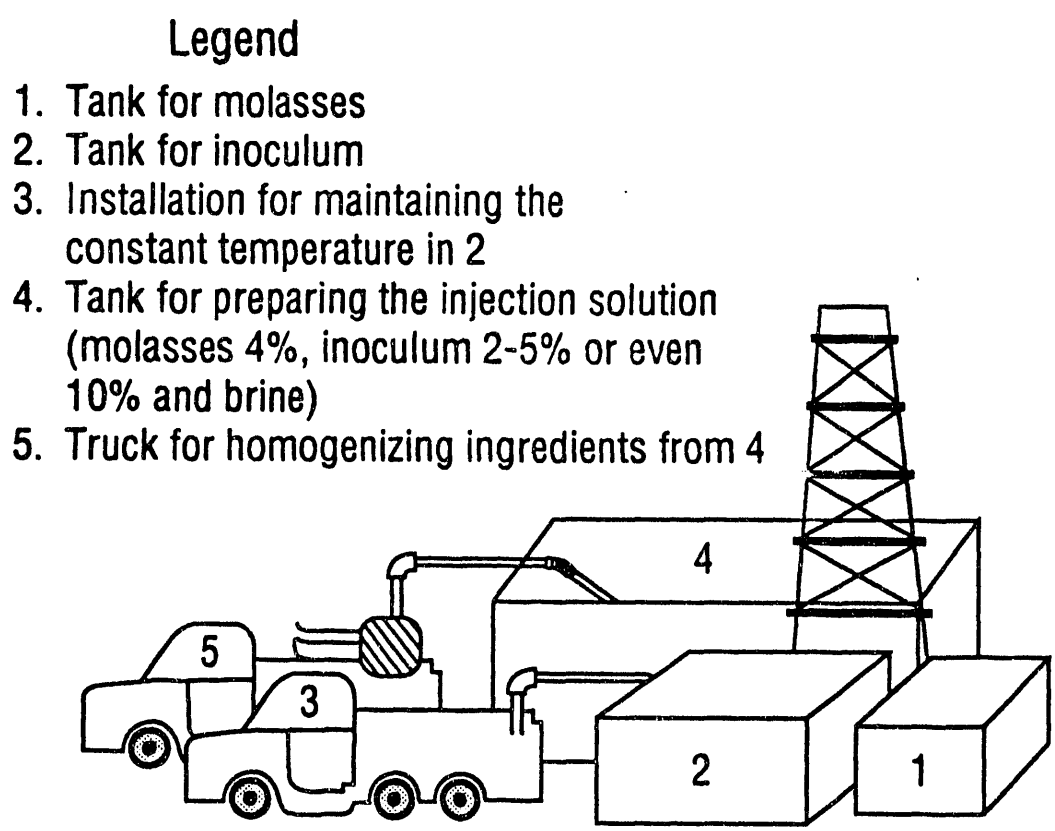


Figure 2. Technical facilities for MEOR processes for microbial wellbore cleanup and microbial well stimulation.

Table 5
Microbiological characteristics of the brine from Caldararu reservoir wells, before and after wellbore clean up treatment

Group of bacteria	Wells treated	Maximum numbers of bacteria/ml, or intensity of bacterial growth	
		Before	6-42 weeks after
Aerobic and facultative anaerobic heterotrophs	3	14.7×10^6	3.5×10^9
Anaerobic bacteria (type *Clostridium*)	3	+++	++++
Sulfate-reducing bacteria	3	++	++
Sulphur-oxidizing bacteria	3	++	+
Iron bacteria	3	++	++

Table 6
Microbiological characteristics of the brine from Caldararu reservoir before and after microbial flooding treatment*

Group of bacteria	Samples analyzed	Minimum/maximum numbers of bacteria/ml, or intensity of bacterial growth	
		Before	6-42 weeks after
Aerobic and facultative anaerobic heterotrophs	4	1.1×10^2/ 3.4×10^3	1.5×10^3/ 4.0×10^7
Anaerobic bacteria (type *Clostridium*)	4	-/++	+++/++++
Sulfate-reducing bacteria	4	+++/++++	+++/+++
Sulphur-oxidizing bacteria	4	+	+/++
Iron bacteria	4	+/++	++/+++

*Molasses in co-produced water after 56 months = 0.01 - 0.02 mg glucose/ml

Table 7
Microbiological characteristics of the brine from Bragadiru reservoir wells, before and after wellbore clean up treatment

Group of bacteria	Wells treated	Maximum numbers of bacteria/ml, or intensity of bacterial growth	
		Before	6-34 weeks after
Aerobic and facultative anaerobic heterotrophs	1	9.2×10^6	2.7×10^8
Anaerobic bacteria (type *Clostridium*)	1	+++	++++
Sulphur-oxidizing bacteria	1	+	+
Sulfate-reducing bacteria	1	++	++
Iron bacteria	1	+ - ++	++

Table 8
Microbiological characteristics of the brine from Bragadiru reservoir before and after microbial flooding treatment*

Group of bacteria	Samples analyzed	Maximum numbers of bacteria/ml, or intensity of bacterial growth	
		Before	6-34 weeks after
Aerobic and facultative anaerobic heterotrophs	8	1.4×10^6	1.2×10^8
Anaerobic bacteria (type *Clostridium*)	8	++/++++	+++/++++
Sulphur-reducing bacteria	8	++/+++	++/+++
Sulfate-oxidizing bacteria	8	-	+/++
Iron bacteria	8	++	++

*Molasses in co-produced water after 34 months = 0.04 mg glucose/ml

Table 9
MEOR field trials in Romania during 1975-1981 ("MEOR first generation")

Period[1]	Reservoir Geological layer	Number of injection wells/ reaction wells	Increased oil production	Remarks[2]
1975-1978	Baicoi Drader	2/5	2.093	-
1976-1977	Tintea Drader	1/3	-	Increased oil production reported at some wells, contrasting with a decrease in oil production at other wells.
1976-1977	Bragadiru Sarmatian III	1/3	-	Increased oil production reported at some wells, contrasting with a decrease in oil production at other wells.
1976-1978	Suta Seaca Meotian III	2/5	Insignificant increase of production reported	-
1978-1980	Vata Meotian I	1/7	3.169	-
1981	Tintea North Dacian	1/5	-	Increased oil production reported at some wells, contrasting with a decrease in oil production at other wells.
1981	Zeama Roce Meotian	1/5	-	Increased oil production reported at some wells, contrasting with a decrease in oil production at other wells.

1) The technology for injection is described by Lazar and Constantinescu [6].
2) The research group do not necessarily agree with these comments by the petroleum field specialists.

Table 10
"Second generation" MEOR field trials in Romania using microbial flooding technology (1987-1991)

Reservoir	Protocol of developing the MEOR treatment	Effects of treatment
CALDARARU (Sarmatian III)		
April 1984	Started water flooding as method of EOR	3 wells for water injection 14 wells for reaction
	Recognized that 1 PV is waterflooded	Oil production continued to decrease
December 1987	Started to inject the bacteria + molasses through well No. 847 and then No. 836 for 218 days	An increase in oil production was reported. For instance, from an average of oil production for Feb 1991 of 0.3 ton/day/well, to 1.3 ton/day/well in Oct. 1991 (Figure 5)
BRAGADIRU (Sarmatian III)		
April 1988	Started water injection for waterflooding of 1 PV (50 m^3/day)	Significantly increased oil production: in wells in the first line of those injected, oil production increased from 36 ton/month to 94 ton/month (Figure 6);
July 1989	Started injection with bacteria + molasses, at least 50 m^3/day from July 1989 to Sept. 1990.	For the first and second line of wells, oil production increased from 68 ton/month (Feb. 1991) to 192 ton/month in Sept. 1991 (Figures 6 and 7)
September 1990	Stopped the injection of molasses and bacteria, but continued with water injection (50-110 m^3/day)	In the first line of wells the average daily oil production increased from 1.28 ton/day (Feb. 1991) to 2.9 ton/day in Sept. 1991

Table 11
MEOR field trials in Romania using wellbore clean up microbial technology

Reservoir and Date of treatment*	Wells cleaned in wellbore area	Effects of treatment
CALDARARU (Sarmatian III)		
March 1988	No. 836 and 847 wells used for water injection as EOR method	The pressure during the water injection reached 60 atm.; after MEOR treatment, it decreased for nearly two years to 20-25 atm.
February 1990	No. 828. Production well that needed to be cleaned up	Oil production increased from 0.1 ton/day to 0.3 ton/day (last observation Oct. 1991) - Figure 3
BRAGADIRU (Sarmatian 3)		
November 1990	No. 1446. Production well that needed to be cleaned up	The daily well production increased from 0.2 ton/day to 1.0 ton/day (last observation Oct. 1991

*After MEOR treatment, the wells were shut for two to three weeks

Going through the procedural steps discussed by Lazar et al. [2], the adapted mixed enrichment cultures (AMEC) were obtained, and were then multiplied to generate the quantities required for the MEOR field trials. This multiplication was made at the installations or at the technical facilities discussed in that paper, and in Figures 1 and 2, of this paper. The conditions at these installations and the technical facilities depended on the local conditions at the reservoir or the treated wells for each oil field unit.

Before making the decision to increase the 25 ℓ of preinoculum received the AMEC was characterized for its ability to generate substances that would play a role in oil release from porous media, for the concentration in cells/ml, for the main types of bacteria, and for residual oil recovery from porous media and crude-oil degradation. The decision to produce the AMEC in the quantities needed for field trials was made after we had obtained these results.

In our recent papers [1,2,5] we discussed some performances of the bacterial inoculum (AMEC) injected into the wells at the Caldararu and Bragadiru reservoirs.

In Tables 3 and 4, we synthesize the major aspects of the performances of the AMEC injected at Caldararu and Bragadiru reservoirs, both at wells treated for microbial flooding recovery and those treated for cyclic microbial recovery, mainly for wellbore clean up. From the data included in these Tables, the following points can be highlighted:

The concentration of bacterial cells/ml can reach values up to 4.5×10^{11};

The main types of bacteria belong to a few genera from the category of mesophilic groups with high ability to adapt to reservoir conditions and which produce, using molasses, substances involved in oil release from porous media. These bacteria belong mainly to genera *Bacillus*, *Clostridium*, and several Gram-negative facultative anaerobic genera with high activity in molasses fermentation. Between the main types of bacteria, a few other genera were identified:

The main types of bacteria, which act as a consortium within AMEC, are very active in producing acids, gases, and biosurfactants, as well as some solvents and biopolymers; all these substances enhance oil release from porous media;

AMEC are very active in releasing residual oil from a porous media; thus, the efficiency of residual oil recovery for the Caldararu reservoir reached up to 33.0 - 34.1% (AMEC for cyclic microbial recovery), and up to 43.0% (AMEC for microbial flooding recovery); for the Bragadiru reservoir the AMEC used caused the release of up to 35.8% for cyclic microbial recovery, and up to 41.3% values for microbial flooding recovery;

The field reports by the petroleum specialists of the oil field unit to which the reservoirs Caldararu and Bragadiru belong were very satisfactory. In the case of the wellbore clean-up technology, the increase in oil production was very good and for microbial flooding recovery, the results were very promising.

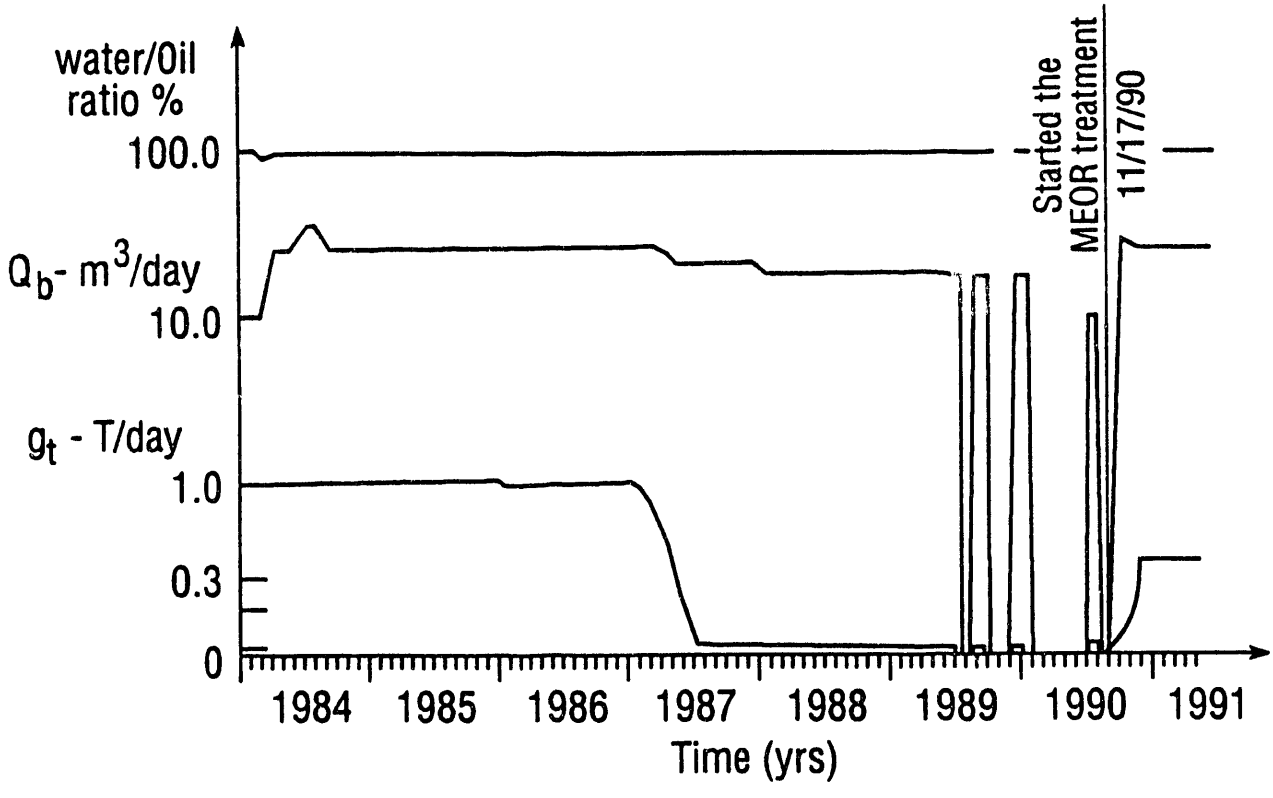


Figure 3. Well 828, Caldararu Reservoir (Sa_3) (wellbore clean-up MEOR technology).

3.3. The protocol of the well injection

Based on recent worldwide experience, published in several Symposia Proceedings [7-11], and a patent [12] as well as our own experience, described in these publications, we found that the MEOR technology used previously for injecting the wells [6] could be greatly improved. Thus, we recently published some new protocols for well injection, both for microbial flooding recovery and cyclic microbial recovery [1-3]. Our practical experience seems to show that following this protocol necessitates the use of a lot of molasses until a PV becomes waterflooded.

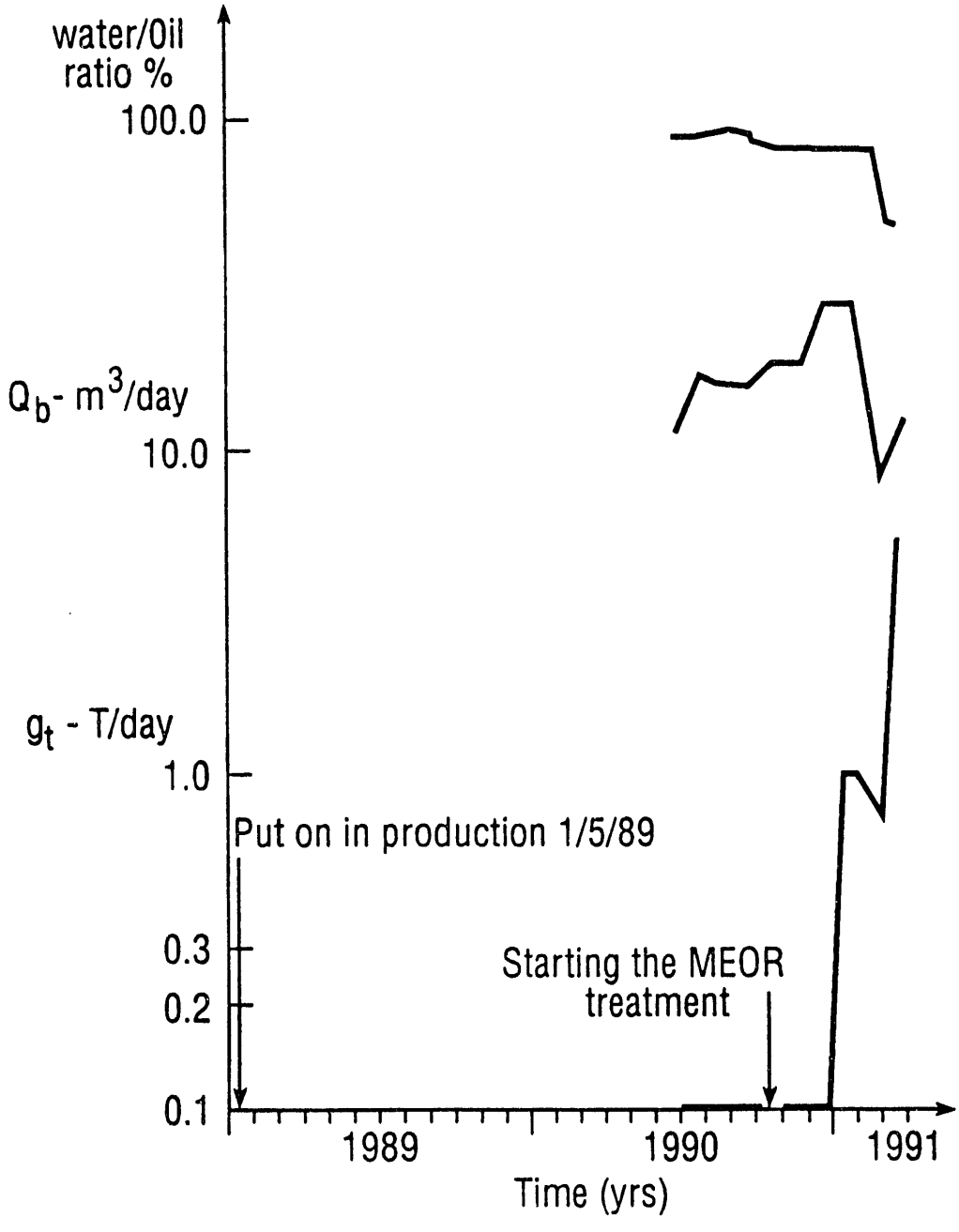


Figure 4. Well 1446, Bragadiru Reservoir (Sa_3) (wellbore clean-up MEOR technology).

Therefore, in a recent patent [13], a new modification of the protocol was proposed. This change is based upon observations of the MEOR field trials over the last 2-4 years and from the necessity to save molasses, which has become expensive (costs have risen tenfold), and so increase the cost-efficiency of the application.

The main idea of this new protocol is to inject brine with 2-4% molasses through the injection well, until this solution reaches the reaction wells, then to stop the addition of molasses and go to waterflooding only, until there is no more molasses in the reaction wells. The molasses injection should be stopped when the concentration in fluids taken from the reaction wells is about 0.04 and 0.05 mg. glucose/ml [13].

Depending on the results, it is then time to decide whether molasses should be injected again with bacterial inoculum (Figure 8). This new technology for well injection will be used at the 3 to 4 reservoirs prepared for microbial flooding recovery in 1993.

At the same time, we will assess whether the bacterial inoculum should be used only in the first stage (Figure 8). The bacterial inoculum can later be used again, when the injection of molasses is resumed (see stage IV of Figure 8), ultimately leading to cheaper applications of MEOR.

3.4. Results of the field trials using the MEOR wellbore clean technology

In previous papers [1,2,5] we presented some preliminary data on the MEOR field trials applying wellbore clean up technology to the Caldararu reservoir. The results, which were extremely encouraging, gave us a lot of confidence in the practical application of the technique. Consequently, we extended this technology to two other production wells in the wellbore area which needed to be cleaned. Thus, during 1991, well 828 at the Caldararu reservoir and well 1446 at Bragadiru reservoir were subjected to wellbore cleanup technology. Evaluations of the microbiology of the brine before and after treatment are shown in Tables 5 and 10 and in Figures 3 and 4. It is impressive how drastically the oil production was improved after treatment on both wells. Because of these results, the oil field specialists are already prepared to extend this technology to another 6 to 8 wells in 1992; if the findings can be reproduced, no doubt the technology will continue to be applied to as many wells as possible which need to be cleaned up in the wellbore area.

Because microbiological technology is much cheaper than chemical technology, it seems likely that the microbiological approach will have priority for application at wells located in reservoirs which fulfill the requirements for this technology. At wells 828 and 1446, which were only in the stage of the brine production and were likely to be abandoned at any time (the same stage of production as, for instance, about 15,000-17,000 wells each year in the United States [14]), our results mean that we may be able to find a cost-efficient MEOR technology for this category of wells.

3.5. Results of the field trials using the MEOR microbial flooding technology

As discussed in previous papers [1,2,5] in the "second generation" of MEOR field trials in Romania, started after 1987, the Caldararu and Bragadiru reservoirs were subjected to microbial flooding recovery technology.

The results of preliminary field trials from Caldararu reservoir have been presented in these papers, together with part of the data for the Bragadiru reservoir. These results cover the years up to 1990; here, we extend these data until the end of the 1991. Both reservoirs are still under observation and we hope that the effects of microbial treatment will be apparent for at least one to three more years.

3.6. Field trials at Caldararu reservoir using microbial flooding technology

Historically, due to decreasing oil production, in April 1984, a MEOR waterflooding operation was started, through three injection wells from a block with 14 reaction wells. Until December 1987, when the microbial flooding was initiated, 313,000 m^3 of brine was injected and 66,000 tons of oil were extracted, but, as the oil production curve shows (see Figure 5, [4]) waterflooding by the EOR method had no effect. The microbial flooding field trials at Caldararu reservoir were carried out between December 1987 and July 1988, where we used wells 847 and 836, through which were injected about 6516 m^3 of brine, about 120 tons of molasses, and about 15 m^3 of bacterial inoculum over 218 days (with some interruptions). From Tables 6, 7, and 9 and in Figure 5, which is a continuation of one already published [1], this experiment seems to have been a success. Many things were learned, even though the increase in oil production was not very spectacular; this information will be of great help for future field trials, planned for 1993 at another three to four reservoirs. However, from the evolution of oil production presented in Figure 5, it is very clear that after waterflooding (EOR method), oil production decreased constantly, but after starting the microbial flooding technology, the decline stopped, and oil production was maintained at a very reasonable level. The reservoir will remain under observation for at least one to two years more.

3.7. Field trials at Bragadiru reservoir using microbial flooding technology

A special plant was built at this reservoir, with adequate technical facilities for the MEOR field trials in a group of wells that were specially prepared and configured in a square geometry, as shown in Figures 1, 6, and 7. In the first group of wells, the smaller square, four new wells were drilled (O_1, R_1, R_2, and R_3) just to obtain this geometric arrangement of wells. Before starting microbial flooding following the protocol set out in a patent of Lazar et al. [12] and in some recent papers [3,4], this group of wells was waterflooded to achieve a level of 1 PV, that is, for the first square of wells around O_1, this would require about 51,000 m^3 of brine.

Following this protocol, microbial flooding was started in July 1989 and continued until September 1990 (with some unexpected interruptions). We then injected brine only, up to about 100 m^3/day, until in March 1991, we had achieved about 70% of 1 PV, representing about 35,870 m^3 brine, 132 m^3 bacterial inoculum, and about 200 tons of molasses. The injection was made at a pressure of 6-9 bars. The radius of action in the reservoir was about 50 m. After March 1991, we injected brine for 160 days, and thus, we reached 1 PV waterflooded. Thereafter, the reservoir was subjected to a waterflooding process, through well O_1, just to keep the pressure in the reservoir sufficiently high for the reaction wells.

The results in Tables 7 and 8 demonstrate that the concentration of bacteria/ml increased markedly, signifying that it was a good level of continuity or communication between the injection well and the reaction wells. Further, this good communication was demonstrated by the presence of low concentrations of molasses in the fluids of reaction wells.

Table 10 and Figures 6 and 7 summarize the results of preliminary field trials as they were reported by the petroleum specialists who supervised these field trials. Thus, mainly from February 1991 to September 1991, a significant increase was reported in oil production (Table 10, and Figures 6 and 7).

From the physico-chemical analyses carried out on the fluids taken from the reaction wells at Caldararu and Bragadiru reservoirs, the most interesting finding is the curve indicating the changes in the pH of the brine (Figure 9). The decline in this curve is a good indication of high gas and acid production

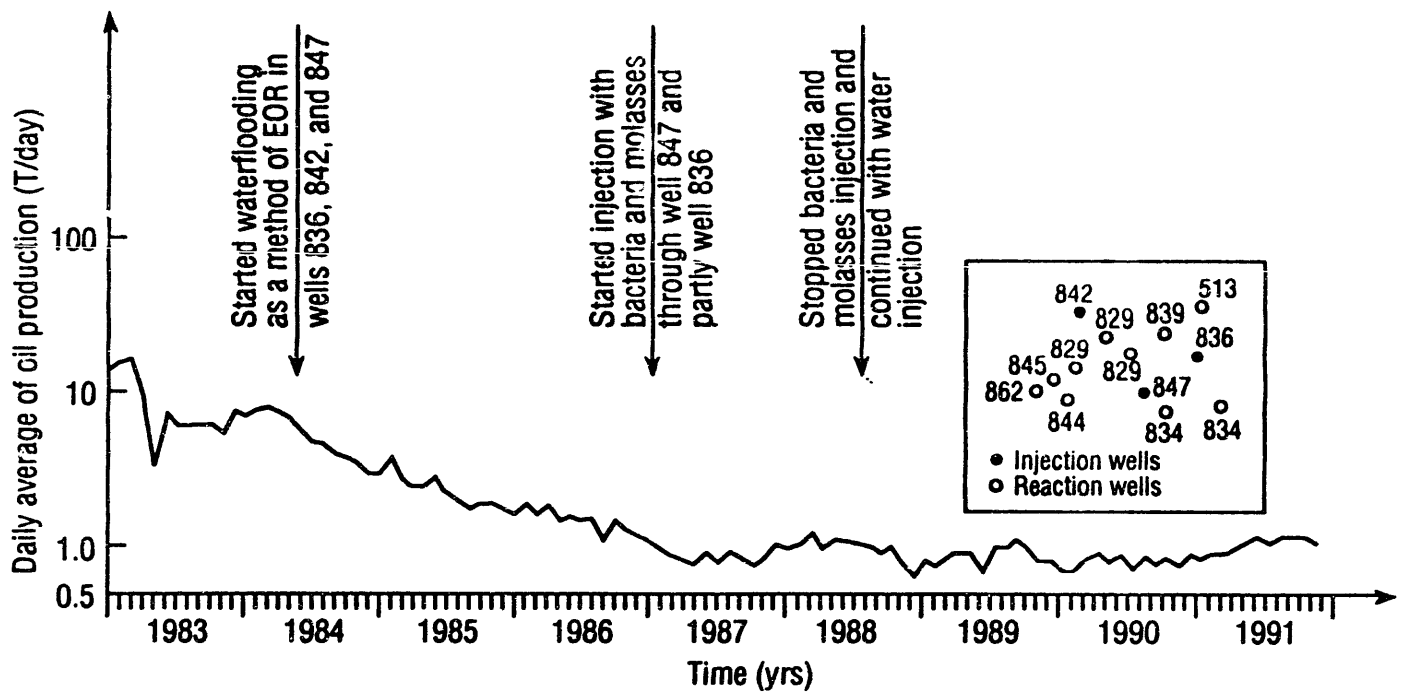


Figure 5. Oil production at the wells of Caldararu Reservoir subjected to microbial flooding.

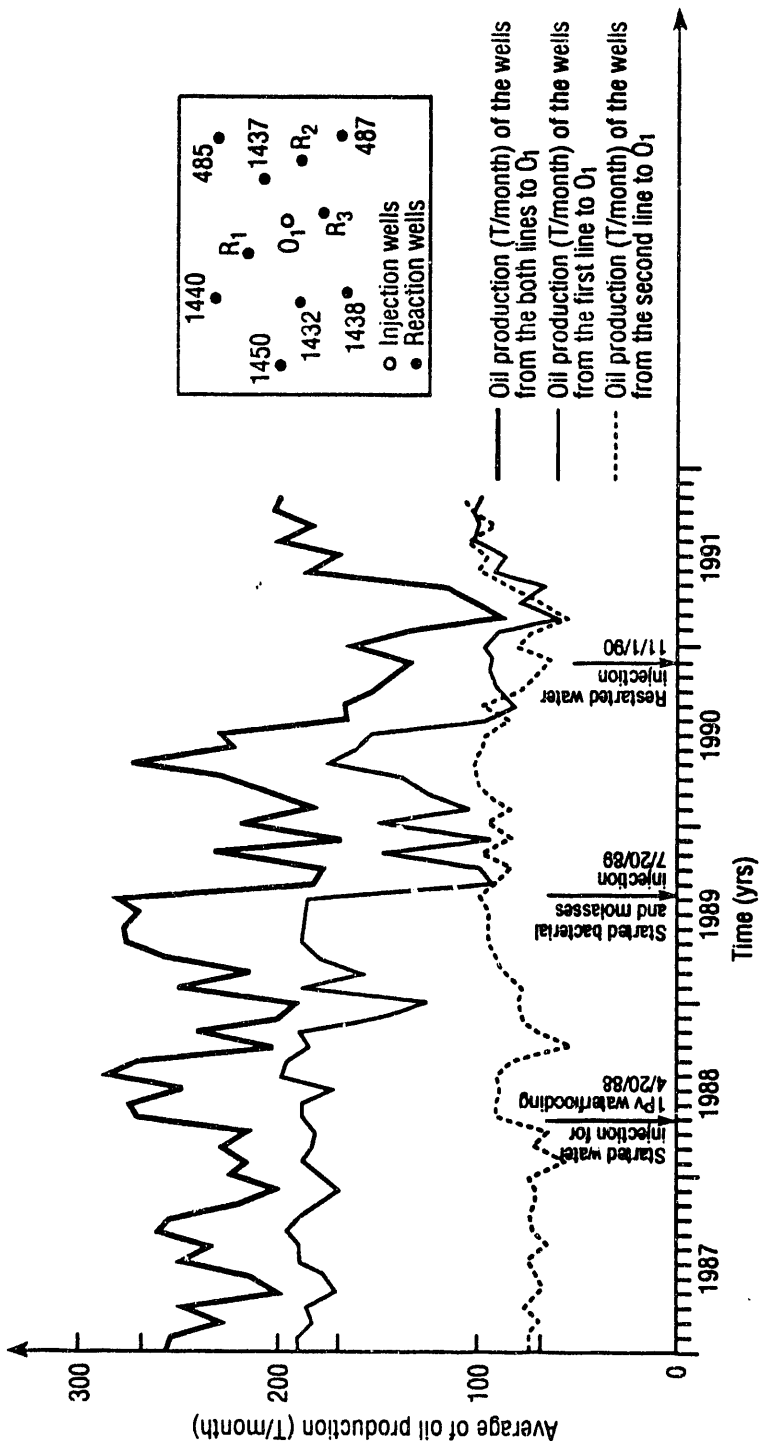


Figure 6. Oil production at the wells of Bragadiru Reservoir subjected to microbial flooding.

in situ by the bacteria after metabolizing the molasses. However, some gases (CO_2) could result from an attack upon rock (releasing $CaCO_3$) by the acid produced by the bacteria. The other physico-chemical characteristics, such as oil viscosity, density/brine mineralization, and crude oil Engler distillation are still under study; so far, it has been difficult to draw conclusions that correspond to the effects.

About two years after stopping the injection of molasses, the bacterial concentration in co-produced water seems to return to the initial values before treatment started, although oil production is maintained at an encouraging level.

If we compare the field trials carried out in the "first generation" (1976-1980) to those carried out in the "second generation" (1987-1981), we find a few clear differences:

In the "first generation" of field trials at seven reservoirs, results were evident at two reservoirs only, with a significant increase of oil production of 5.262 tones oil (Table 9);

In the "second generation" of field trials, the results are not as spectacular as in the two "first generation" reservoirs; however, results were positive in both reservoirs tested and seem to last for longer. Nevertheless, with all improvements incorporated in this "second generation" of field trials, we expected to have better results. The final conclusions will be drawn in one to two years for the Caldararu and Bragadiru reservoirs, to

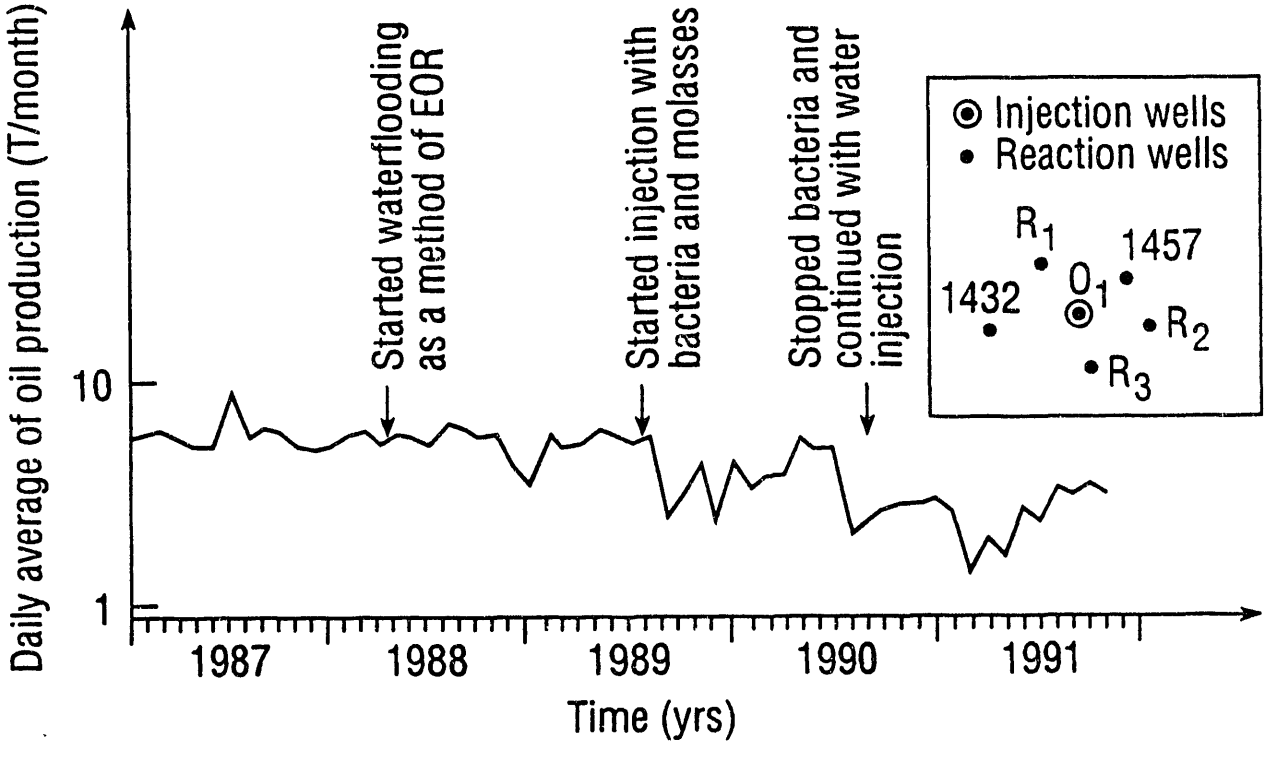


Figure 7. Oil production at the wells of Bragadiru Reservoir subjected to microbial flooding (first line wells).

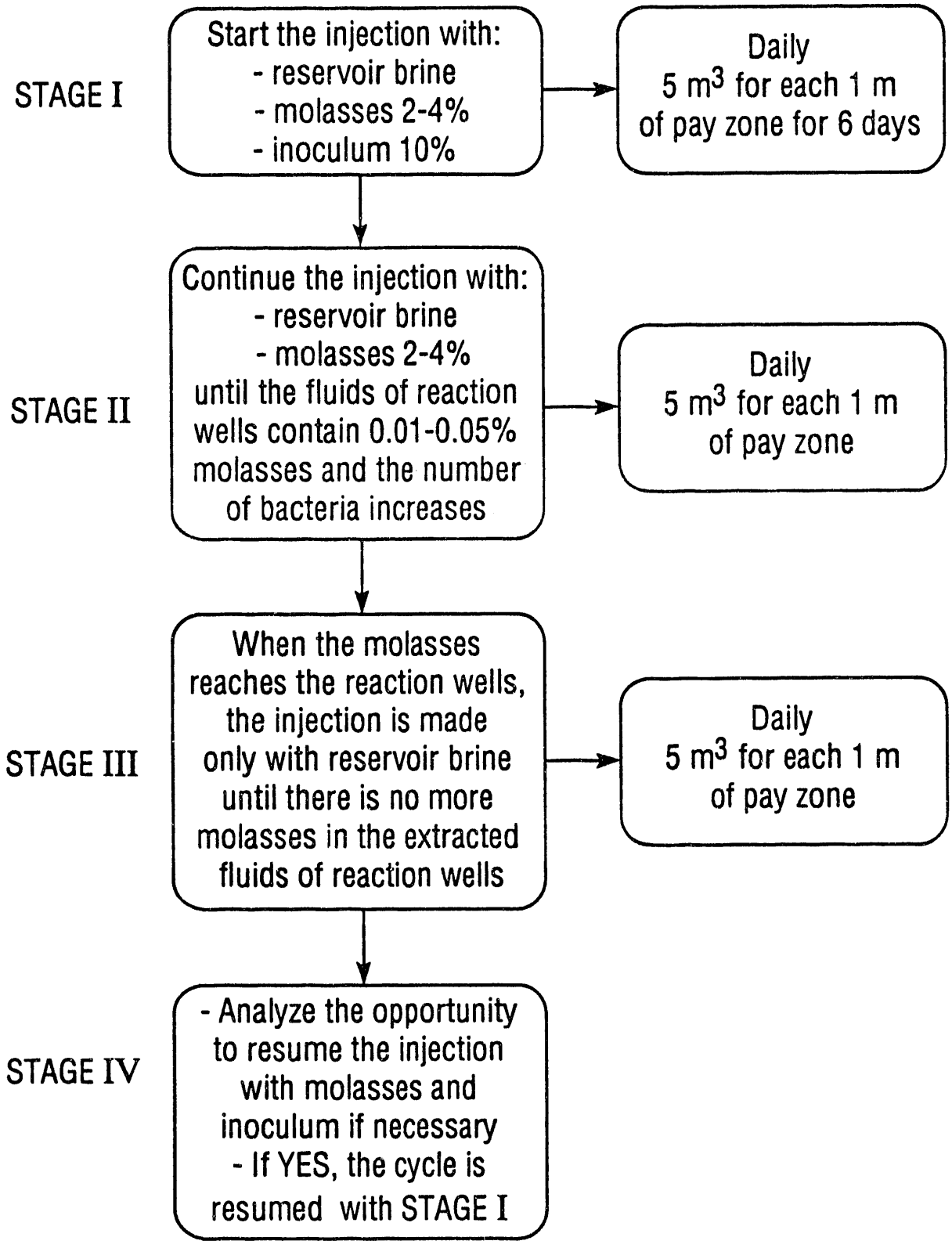


Figure 8. A new protocol of the microbial waterflooding technology of well injection.

which, in 1993, we shall add another three to four reservoirs. These are now being prepared for microbial flooding technology using an improved protocol for injecting the wells.

The findings on the wellbore clean up MEOR technology are of great interest and are the first to gain the confidence of the specialists working in the oil fields. So, probably in the future, this technology will be used for as many wells as possible which require a wellbore clean up treatment. In the second part of this year, we plan to apply such MEOR technology to about six to eight wells.

4. CONCLUSIONS

After about twenty years of activity in the field of MEOR in Romania, useful experience has been gained, which, after 1985-1986, resulted in several improvements to the MEOR technology used during the "first generation" of field trials (1976-1980). All these improvements, which were incorporated into the MEOR field trials of the "second generation" (1987-1990), deal with:

Obtaining the bacterial inoculum (AMEC) with a very simple methodology, published in several patents and summarized by Lazar et al. [2];

Basing the AMEC on a few important groups of bacteria, generally with high ability to adapt to reservoir conditions and to use molasses with the production of substances with a role in oil release. These bacteria belong to the genera *Clostridium*, and *Bacillus*, together with some Gram-negative bacteria of the genus *Pseudomonas* and family *Enterobacteriaceae*;

Improving the methodology of multiplying the selected AMEC. The technical facilities for producing the bacterial inoculum in the quantities required for field trials are also presented in some patents and some recent papers by Lazar et al. [1,2,13];

Establishing a new protocol for well injection as well as for increasing the amounts of bacterial inoculum and molasses used, and specifying the times of injection of these ingredients. The protocol of well injection used at the

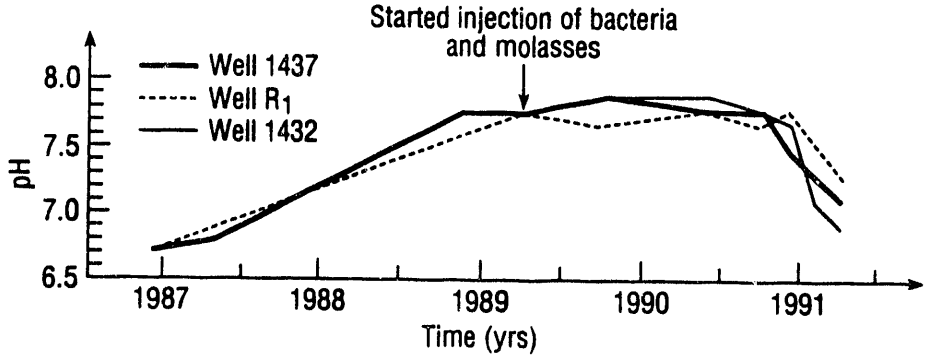


Figure 9. Evolution of pH at the Bragadiru Reservoir subjected to microbial flooding.

Caldararu and Bragadiru reservoirs could be improved from the practical experience gained during work there over the last three to four years. For this purpose, a new proposal for a patent was submitted with a new protocol for microbial flooding that will save molasses and use less bacterial inoculum compared with the protocol applied at the Caldararu and Bragadiru reservoirs;

Using wellbore clean up at four wells, the success of the MEOR technology has become apparent, increasing the confidence of people from the oil field units. Consequently, in the near future, such technology will be extended to another six to eight wells. In the case of MEOR microbial flooding technology, the results obtained at the two reservoirs are encouraging, and generally, have been better than the results in the "first generation" of field trials;

Starting from these promising results, oil field specialists in Romania decided to extend microbial flooding in 1993, to at least three to four more reservoirs. Finally, we would emphasize that the same intensive consideration is also being given to other aspects of MEOR technology, such as obtaining biopolymers and biosurfactants.

5. ACKNOWLEDGMENT

We would like to thank several petroleum specialists working at the oil units at the Caldararu and Bragadiru reservoirs for their help. We are also grateful to members of the PETROM RA Institute for Research and Technology Cimpina, and the Technical Division of the Petroleum Ministry of Romania, who expressed confidence in MEOR and supplied most of the funds for our research.

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Microbial-Enhanced Waterflooding Field Pilots

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Abstract

To determine the feasibility of improving oil recovery and the economics of microbial enhanced waterflooding in mature oil wells in the United States, two field pilots have been conducted. Candidate fields were screened to determine whether they had any potential for a microbial system developed at the National Institute for Petroleum and Energy Research (NIPER), and microbial compatibility tests were conducted in the laboratory to select the target fields. Both field projects were conducted in fluvial-dominated deltaic sandstone reservoirs, designated by the U.S. Department of Energy as Class I. A specific microbial formulation was selected that was compatible with the reservoir's environment, and had been shown to recover oil after waterflooding in Berea sandstone and field cores. A 20-acre pilot test was started in October 1986, and completed December 1989. The results demonstrated that microorganisms could be injected into an ongoing waterflood and thereby increase oil production by at least 13%. A larger test (520 acres) is in progress in the same formation to evaluate the feasibility of commercial application of the technology; microorganisms and molasses were injected from a centralized injection station in June 1990. All 19 injection wells were treated and oil production is being monitored from the 47 production wells. Injection pressures and volumes have continually been monitored since the start of the project. No operational problems were encountered. Based upon the data obtained to date, the oil production decline curve has improved, and monitoring of oil production will continue through December 1992.

1. INTRODUCTION

A microbial-enhanced waterflood field project, sponsored by the U.S. Department of Energy (DOE), Microbial Systems Corp. (MSC), and INJECTECH, Inc., and conducted in cooperation with the National Institute for Petroleum and Energy Research (NIPER), was initiated in October of 1986. The purpose of the project was to determine the feasibility of injecting a microbial formulation in a mature, ongoing waterflood, and determine if such an injection could increase oil production rate.

A DOE Fossil Energy report, "Oil Research Program Implementation Plan" has stressed the need for near-term oil recovery activities by independent petroleum producers for declining oil fields and stripper wells [1]. According to that study, these activities are particularly important because independent operators produce about 40% of the total oil recovered in the United States, but cannot afford to conduct needed EOR research. Microbial methods for improving oil recovery are potentially cost-effective and particularly well suited for today's economic climate. The technology is flexible, relatively inexpensive, and can be applied by independent producers. Microbial formulations can be used in a variety of methods including well simulation treatments, permeability

modification treatments, and microbial-enhanced waterflooding. Well stimulation treatments are inexpensive and easy to implement and can provide rapid recovery of nominal investment costs. Microbial-enhanced waterflooding has significant potential for increasing production from aging oil fields that are currently under waterflood. The incremental cost for injecting microbes and nutrient is relatively small in an existing waterflood, which may make this recovery method applicable at low oil prices when more expensive methods are not economically feasible.

The concept of the use of microorganisms to recover oil from depleted petroleum reservoirs is not new. Field and laboratory research has been performed, and patents have been granted for this technology since the late 1940s. Early microbial enhanced oil recovery (MEOR) patents by Zobell [2], Hitzman [3], and Updegraff and Wren [4] described the use of microorganisms in reservoirs to produce chemicals that could help to mobilize oil. Several literature reviews on MEOR have been published [5-8].

Laboratory research has demonstrated that the products from microbial fermentation of nutrients can change the interfacial properties between oil and water, selectively plug high-permeability zones to improve sweep efficiency, and increase wellhead pressures in single-well injections. Some microbial species can also significantly improve oil production by helping to remove suspended debris and paraffins from the near wellbore region.

Microorganisms most commonly used for MEOR field processes that rely on improving the efficiency of microscopic oil displacement are species of *Bacillus* and *Clostridium*. These species have a greater potential for survival under petroleum reservoir conditions than other species because they produce spores, which are dormant, resistant forms of the cells that can survive under stressful environmental conditions. *Clostridium* species produce surfactants, gases, alcohols and solvents, whereas some *Bacillus* species produce surfactants, acids, and some gases.

In microbial-enhanced waterflood applications, it is important that the microbes be capable of moving through the reservoir matrix and producing chemical products that can mobilize oil. The relative rates of transport of the nutrient and microorganisms will affect the injection strategy and design of the microbial system.

A microbial treatment requires careful design and sound reservoir engineering practice, as does any enhanced oil recovery (EOR) method. The methodology for designing and optimizing MFOR field tests has yet to be established; however, the literature and laboratory experience indicate that certain procedures are necessary to implement a microbial-enhanced waterflood. These particular field experiments were designed to use microorganisms that produced chemicals (surfactants, gases, alcohols, and fatty acids) for improved oil mobilization, and had the ability to transport through porous media. Detailed information about the Mink Unit Project through January 1990 has been published previously [9].

2. FIELD TEST DESIGN - MINK UNIT

The Mink Unit site, which includes both the Candy and Sallie Mink leases, selected for the project is located in Delaware-Childers field in Nowata County, Oklahoma (Figures 1 and 2). This particular part of Delaware-Childers field was owned by B & N Oil Company when the project was initiated in 1986. The legal description of the Mink Unit is Section 36, Township 27N, Range 16 E of Nowata County.

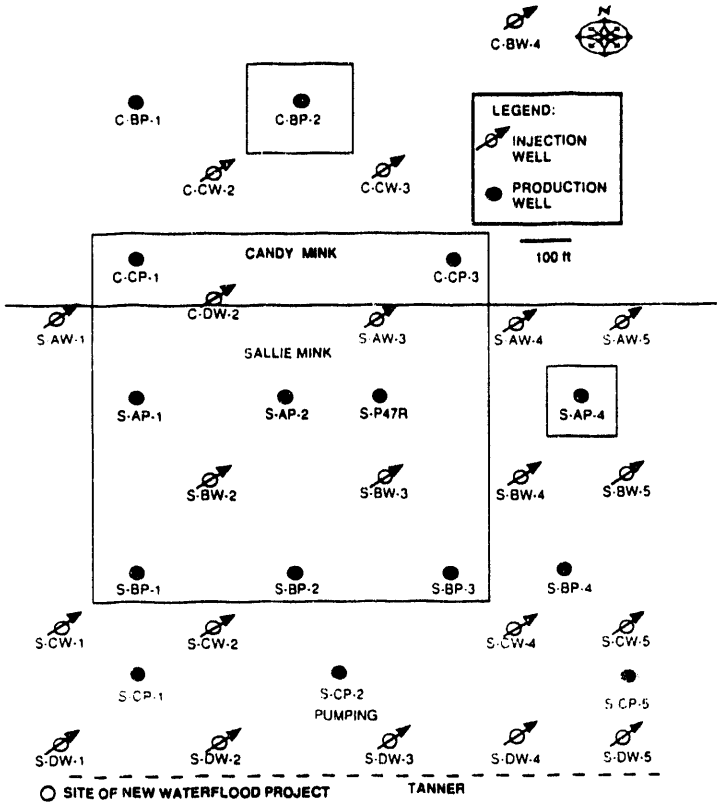


Figure 1. Map of Mink Unit - Delaware-Childers field (S36-T27N-R16E).

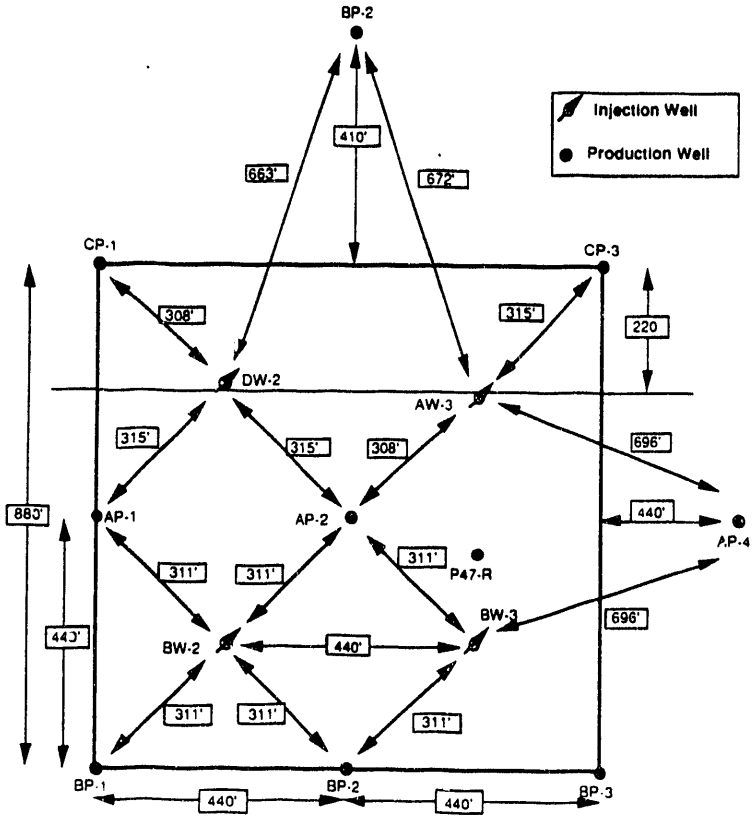


Figure 2. Pilot area of Mink Unit showing well spacing in feet.

Table 1
Reservoir properties for Mink Unit

Formation	Bartlesville Sandstone
Depth, ft	600
Average net pay thickness, ft	30
Average permeability, md	90
Porosity, %	20
Average formation temperature, °F	75
Number of injection wells	21
Number of production wells	15
Average water injection rate, bbl/day	40/well
Average injection pressure, psi	530
Average oil production, bbl/day	6.4
Oil gravity, °API	34
Oil viscosity, cP @ 77°F	7
Total dissolved solids of injection water, %	0.03
Average total dissolved solids of produced water, %	0.5
Average oil saturation, % (at start of project)	30

One waterflood, initiated in March 1954, was the Sinclair Oil and Gas Company's Tanner Flood, which encompassed about 1,200 acres and included the Mink leases, the site of the microbial field experiment. Surface water from the nearby Verdigris River has continued to be the source water for this flood since its initiation. The flood has been in continuous operation, although under various owners, to the present time. Fortunately, more field information exists than would normally be expected for a shallow field which has been producing for over 80 years. This results, in part, from the field size, pioneering secondary recovery efforts, and the close proximity of a petroleum research facility, founded in 1917 as the Bureau of Mines Petroleum Experiment Station, in Bartlesville, Oklahoma.

The Mink leases were determined to have an average porosity of 20%, an initial average oil saturation at the start of the project of 32.6%, and a combined net pay bulk volume of 2,900 acre-feet. The estimated cumulative oil production from the two leases has been 341,217 bbl through 1986. The project area has a surface area of 17.78 acres and a net pay bulk volume of 516 acre-feet.

In 1988, as a result of the sale of this oil field, cores were drilled on the Mink Unit and Brown leases of this field, and the resulting information was provided by the new owners to NIPER. Based on this new information, the average permeability is 90 millidarcies, which was higher than some of the earlier core analyses, and the average porosity is 19.1%.

With an estimated irreducible oil saturation of 25%, the recoverable oil within the leases by waterflooding was 76.3 bbl/acre-foot or about 40,000 bbl in the pilot area, at the initiation of the microbial field project. The Mink Unit covers a 160-acre area of which 110 acres are productive and contain 21 injection wells and 15 production wells drilled on 5-acre spacing (Figure 2). Only one of the producing wells is being pumped. Well completions are open-hole. Table 1 lists the average reservoir properties.

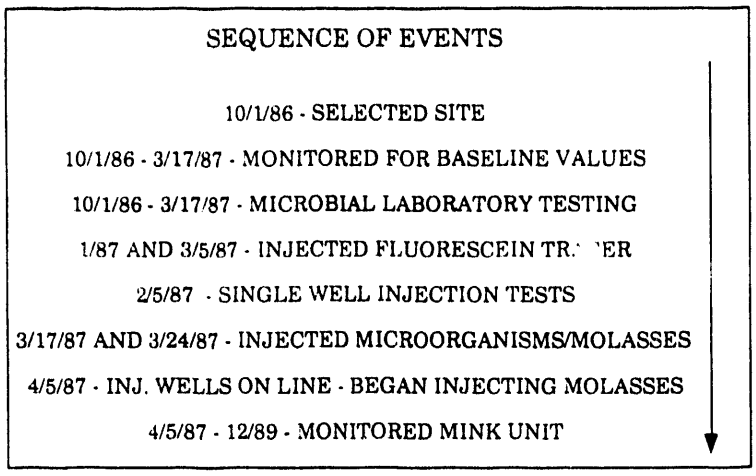


Figure 3. Sequence of events in the Mink Unit Project.

3. FIELD OPERATIONS - MINK UNIT

A chemical tracer study was implemented during the baseline period (December 1986) to determine: (1) the flow patterns of the injected fluids in the Mink Unit; (2) if any gross channeling existed; and (3) if there was communication among all producing wells and the four treated injectors. Each producing well was sampled daily for the first 5 days after tracer injection, then biweekly sampling continued until no fluorescein was detected in the production wells. Samples were protected from light and transported to NIPER where the fluorescein concentration was determined using a spectrophotometric method. The tracer studies seemed to indicate a northeasterly flow pattern (Figure 1) because wells C-CP-1 and C-CP-3 and S-AP-4 received fluorescein in greater amounts and more quickly than the other wells. The middle well, S-AP-2, received the highest amount of fluorescein, which was expected since this well is affected by all four injection wells.

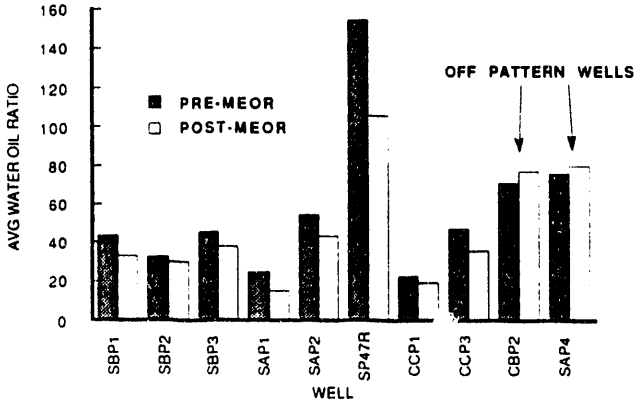


Figure 4. Average water-oil ratios of monitored Mink Unit producers.

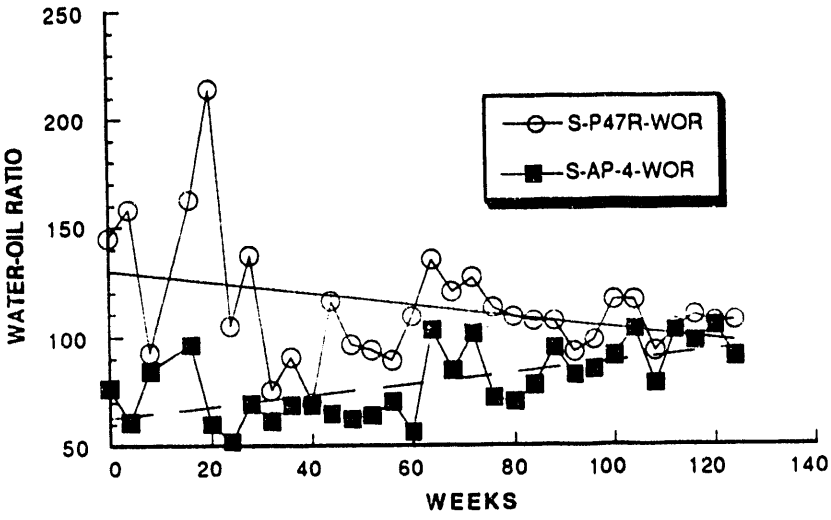


Figure 5. Average WOR of S-P47R and S-AP-4 (off-pattern) wells.

Fluorescein was again observed about 1.8 years (22 months) after injection. The persistence of this response indicated that the tracer had just transported through the matrix of the formation, and that the earlier response of tracer was due to low-volume, high-permeability streaks in the formation. Of the wells sampled, the fluorescein appearance again indicated a northeasterly flow pattern, because C-CP-1 and C-CP-3 showed fluorescein in high amounts, as did S-AP-2, the middle well.

The tracer results obtained are consistent with reports from a micellar-polymer pilot conducted on a nearby lease [10]. Chemical tracers (ammonium thiocyanate and isopropyl alcohol) were used in that study; however, no breakthrough of the tracers was ever detected in the produced water. Later, after injection of the micellar-polymer solutions, polymer was detected in off-pattern wells to the northeast of the pilot site, which indicated a directional

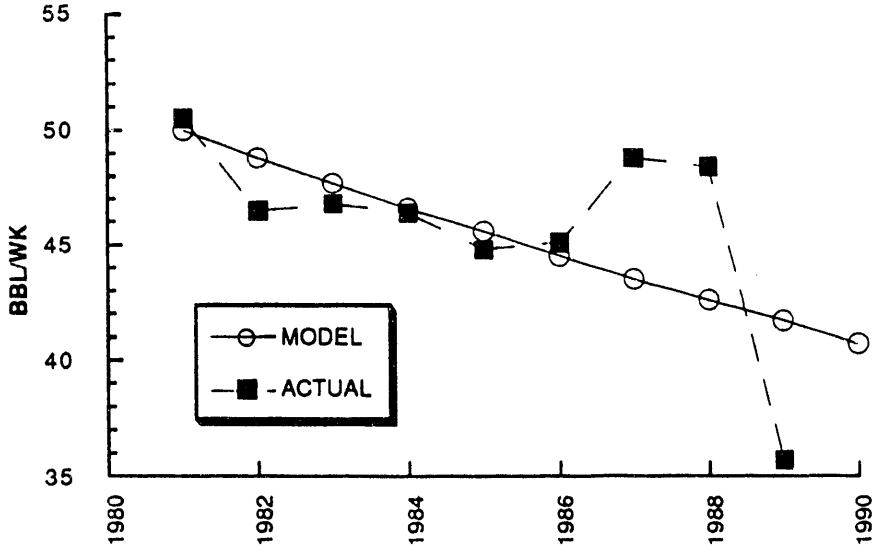


Figure 6. Predicted and actual average oil production for Mink Unit during 1981-1990.

permeability flow from the southwest to the northeast. The Bartlesville sandstone is a Cherokee Group, Desmoinesian Series, Middle Pennsylvanian System fluvial-dominated deltaic deposit [11]. Ultimate recovery of oil from reservoirs in the Cherokee Group is affected by facies, bedding boundary and other permeability barriers, and diagenetic changes. Because of these factors, permeability trends such as those observed in Delaware-Childers field, would be fairly common.

3.1. Injection of NIPER Bac 1 and molasses

Twenty-six gallons (0.65 bbl) of a microbial formulation, NIPER Bac 1, were injected into each of the four targeted injection wells, C-DW-2, S-BW-2, S-AW-3, and S-BW-3 (Figure 2). Wells C-DW-2 and S-BW-2 were treated on March 19, 1987, and wells S-AW-3 and S-BW-3, on March 23, 1987. Twenty gallons of molasses diluted to a concentration of approximately 4% was injected into each well periodically during the microbial injection. The molasses and microorganisms were injected by means of a header bypass system. The four treated injection wells were shut-in until April 3, 1987, when water injection was resumed. The other 17 injection wells in the Mink Unit were still in operation during the shut-in period. After water injection was resumed, the injection wells were backflushed to determine if microbial activity could be observed. Samples of water backflushed from the treated injection wells foamed when shaken, indicating surfactant production and that the microbial populations were viable. Subsequently, the four injection wells received the equivalent of 2 gallons of undiluted molasses per well per day until September 21, 1989.

4. PROJECT EVALUATION - MINK UNIT

The sequence of events in this project is briefly outlined in Figure 3. Field sampling for baseline values began in November 1986, and continued to March 17, 1987. The data from these studies showed that the total dissolved solids (TDS), pH, oil viscosities, and microbial counts were consistent during this period. Field data, including injection pressures and volumes, oil production rate, and water/oil ratios all remained fairly constant during the baseline monitoring period. Detailed results from the studies were presented in the Mink Unit Final Report [9].

Injection pressures at the microbially treated injection wells have not increased since the beginning of the microbial treatment. Injection pressure monitoring was critical to this microbial-enhanced waterflood experiment. In NIPER laboratory coreflooding experiments, no facial plugging was ever observed by NIPER Bac 1. Later coreflood experiments with similar microorganisms indicated that microorganisms and their products transport at reasonable rates through porous media [12]. Based upon laboratory and field results, we conclude that no adverse plugging effects have occurred because of the microbial injection.

The average water-oil ratios (WOR) at all monitored production wells in the Mink Unit have decreased when compared to the averages during the baseline period (Figure 4). These WORs have high standard deviation values, primarily because of gas production in the wells, which cause large fluctuations, but the overall averages have definitely decreased, and in wells S-P47R and C-CP-3, the decrease is significant. Note that in the two off-pattern wells, S-AP-4 and C-BP-2, the WOR has not decreased; thus, the microbial treatment has probably affected those wells closest to the injectors. Figure 5 presents a graph of the WOR for S-P47R vs. the WOR for S-AP-4; the WOR for S-P47R is decreasing, while that of the off-pattern well, S-AP-4, is increasing.

Table 2
Predicted and actual oil production rates for the Mink Unit

Year	Production Predicted avg bbl/wk	Production Actual avg bbl/wk
1981	50.0	50.5
1982	48.8	46.5
1983	47.7	46.8
1984	46.6	46.4
1985	45.6	44.8
1986	44.5	45.1
1987	43.5	48.8
1988A[1]	42.6	48.2
1988B[2]	42.6	46.5
1989	41.7	36.3
1990	40.7	26.3

[1]1988A - Jan. 1 - May 31.
[2]1988B - Jun. 1 - Dec. 31.

Oil production increased since the microbial injection through May 1988 (Figure 6). After the infill drilling and hydraulic fracturing occurred, the wellhead pressures at some of the nearest Mink Unit producers were much lower, as was the total produced fluid. The MEOR injection had a positive effect on oil production until the drilling and hydraulic fracturing activity and completion of the water injection plant on the nearby Tanner lease (Figure 1). Table 2 and Figure 6 show the predicted and actual average production for the 1981-1990. Since that time, actual oil production has dropped significantly below the predicted decline curve.

4.1. Economic analysis

Limited economic analyses of this field pilot showed that the major cost of a microbial-enhanced waterflood would be the nutrient support for the microorganisms. When determining the cost per incremental barrel of oil for the Mink Unit, the following assumptions were made: (1) no cost was assigned for the research and development of the microbial formulation; (2) the cost for equipment for this particular microbial injection was less than $500; (3) we were not overfeeding the microbial population; and (4) we also cannot assume that the total effect of the microbial injection has been attained. Since the chemical tracer just began to appear 1.8 years (22 months) after injection, based on preliminary data from early breakthrough of tracer, microorganisms should have begun to appear in the production wells about 0.6 - 0.8 years (7-10 months) after the tracer appearance. Unfortunately, this would have been about the time that infill drilling near the Mink Unit began, and our sampling period ended.

During the 14 months of microbial/nutrient injection before infill drilling and hydraulic fracturing activity, 577 incremental bbl of oil were obtained when

compared to the predicted oil recovery by waterflooding alone. A total of 18.7 tons of molasses was injected during this period. Using a nutrient cost of $100/ton, this is equivalent to $3.24 per bbl of incremental oil. This does not take into account any other injection costs, although for this particular project, the costs were fairly minimal. However, this cost also does not include any projected recoveries beyond the time of infill drilling. Since fluorescein was detected in the Mink Unit producing wells after the infill drilling in the Tanner lease, one may assume that the microbial treatment had not yet transported through the formation matrix; thus, the complete effect on incremental oil production that may have occurred would have been masked.

5. FIELD TEST DESIGN - PHOENIX SITE

In May 1988, Comdisco Resources, Inc., purchased property in the Delaware-Childers oil field from B & N Oil company. The Mink Unit leases were a part of this purchase. After much negotiation, an agreement was executed on April 18, 1989, by Microbial Systems Corporation (MSC) and Comdisco Resources, Inc. (Comdisco) that financially compensated the project for relocating the planned expansion of the project in three nearby leases in the B & N property of Delaware-Childers field to an alternate site. With DOE's approval, MSC and NIPER began to search for the new site, which ideally would have the same or similar properties to that of Delaware-Childers field.

Compatibility tests were conducted with reservoir fluids from three nearby waterfloods and selected microorganisms from the NIPER microbial culture bank. Based upon these studies, a new field site was selected. The site selected for the expanded MEOR project site is in Section 8, Township 24 North, Range 17E of Rogers County, Oklahoma. This site is part of Chelsea-Alluwe field in the Bartlesville formation and was initially developed soon after Delaware-Childers field. It is currently being waterflooded and owned by Phoenix Oil and Gas, Ltd. An isopach and top of structure map indicated fairly uniform gross thickness of the Bartlesville Formation, over the area of about 32-38 ft. Table 3 lists the reservoir data for the five leases in this site. This field is in an isolated area, with virtually no other oil-producing leases nearby. Porosity was obtained from the compensated density logs to estimate the average porosity and net pay thickness. Four core analyses are available from wells which also were logged and the data compared. Porosities from the logs averaged about 1% low to the core analysis and were adjusted accordingly. The net pay thickness was estimated to be 60% of the gross thickness. Using these data, the gross formation, net reservoir and pore volumes were estimated for the portion of each lease under production. Estimates of original oil saturation from core analyses and from the Delaware-Childers field were used to estimate the oil currently in place for each individual lease. An average porosity of 20% was assigned. The recoverable oil in place values at discovery are the same for each property when estimated in bbl/acre-ft. Figure 7 shows a map of the Phoenix site, and Table 4 lists the reservoir properties.

6. FIELD OPERATIONS - PHOENIX SITE

Figure 8 shows the sequence of events from the Phoenix field project. Monitoring of oil production will continue through December 31, 1992.

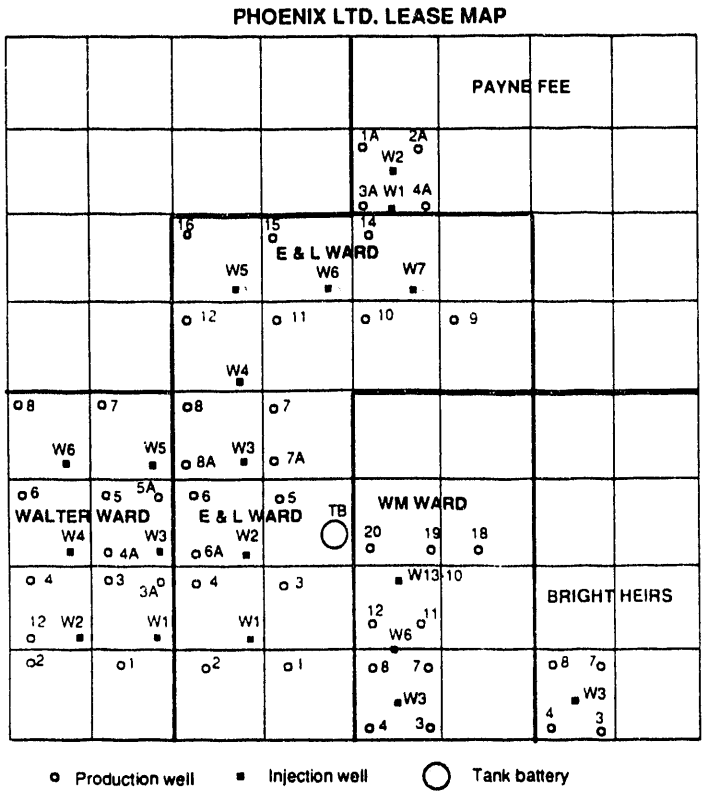


Figure 7. Map of Phoenix Field site.

Fluorescein was injected as a tracer on June 6, 1990. Eighty-five bbls of a solution containing 126 ppm fluorescein were injected. Wellhead samples were collected from all 19 injection wells at 2-hr intervals the first day to determine the injected tracer concentration. Table 5 and Figure 9 show the data obtained from the tracer study. Twenty-one producers were sampled 24 hr after

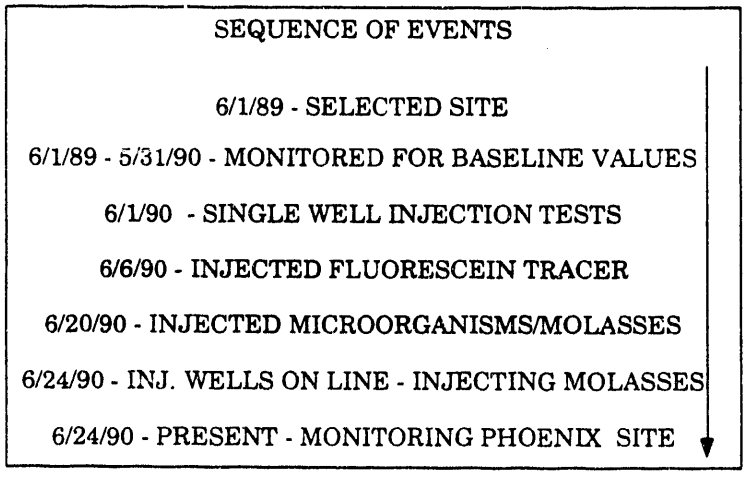


Figure 8. Sequence of events for the Phoenix project.

Table 3
Reservoir data for leases in the Phoenix site

	E & L Ward	Walter Ward	Wm Ward	Bright Heirs	Payne
Surface area, acres	160	80	27.5	10	10
Net Reservoir Acre-ft	3,393	175.8	596	189.6	204
Pore Vol., @20% bbls	5,264,812	2,721,317	924,795	294,196	316,540
OOIP @ S_o 65%, bbls	3,422,128	1,768,856	601,117	191,278	205,751
OIP @ Ab. S_o 25%, bbls	1,316,203	860,329	231,199	73,549	79,135
Init. Recov. Oil, bbls	2,105,925	1,088,527	369,918	117,729	126,616
Prod. 7/53 to 12/88, bbls	461,066	266,662	95,896	20,196	-
Current Recov., bbls	1,644,859	821,865	274,022	97,533	-

injection of tracer, sampled daily, then weekly, once a month, and finally, intermittently. Since the second day of sampling, the tracer response has never been higher than 0.30 ppm for all but one well (WM-20). The pattern of the fluorescein response seems to follow the same trend as that observed during the monitoring of the Mink Unit. There was an initial quick response of tracer from some of the nearest production wells; the response then leveled out to very low

Table 4
Reservoir properties for the Phoenix field site

Formation	Bartlesville Sandstone
Depth, ft	400
Number of injection wells	19
Number of production wells	47
Net pay thickness, ft	19-23
Well spacing, acres	2-5
Average formation temperature, °F	66
Average permeability, md	16
Average porosity, %	20
Average water injection/well, bbl/d	111
Average oil production/well, bbl/d	1
Oil gravity, °API	34
Est. average original S_o, %	65
Est. average irreducible S_o, %	25

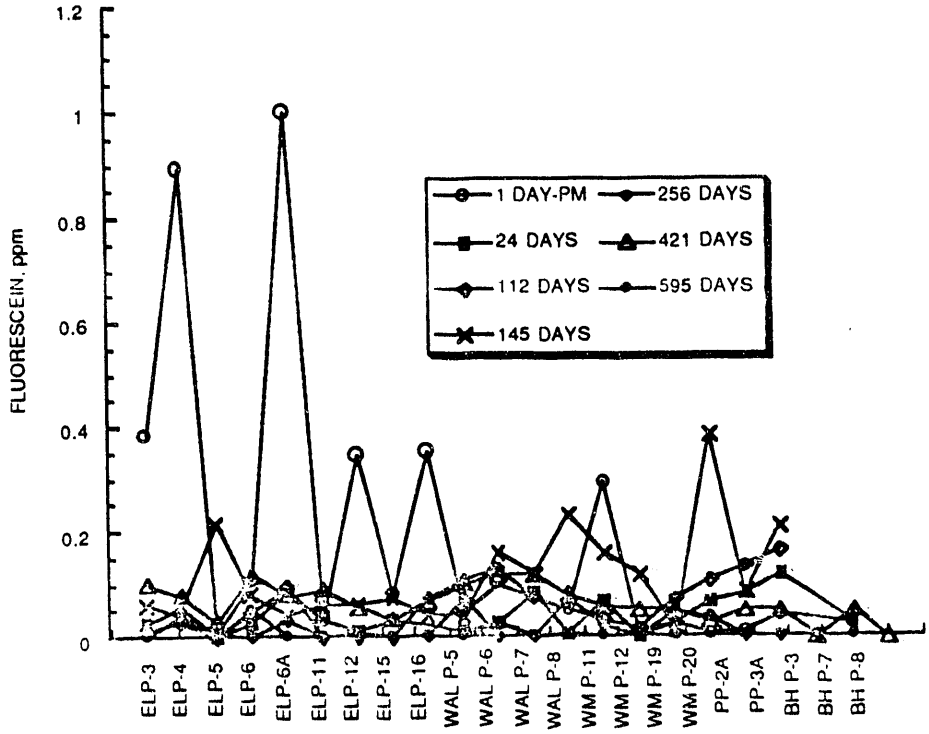


Figure 9. Tracer response in selected Phoenix producers.

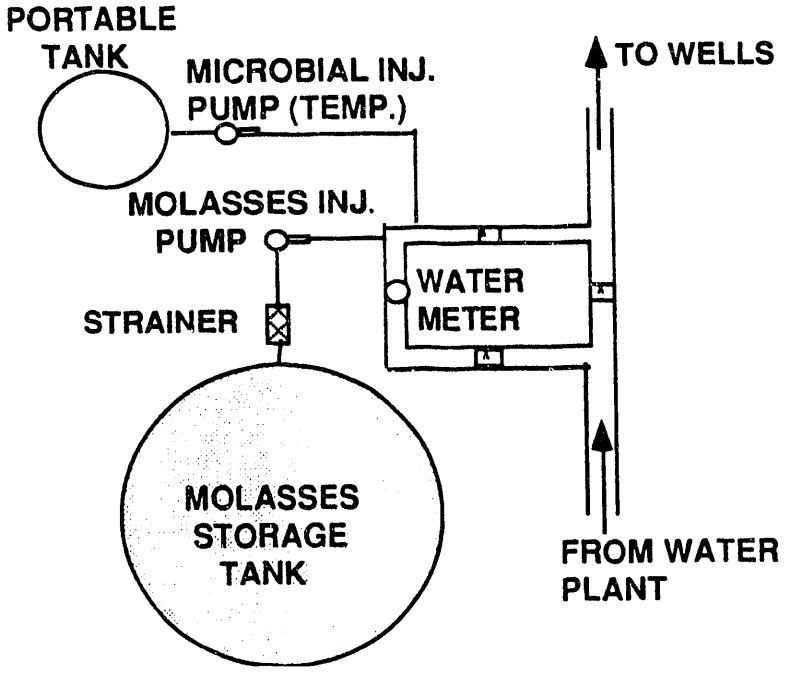


Figure 10. Map of the Phoenix Field site injection station. Injected water is recycled produced water.

values. Fluorescein values seemed to peak at 145 days, and were monitored for 599 days post-injection.

To determine the commercial feasibility of microbial-enhanced waterflooding, a method of microbial and molasses injection had to be designed for the entire field. Figure 10 shows a schematic of the centralized injection station used for the Phoenix field site. After the initial injection of the microbial formulation, molasses was injected continuously from the station.

The microbial formulation consisting of NIPER 1A and NIPER 6 was selected for this field injection. NIPER 1A is a variation of the same *Bacillus licheniformis* strain that was used in the Mink Unit project, and produces surfactant and acid from molasses. However, its growth in the reservoir brine from the Phoenix field site was never as optimal as that from the Mink Unit. NIPER 6 is another species of *Clostridium*. There are two potential reasons for this: (1) The Mink Unit injection water was fresh, and thus the fluids for cultivation of NIPER 1A had always been relatively low (0.5%) in salinity. The Phoenix brine is recycled produced water and has a salinity of around 2.9%. An analysis of the Phoenix produced water is shown in Table 6. (2) There is a higher concentration of iron in the Phoenix brine that may be inhibiting the growth of NIPER 1A. We selected another *Clostridium*, NIPER 6, to be injected with NIPER 1A. NIPER 6 was smaller in size than NIPER 3 *Clostridium* that was used in the Mink Unit. Since the Phoenix had a lower average permeability, we felt this would be a better microbial species for injection. After conducting several corefloods, we felt the other two microorganisms used in the Mink Unit had relatively little effect in terms of improved oil production, so they were not injected into this site.

On June 20, 1990, approximately 100 bbls of NIPER 1A and NIPER 6 were injected in 4% molasses. This injection was followed by continuous injection of 40 gallons of molasses per day, which was increased after one year, to 80 gallons per day until December 31, 1991.

The objective of the expanded Phoenix project was to determine how the process could be expanded for a whole field, and to determine if it is economically feasible to conduct microbial-enhanced waterflooding. The monitoring for this field site was designed to be minimal; including only those field parameters that would affect oil production. In addition to oil production from the field, individual injection pressures and volumes were monitored. Since this was a recycled flood, produced volumes could not be monitored.

7. PROJECT EVALUATION - PHOENIX SITE

The tracer concentrations observed in the selected producing wells indicated a very quick show in some wells, probably from very low-volume, high permeability stringers in the formation. This very quickly decreased to baseline levels. These data were corroborated by our observations of tracer response in the Mink Unit.

Injection well volumes and pressures did not significantly change after microbial injection (Figures 11 and 12). These are important parameters when monitoring a microbial injection. Significant plugging problems have not occurred.

Figure 13 shows the oil production from the Phoenix leases. The hyperbolic decline curve analysis indicates that oil production has increased after microbial treatment. An incremental 2,508 bbl of oil have been produced over the projected hyperbolic decline curve through September 1992, which corresponds to approximately 14% improvement in oil production.

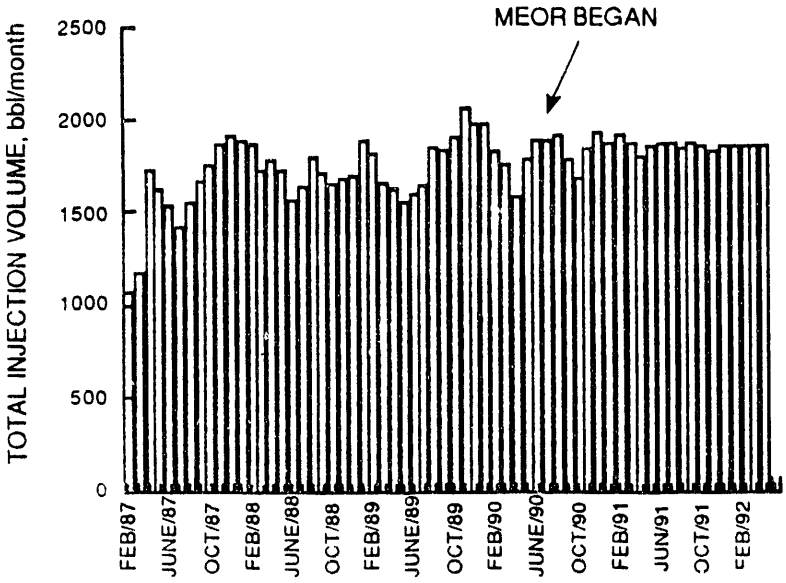


Figure 11. Total injection volume at the Phoenix field site.

7.1. Economic analysis

The total amount of molasses injected was 104 tons. From the oil production data above, an incremental 2,508 bbls of oil were obtained through September 1992. Using $20/bbl as the cost of oil, this amounts to $50,160 gross income. At a cost of $100/ton, $10,332 was spent for injected nutrient. The cost of the centralized injection station facilities was $2,500. Thus, a total of $12,832 was spent for the microbial flooding, leaving $37,328 of additional income over the cost of the MEOR treatment.

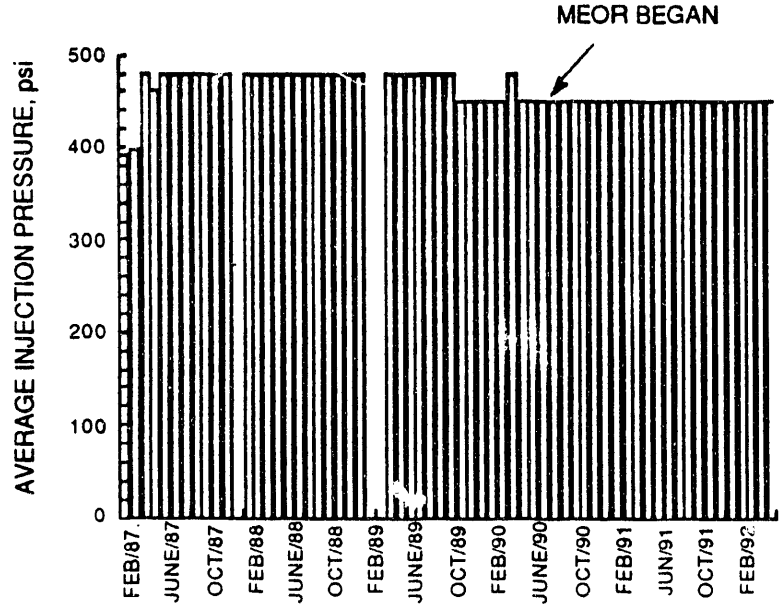


Figure 12. Average injection pressure at the Phoenix field site.

7.2. Environmental considerations

Before the start of injection of the Phoenix field site, various state agencies were contacted regarding any potential environmental impact of the project. Letters were received from the Oklahoma Historical Society, the Oklahoma Fish and Wildlife Society, and the Oklahoma Archaeological Society that indicated there would be no significant impact. An environmental contingency plan was in place in the event of an unwanted release of microorganisms or spill.

8. CONCLUSIONS

These microbial-enhanced waterflood field projects demonstrated the feasibility of microbial technology in a manner that an independent operator could implement, and using existing infrastructure and facilities. It is noteworthy that no operating problems were encountered before or during either project. No corrosion problems were experienced; in fact, the sulfate-reducing bacterial populations remained relatively low compared to the baseline counts. There were no problems with injectivity. During the Mink Unit project, laboratory and field data were correlated to develop and document a methodology for conducting microbial-enhanced waterflood field projects. This particular microbial formulation, NIPER Bac 1, and molasses injection improved oil production rates by about 13% and decreased water/oil ratios for producing wells nearest the injection wells up to 35%. The Phoenix project demonstrated the commercial applicability of microbial-enhanced waterflooding, and showed an improvement in oil production as of August 1992 of 11%.

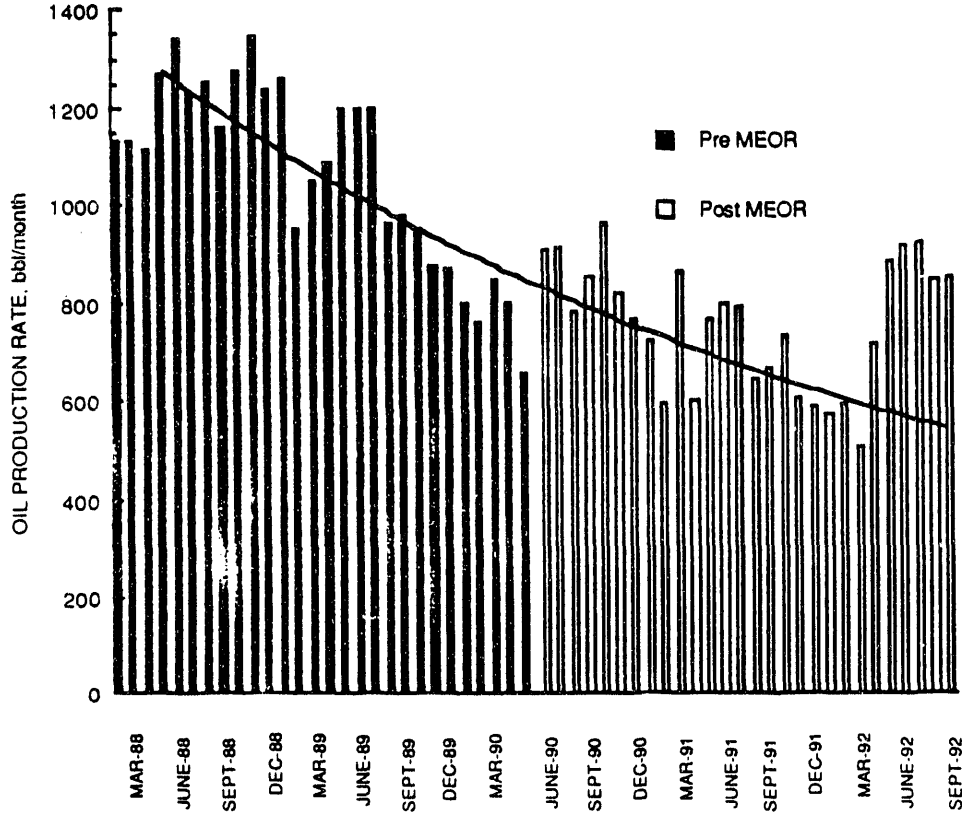


Figure 13. Oil production from the Phoenix field site.

Table 5
Fluorescein concentrations observed at selected producing wells at the Phoenix field site

Post Inj. days*	EL-3	EL-4	EL-5	El-6	El-6A	EL-11	El-12	EL-15	El-16
1 a.m.	0.000	0.463	0.128	0.106	1.000	0.058	0.324	0.346	0.186
1 p.m.	0.383	0.893	0.005	0.106	1.000	0.032	0.346	0.080	0.351
2	0.351	0.542	0.021	0.117	0.484	0.058	0.250	0.096	0.261
3	0.170	0.362	0.016	0.048	0.229	0.016	0.191	0.053	0.112
4	0.186	0.292	0.021	0.058	0.221	0.011	0.112	0.043	0.101
5	0.133	0.239	0.011	0.133	0.197	0.016	0.122	0.043	0.074
6	0.170	0.255	0.005	0.043	0.149		0.069	0.021	0.053
7	0.064	0.197	0.027	0.032	0.133	0.032	0.090	0.021	0.053
15	0.129	0.178	0.017	0.112	0.123	0.039	0.062	0.079	
24	0.028	0.056	0.000	0.028	0.090	0.034	0.011	0.034	
51	0.079	0.146	0.022	0.062	0.006	0.011	0.006	0.039	
85	0.067	0.084	0.028	0.120	0.140		0.050	0.028	0.275
112	0.017	0.028	0.000	0.045	0.095	0.000	0.000	0.000	0.073
145	0.062	0.039	0.213	0.079	0.039	0.062	0.062	0.073	0.039
256	0.010	0.031	0.005	0.000	0.031	0.000	0.005	0.000	0.000
299	0.037	0.049	0.000	0.104	0.061	0.000	0.000	0.000	0.000
421	0.101	0.079	0.028	0.118	0.079	0.090	0.056	0.034	0.067
500	0.023	0.016	0.000	0.035	0.041	0.000	0.000	0.000	0.004
595	0.000	0.041	0.000	0.053	0.000	0.000	0.000	0.000	0.000

*Start date - 6/6/90

Post Inj. days*	WL-5	WL-6	WL-7	WL-8	WM-11	WM-12	WM-19	WM-20	PP-2A
1 a.m.	0.053	0.064	0.027	0.064	0.505	0.016	0.096	0.043	0.053
1 p.m.	0.048	0.101	0.074	0.053	0.292	0.005	0.027	0.005	0.011
2	0.037	0.080	0.074	0.058	0.128	0.011	0.000	0.027	0.043
3	0.005	0.053	0.016	0.085	0.064	0.011	0.032	0.021	0.011
4	0.043	0.058	0.027	0.021	0.043	0.021	0.064	0.005	0.011
5	0.027	0.085	0.016	0.048	0.069	0.011	0.080	0.011	0.011
6	0.037	0.058	0.043	0.032	0.043	0.011	0.096	0.058	0.005
7	0.085	0.005	0.133	0.000	0.016	0.016	0.000	0.011	0.032
15	0.056	0.062	0.051	0.157	0.123			0.023	0.039
24	0.011	0.028	0.084	0.006	0.067	0.000	0.028	0.067	0.084
51	0.000	0.090	0.011	0.123	0.000	0.000	0.006	0.000	0.006
85	0.079	0.129			0.067	0.011	0.056	0.039	0.107
112	0.107	0.129	0.073		0.034	0.017	0.073	0.107	0.135
145	0.039	0.157	0.118	0.230	0.157	0.118	0.017	0.382	0.084
256	0.072	0.000	0.000	0.072	0.021	0.000	0.046	0.036	0.000
299	0.055	0.129	0.049	0.086	0.031	0.012	0.037	0.037	0.049
421	0.101	0.118	0.118	0.079	0.056	0.051	0.051	0.028	0.051
500	0.023	0.078	0.004	0.000	0.000	0.000	0.016	0.000	0.000
595	0.000	0.029	0.000	0.004	0.000		0.000		0.000

Table 5 - continued
Fluorescein concentrations observed at selected producing wells at the Phoenix field site

Post Inj. days*	PP-3A	BH-7	EL-RET.	WALT.-RET.	P/BH-RET
1 a.m.	0.043	0.053			
1 p.m.	0.043	0.027			
2	0.021	0.117			
3	0.011	0.043			
4	0.011	0.027			
5	0.021	0.043	0.048	0.096	
6	0.016	0.016	0.009	0.016	0.005
7	0.053	0.032	0.058	0.101	0.122
15	0.056	0.056	0.056	0.090	0.090
24	0.118	0.028			
51	0.039		0.006	0.006	0.000
85	0.017		0.039	0.067	0.056
112	0.163		0.028	0.095	0.107
145	0.208		0.000	0.140	0.281
256	0.000		0.000	0.036	0.041
299	0.055	0.012	0.043	0.037	0.000
421	0.051	0.051	0.051	0.073	0.073
500	0.016	0.004	0.023	0.016	0.000
595	0.000		0.010	0.010	0.000

*Start date - 6/6/90

Table 6
Trace mineral analyses from Phoenix produced water; all in mg/liter

	Injection Water	E & L #6
Cation		
Sodium	8,080	9,813
Calcium	764	1,240
Magnesium	422	362
Strontium	50.7	0
Barium	153	0
Iron	40.2	96
Anion		
Chloride	14,700	17,702
Sulfate	<0.1	0
Bicarbonate	869	1,171
Carbonate	0	0

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Microbial Characteristics and Metabolic Activity of Bacteria from Venezuelan Oil Wells

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Abstract

Microbial enhanced oil recovery has appeared recently as an alternative to obtain better performances in oil production. The new alternative needs detailed studies about the ability and capacity of bacteria associated with formation waters, or with percolated/injected waters during secondary crude oil recovery.

In the present work, we analyzed the morphological characteristics and ability to use organic substrates of bacteria. Bacteria were isolated from water associated with some shallow, biodegraded oil wells, with average pressure of (falta) and temperature of 60°C, located on the Eastern Coast of Maracaibo Lake. Aliquot samples of 14 oil wells were taken, incubated at 32°C and 50°C during 15 days in mineral media supplemented with yeast and glucose extracts for bacteria recovery. A total of 150 colonies was isolated and their micro-macro morphology were observed; also 16 biochemical tests were performed accordingly with the particular characteristics of origin of these bacteria, grouped in sugar fermentation, protein hydrolysis, mineralization of nitrogen and phosphorous compound, high NaCl concentrations, and presence of haemolotical and amilolitical enzymes. These tests allow knowledge of the ability of bacteria to produce metabolites that improve crude oil recovery. Statistical grouping tests were performed to classify the bacteria in toxonomic and morphological groups, and their presence and abundance in each analyzed oil well. The bacterial colonies formed seven groups, with non-homogenous distribution in the different oil wells. The results suggest that the source of the bacterial groups are not the same, or their presence in the oil wells do not have a common origin; this study also allows us to know the kind of energetic resource that must be used in each particular oil well.

1. INTRODUCTION

The increasing difficulties of extracting oil from subsoils by conventional methods has forced the search for new alternatives and techniques for the extraction of crudes. Recently, microorganisms have been used to enhance oil recovery, and much research carried out to understand their physiological characteristics and ability to produce metabolites at pressures and temperatures similar to the original conditions of the oil reservoir.

Microbial enhanced oil recovery (MEOR) involves the development of new techniques, with the aim of enhancing oil recovery. Several groups have obtained better performances using autochthonic microorganisms from the reservoir under exploitation; it is important to culture and observe the microorganisms in the laboratory to determine their metabolic activity and production of organic compounds using profitable, easy-to-obtain substrates, with high efficiency.

The following characteristics of the microorganisms can be evaluated in culture: i) efficiency in the use of substrates with a high content of hydrocarbons, nitrogen compounds, and phosphorous; (ii) growth at temperatures

above 50°C; (iii) ability to grow under low oxygen concentrations or without oxygen; and (iv) efficiency in the production of surface-active organic compounds, alcohol, organic acids, and gases.

In the present work, we characterized and analyzed the metabolic products of bacteria associated with the formation waters of two representative reservoirs from a zone of intense oil extraction. Our aim was to assess their efficiency and potential use as agents in MEOR.

2. STUDY SITES

Studies were performed in two different zones of crude oil exploitation. Area I, which is located on the eastern shore of the Maracaibo Lake (Figure 1), with 14 oil wells in production from Miocene reservoirs, averaging 4000 feet deep, and characterized by meteoric origin waters, partially biodegraded crude, and mechanically pumped oil. The temperature of the oil reservoirs was between 50-60°C, and the pressure in the well heads was 125 psi (Figure 2).

Area II is located in the Eastern Venezuelan Basin, at TRICO Field (Figure 3), where 3 oil wells in production of Miocene reservoirs were studied. The wells

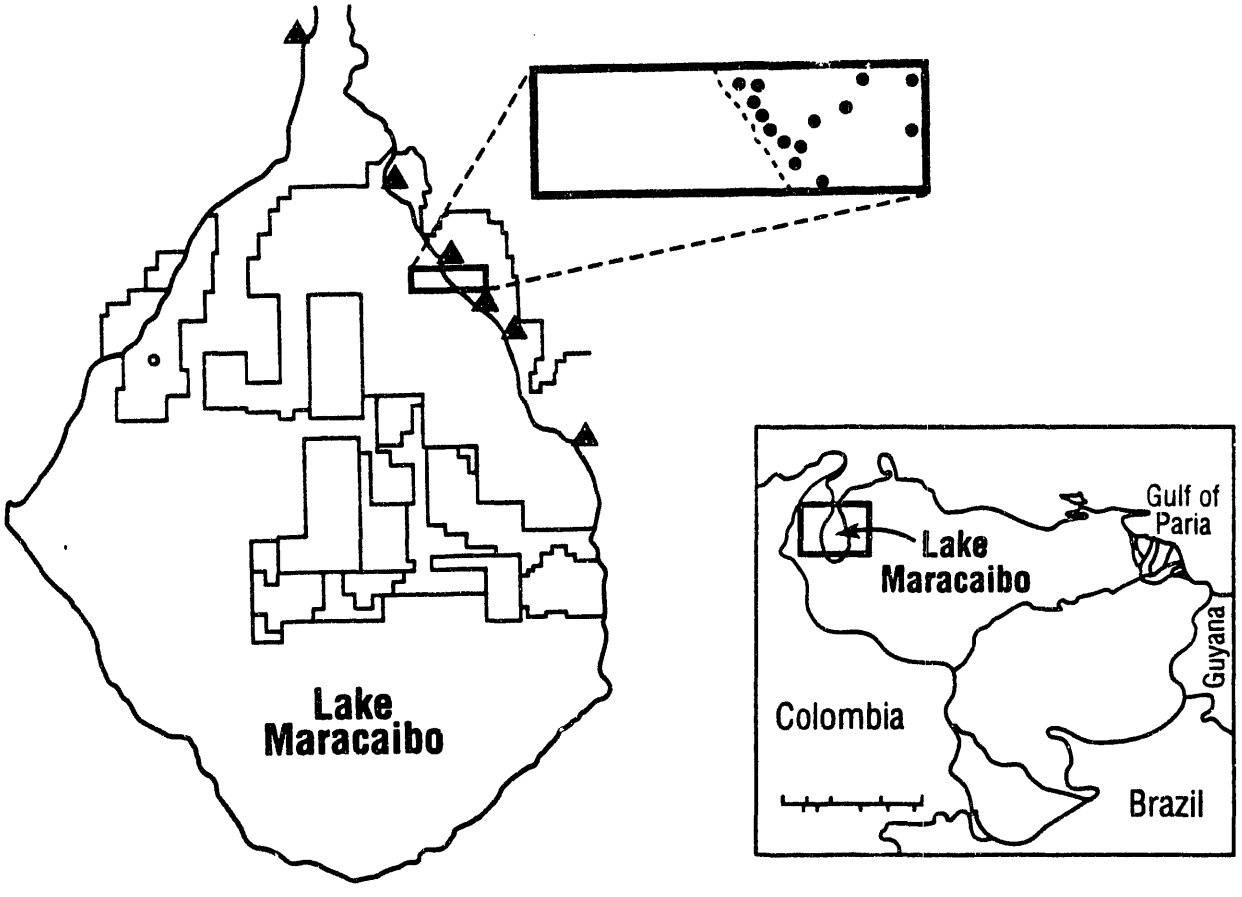
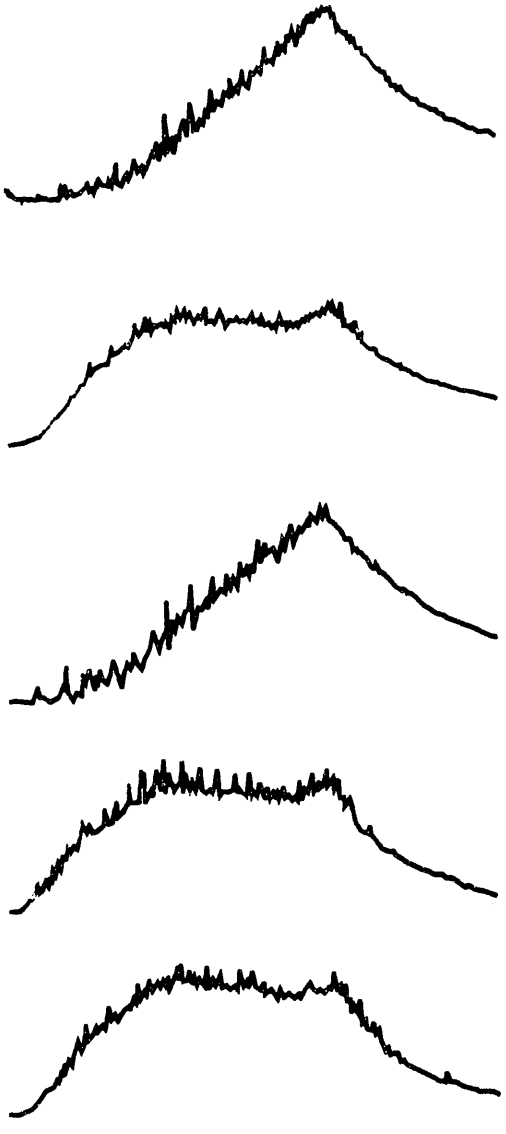


Figure 1. Study area I. Maracaibo Lake is shown on the small map (right) of Venezuela. The enlarged diagram of the lake shows study area I, with details (left) of the sites of the 14 wells.

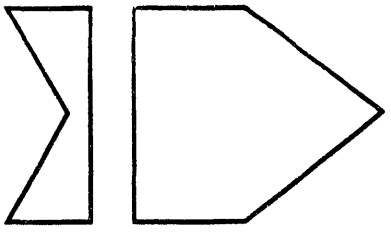
Table 1
Biochemical and morphological characteristics of bacteria associated with formation waters, held at 50°C

ASSAYS	FUNCTIONAL GROUPS															
	C1	C2	C3	C4	C5	C6	C7	T2	T3	T4	T5	T6	T7	T8	T9	
UREA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
ALPHA HEM.	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-	
BETA HEM.	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	
CITRATE	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	
STARCH	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	
HIDROL. GEL.	+	+	+	+	-	+	-	-	-	-	-	+	-	+	-	
ACETATE	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	
GLUCOSE	+	+	-	+	-	+	-	-	-	-	+	-	-	+	-	
LACTOSE	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	
NaCl 1%	+	+	+	+	-	-	-	+	-	+	+	-	-	+	-	
NaCl 3%	+	-	+	+	-	-	-	+	-	+	+	-	-	+	-	
NaCl 5%	+	-	+	-	-	-	-	+	-	+	+	-	-	+	-	
CATALASE	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	
GRAM STAIN	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	
COCCI	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	
SMALL RODS	-	-	-	-	-	+	+	+	+	+	+	-	-	-	+	
BIG RODS	+	-	+	-	-	-	-	-	-	-	-	+	+	+	-	
SPORES	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	

were 4500-5000 feet in depth, and were characterized by connate waters, marine and mature crudes without evidence of biodegradation, and mechanically pumped oil. The temperature of the oil reservoirs was about 55°C, and pressure in the well heads was 125 psi (Figure 4).



HIGH RESOLUTION GC
n-paraffin fraction



METEORIC WATERS
STIFF DIAGRAM

Age: Miocene
Temperature: 50 - 60 °C
Pressure: 125 psi
Porosity: 20%
Permeability: 450 md

Figure 2. Characteristics of the reservoir and associated fluids of study area I.

3. METHODS

The samples were taken from the well head through the valves into sterile 1ℓ capacity glass vessels; during transport to the laboratory, they were kept at the same temperature as the wells. A subsample of 100 ml of crude water mixture was taken from each sample, and inoculated into 300 ml of mineral medium with 0.6% glucose as a carbon source for the microorganisms. All samples were incubated at 50°C for 7 days.

After incubation, triplicate samples were sprayed on mineral agar plates and incubated at 50°C to observe colony formation units (CFU); a significant number of CFUs were isolated for further macro- and micro-morphological observations, including Gram-stain test, appearance (as cocci, small rods or big rods), and spore formation.

The content of n-paraffins (Figures 2 and 4) in the samples was determined by gas chromatography. Isolated CFUs were cultured under aerobic and anaerobic conditions.

Ten biochemical tests (including the use of urea, alpha and beta hemolysis, citrate, starch, gelatine, acetate, glucose and lactose as substrates, catalase production, and activity under three concentrations of NaCl) were performed on isolated CFUs, to determine the ability of bacteria to use different organic and inorganic chemical compounds as substrates, and to evaluate the production of surface active compounds. Cluster analysis was applied to obtain classifications of bacteria in taxonomical and morphological functional groups.

The kinetics of growth were analyzed during incubation for 168 hours at 50°C using mineral medium, with 0.6% of soluble starch as sole source of carbon. Samples were taken at 3, 6, 9, 24, 48, and 168 hours for counting colony forming units.

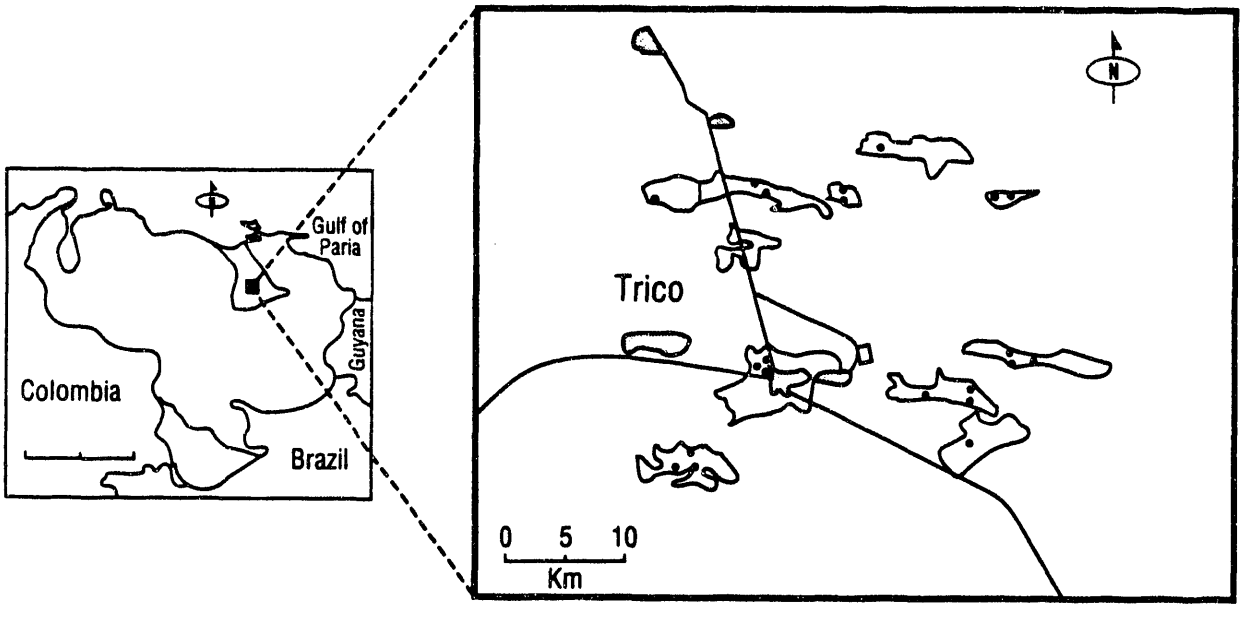


Figure 3. Study area II in the Eastern Venezuelan Basin at TRICO Field. The study area is shown on the small map (left) with details (right) of the sites of the 21 wells.

4. RESULTS

One hundred and fifty CFUs from study area I and 66 from the study area II were isolated after incubation of the samples. From biochemical and morphological analyses on the isolated CFUs, seven functional groups from study area I and eight from study area II were derived (Table I).

From study area I, the functional groups C1, C2, C6, and C7 are potential producers of surface active metabolites, due to their response as alpha-hemolytic agents. All bacterial groups from this area showed aminolytic activity, producing surface active metabolites from complex organic compounds, such as dextrine, a substrate that is easy to obtain and economical. Also, all bacteria from the C series were capable of mineralizing urea; only the C5 and C6 groups could not degrade peptide linkages. Citrate use as an energy source was recorded for the C2, C3, and C6 groups, while acetate was used by C3, C5, and C7. Tolerance to various levels of NaCl indicated that only the C1 and C3 groups could grow under all the concentrations tested; the C4 group grew at 1 and 3%, while the C2 group showed growth at 1% NaCl.

No functional group from study area II showed hemolytic activity, but all bacterial groups from this zone had the ability to degrade starch. Similar to study area I, all bacteria from study area II series mineralized urea. Gelatin hydrolysis (degradation of peptide links) was recorded only for the T6 and T8 groups, and none could use citrate as a carbon resource; only the T3 group had the ability to use acetate. The T2, T4, T5, and T8 series showed tolerance to NaCl concentrations between 1 to 3%.

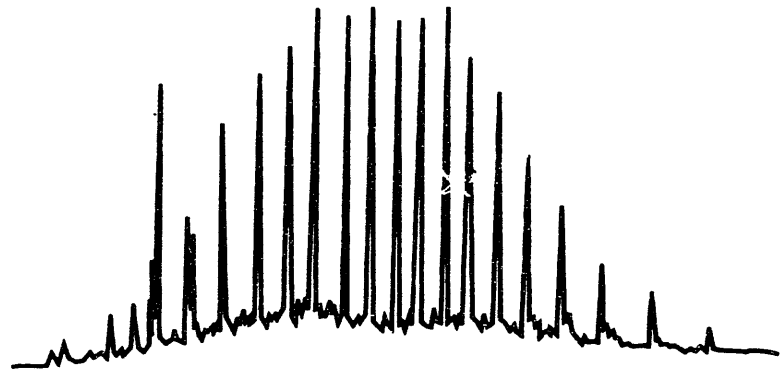
Figures 5 and 6 show, respectively, the growth kinetics of all functional groups using starch as sole carbon source for the study area I series and the study area II series.

Bacterial groups from study area I showed several changes in their growth kinetics during the experiment (Figure 5); at 8 hours, values were the highest for the C1 and C3 groups, and lowest for C7. At 24 hours, almost all bacterial groups showed a trend to maintain the same number of individuals, with a slow increase in growth rate at 30 hours. From then until 48 hours, three well-defined groups could be distinguished: the C1, C5, C2, and C4 series with the highest growth, the C6 and C7 series with the lowest growth, and the C3 series with intermediate growth. This distribution persisted without change until the end of the experiment at 168 hours.

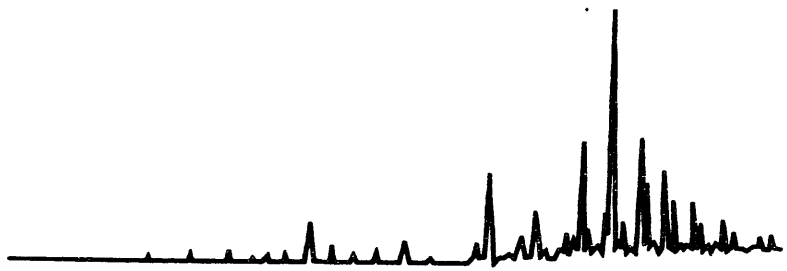
Three groups also were distinguishable from study area II during the first 6 hours of experiment (Figure 6): the T2, T3, T4, and T8 series had the highest values, and the T5 and T6 series had the lowest values. After that, growth was slow until 48 hours, when two new groups were formed, with the T2 and T5 series showing the highest values, and the remaining series with low values. The trend of the two groups was maintained until 168 hours, except for the T2 series, which showed a reduction in its growth; the highest rates of growth were reached by the T3, T5 and T6 series, while the remaining series had low values.

As a way of interpreting the microbial interactions, the similarity of their origin and behavior of the various bacterial groups found in the formation waters of the oil wells, we analyzed data on presence-absence obtained for Study Area I by the frequency of appearance of each bacterial group in the 14 oil wells. The results are shown in Table 2, with the percentages of bacterial groups per oil well and the frequency of appearance of each functional group.

The highest percent of bacterial groups appeared in oil well 1 (71.40%), with 5 functional groups present, and oil well 7 with 4 groups (57.12%), while for oil wells 9, 10, and 11, only one group was present. The rest of the oil wells had

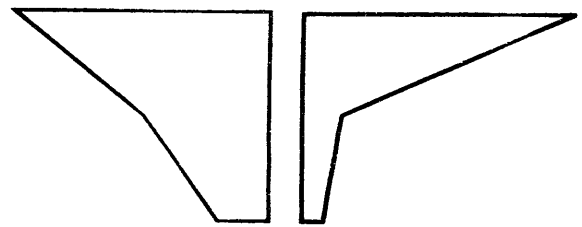


HIGH RESOLUTION GC
n-paraffin fraction



GC-MS TERPENOIDS
m/e 191

CONNATE WATERS
STIFF DIAGRAM



Age: Miocene
Temperature: 55°C
Pressure: 125 psi
Porosity: 25 - 30%
Permeability: 600 md

Figure 4. Characteristics of the reservoir and the associated fluids of study area II.

Table 2
Frequence of appearance of different groups of bacteria from study area I

WELLS	BACTERIAL GROUPS							% OF TOTAL BACTERIAL GROUPS
	C1	C2	C3	C4	C5	C6	C7	
1		X	X	X	X	X		71.40
2				X		X		28.56
3		X	X					28.56
4		X	X	X				42.84
5					X		X	28.56
6			X		X	X	X	57.12
7	X	X		X				42.84
8			X					14.28
9		X						14.28
10				X				14.28
11	X	X	X					42.84
12			X	X			X	42.84
13			X		X			28.56
14		X	X	X				42.84
	13%	47%	60%	47%	27%	20%	20%	

two to three groups. Figure 7 shows the distribution of the various bacterial groups per oil well.

Figure 8 is a graphic representation of the frequency of appearance of the different bacterial groups characterized for study area I. The C3 group had the highest frequency (9 oil wells, 60%), and the C1 group was represented in only two oil wells (8 and 12, 13%). The C2 and C4 groups showed a high distribution index being present in seven oil wells.

5. DISCUSSION

The need for good recovery of crude oil from reservoirs has led to the use of microorganisms as an alternative approach. Beckman [1] and Zobell [2] were the first authors to suggest the importance of microorganisms in enhanced oil recovery (EOR). In recent years, the research in this field has increased; many researchers have isolated bacteria from different environments, and have studied their metabolic production and capability to tolerate extreme conditions, similar to those of the reservoir, and their ability to produce metabolites potentially useful for EOR [3-9].

However, few researchers have studied the autochthonic microorganisms in the oil wells, as a way of assessing the ability of the bacteria present in the formation waters to produce metabolic compounds potentially useful in EOR. In the present work, a number of microorganisms from the formation waters of oil wells were characterized, using biochemical and morphological tests, and statistical methods, revealing seven functional groups in the oil wells of study area I, and eight groups from study area II.

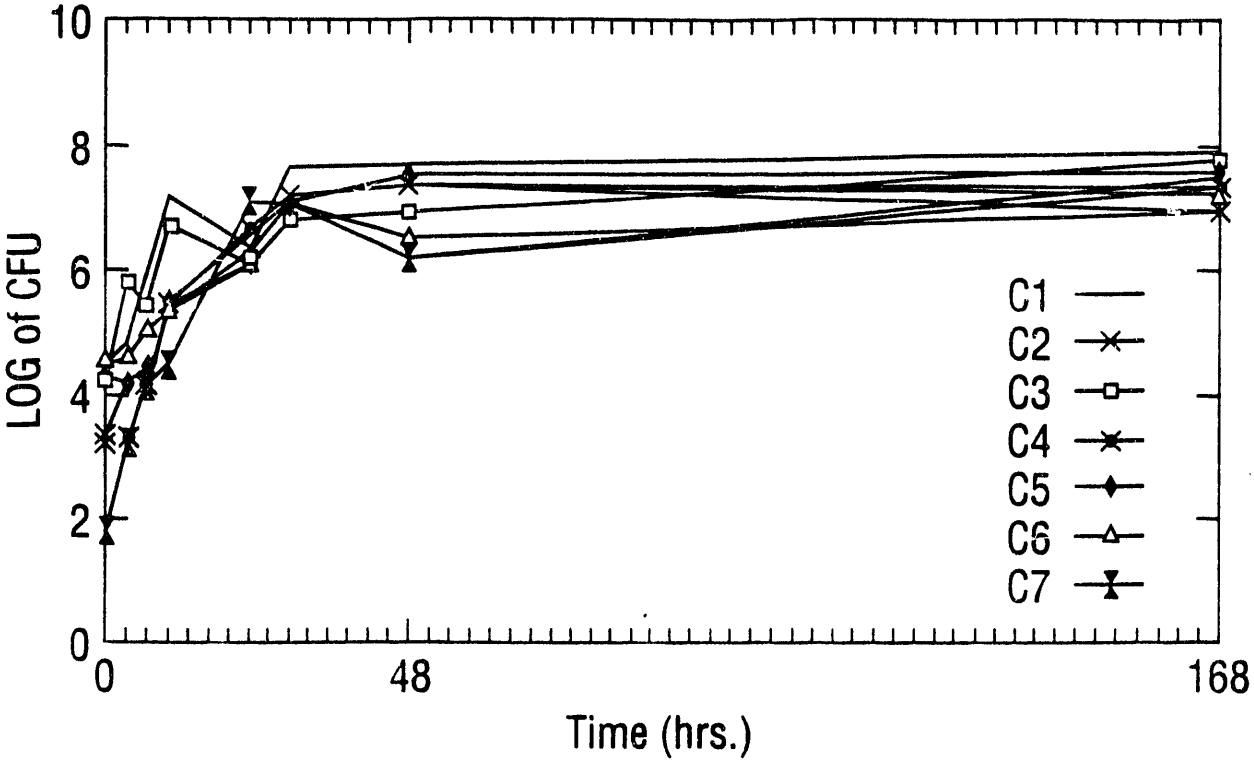


Figure 5. The kinetics of growth at 50°C of bacteria from study area I.

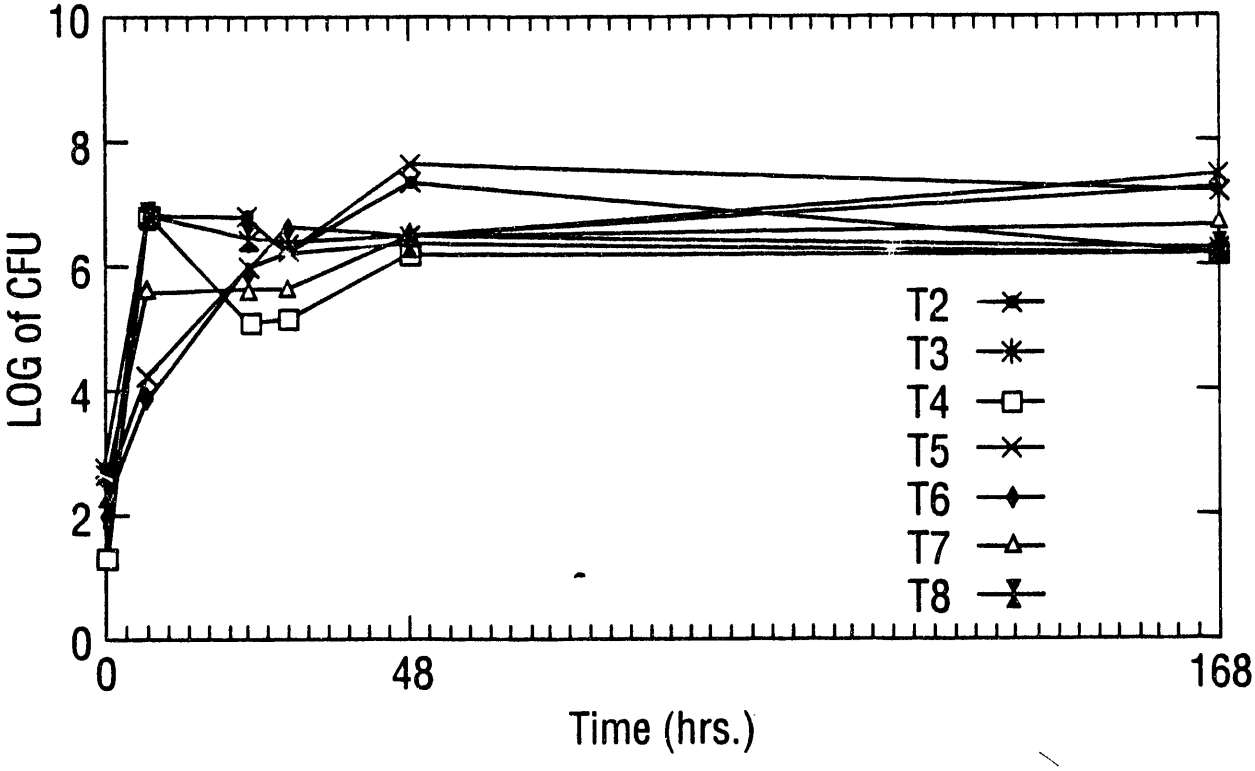


Figure 6. The kinetics of growth at 50°C of bacteria from study area II.

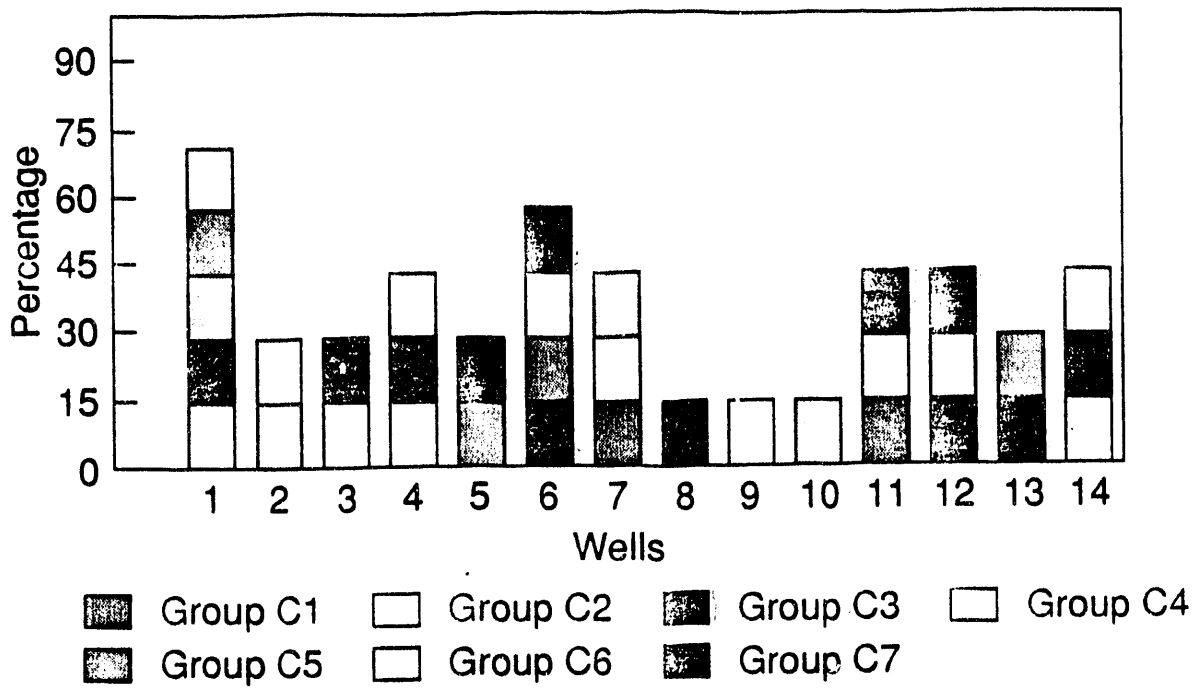


Figure 7. The appearance of different bacterial groups in the 14 wells of study area I.

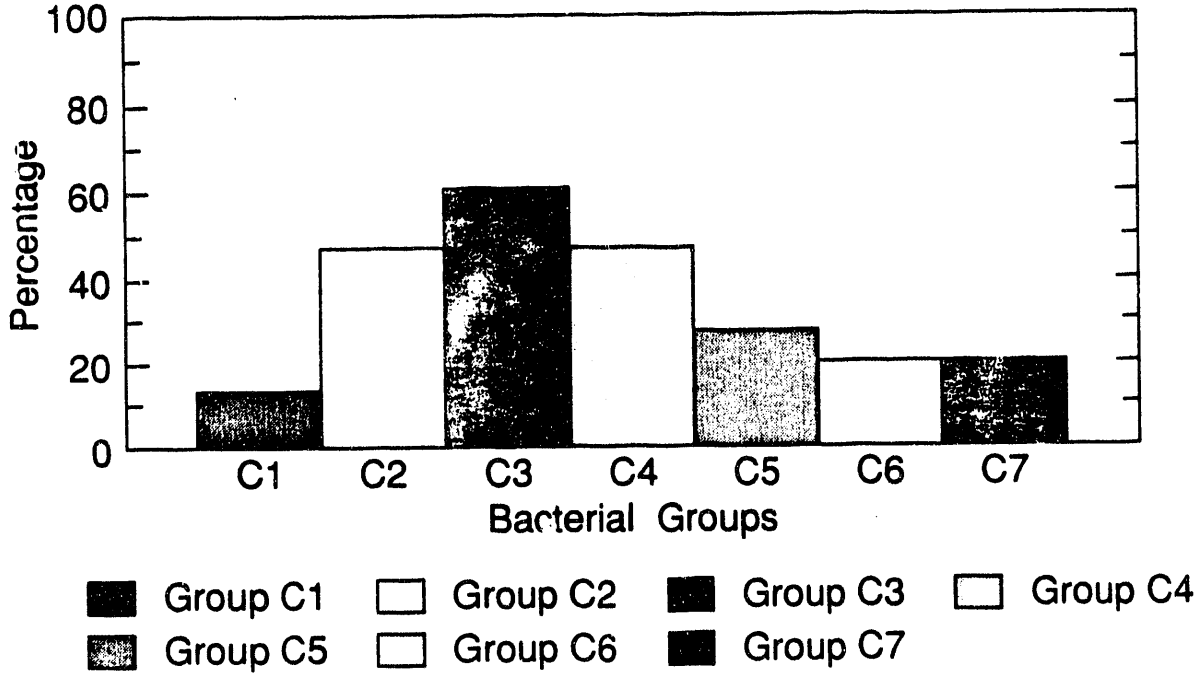


Figure 8. The frequency of the appearance of different bacterial groups in the wells of study area I.

Tests of growth were performed on each functional group, using starch as a unique energy source, this compound, a carbohydrate formed by dextrines from three maltose molecules, is quickly degraded by microorganisms. By using an iodine solution, the presence of starch could be detected in the culture media used in growth experiments. The results of kinetic growth studies showed that starch was quickly used by all the functional groups with the levels of starch exhausted within 30 to 48 hours, although the population of bacteria remained stable. This result suggests that there is not absolute dependence on the carbon source; it is utilized only to activate bacterial metabolism. The most important factor seems to be the presence of mineral substrates, which suggests that the microorganisms are more likely to be lithotrophes rather than organotrophes.

It is interesting to note that these microorganisms, that do not depend on organic substrates, represent a significiant alternative to be used in EOR. The results from [10] on the degradation of light crude oil by bacteria isolated from formation waters, indicated that the supply of organic chemical compounds in the environment prevents bacterial attack to the crude oil paraffins, but allows their growth and metabolism in the presence of crudes.

Analysis of changes in surface tension in filtered culture free of bacteria, after 168 hours of culture, showed a drastic reduction of surface activity in comparison with control samples.

Taking into account the results of the statistical clustering analysis, we conclude that there are several bacterial groups present in different oil wells with a high distribution frequency, such as the C3 group that appeared in nine oil wells, followed by the C2 and C4 groups, present in seven oil wells. However, these three groups are coincident only in three out of the 15 oil wells under study, which suggests that probably there is a similarity in origin of the bacteria associated with the formation waters of these three oil wells. These differences in the frequency of bacterial groups suggest that the wells which have similar bacterial groups probably have similar formation water.

Further detailed studies, taking into account these characteristics and similarities, could allow the design of ecological models to follow established predictions about the characteristics of the reservoirs.

6. CONCLUSIONS

All the bacterial groups studied were facultative aerobes-anaerobes, and all bacterial colony formation units showed rapid growth at 50°C. We observed optimum utilization of starch as a carbon source, and the production of organic compounds such as surface-actives, acids, and alcohols was recorded.

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A Nutrient Control Process for Microbially Enhanced Oil Recovery Applications

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Abstract

When applying MEOR in a waterflooded oil reservoir, it is desirable to stimulate the required biological effect (i.e., biosurfactant production, selective plugging or gas production) distal from the wellbore, thereby enhancing areal and vertical sweep efficiency. Current methodology of injecting a complete growth media (e.g. molasses) allows excessive use of the nutrients near the wellbore, which can result in propagation of inhibitory end-products or incomplete nutrients downstream, causing plugging in the vicinity of the wellbore. In addition, the reservoir acts as a chromatographic column which can selectively retain required nutrients near the wellbore. Phillips Petroleum Company (PPCo) developed a nutrient injection process that takes advantage of the chromatographic separation of nutrients to lessen the effects of microbial growth near the wellbore. The process requires the identification of those nutrients which are not present in the injection brine and which limit the desired metabolic activity (e.g. nitrogen, phosphorus, carbon). Once these nutrients have been identified, their retentive characteristics are determined and they are injected sequentially in order of their decreasing quantitative formation retainability (QFR). This procedure allows the nutrients to combine at depth at which point a complete medium is generated and the indigenous or injected microorganisms can affect the desired activity. A one-dimensional, time-dependent, adsorption-desorption, mathematical model developed at PPCo. was used to determine retentive characteristics from laboratory core results.

One such nutrient control process has been designed for use at the North Burbank Unit (NBU) operated by PPCo. The process is designed to selectively plug high permeability zones, thereby increasing sweep efficiency. A two-dimensional version of the time-dependent adsorption-desorption model was used to design the injection protocol (i.e., slug size and concentrations). A single injector pilot is underway to test the efficacy of this process in establishing plugging distal to the wellbore.

1. INTRODUCTION

In the design of a microbially enhanced oil recovery (MEOR) process, microorganisms and nutrients are injected into a reservoir to stimulate the *in situ* production of chemicals (e.g. biopolymer, biosurfactants, acid or gas) that can be used to recover oil remaining in the reservoir. It was suggested that as an alternative to injecting microbes, indigenous microorganisms can be stimulated by the injection of nutrients alone [1,2]. Nutrients currently used in MEOR processes are predominantly molasses and corn syrup products which are inexpensive, readily available, and environmentally safe. Recently, urea was proposed as a nutrient in a novel biomineralization process [3].

Although widely used, molasses has several disadvantages for MEOR. Since molasses is a waste product of the refining of sugar cane or sugar beets, its chemical composition is variable and undefined. Consequently, the predictability and consistency of the expected biological products decreases and also it is difficult to predict the transportability and utilization of these undefined

nutrients at depth in the reservoir. In addition, molasses contains suspended particles (i.e. fibers, plant debris) that could reduce injectivity into the formation [4,5].

Other processes that use more compositionally uniform nutrients, such as corn syrups, sucrose, glucose, or urea are usually injected in combination with an ammonium and phosphorus source. Ammonium and phosphorus are often amended to oil field brines to provide a complete growth medium. However, the potential disadvantages of injecting a complete growth medium are twofold: 1) a complete medium will stimulate microbial growth and metabolism immediately upon injection (i.e. near the wellbore) thus inhibiting in depth penetration of nutrients, and 2) preparation of a complete medium will permit the aboveground feedstocks to deteriorate while waiting to be injected.

The process described in this paper attempts to overcome these problems through sequential injection of the individual nutrients comprising a complete growth medium [6]. Furthermore, these nutrients are injected in order of their decreasing QFR, thereby, using the differential chromatographic separation by the reservoir of the various nutrients (i.e. phosphorus, nitrogen, carbon). Hence, individual nutrients propagate in the formation at different velocities and combine at depth. Process steps are described which include the use of one- and two-dimensional models, based on the time-dependent, adsorption-desorption kinetics of nutrients, to design the injection protocol.

2. CONCEPTUAL MODEL

Conventional process: During a conventional MEOR process, a complete growth medium such as molasses, which contains all the major nutrients necessary for microbial growth including carbon, nitrogen, and phosphorus, is injected into the reservoir to feed either indigenous or injected microbes (Figure 1A). Because the medium is complete, once it reaches the wellbore region it immediately stimulates microbial growth and metabolism. In fact, the potential exists for microbial growth and metabolism of molasses above ground, although microbial growth is partially inhibited by the high osmotic pressure of the concentrated sugar solution. However, once this concentrate is diluted in the injection stream, conditions in and near the wellbore become amenable for bacterial growth and metabolism.

In a reservoir containing layers or zones of differing permeability, a major problem arises when the water used to displace the oil to the producer begins to channel through the high permeability layers producing large volumes of water. Then, it is desirable to find ways to plug off these watered-out sections and redirect water flow into lower permeability regions with higher oil saturations. One way to do this would be to promote adequate microbial growth and polymer formation in the high permeability, watered-out zones. However, if growth and polymer production is initiated at or near the wellbore, then, due to depletion of nutrients at this point, the plug will not penetrate far (Figure 1B). In addition, this shallow zone of growth can act as a sink to prevent the subsequent propagation of additional nutrients deep into the reservoir. Assuming some cross flow exists between layers of differing permeability, the flow will be diverted only near the well-bore; consequently, once the plug is bypassed, flow will return to the high permeability zone (Figure 1C). Since flow in a reservoir occurs radially, the potential target or volume of bypassed oil increases as the square of the distance from the wellbore. Therefore, a process which forms a plug near the wellbore will not recover as much oil as one which creates a plug deeper in the formation.

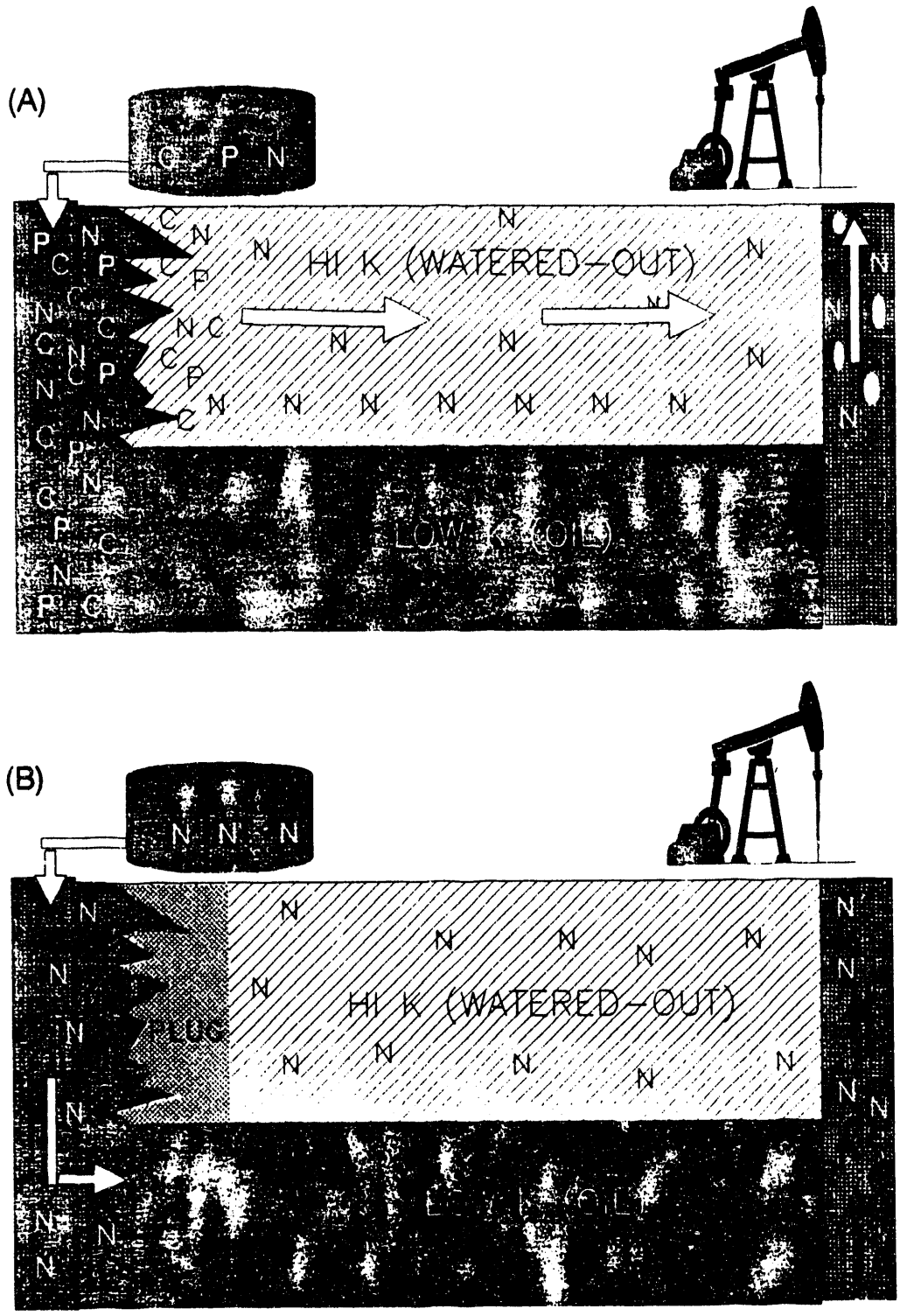


Figure 1. Injection of a complete growth medium (molasses) into a reservoir; C, carbon source; P, phosphorus source; and N, nitrogen source. (A) complete nutrients injected into high permeability (Hi K) layer already equilibrated with a nitrogen source; (B) plug (biomass) forms near the wellbore soon after complete nutrients are injected, thereby diverting subsequent water injection into the low permeability (low K) layer to recover oil, (C) since plug does not form in-depth, water drive bypasses around it leaving a substantial target of oil unrecovered.

Nutrient control process: One way to eliminate microbial growth and metabolism of injected nutrients is to leave out at least one nutrient necessary for microbial growth. Since microbial growth requires the presence of a usable carbon, nitrogen, and phosphorus source, it is desirable to determine which of these nutrient(s) are deficient in the reservoir fluids. In many cases, the nutrients limiting microbial growth and metabolism will be a source of carbon and/or phosphorus. Many connate brines and injection waters already contain ample amounts of nitrogen as ammonium ions [7]. In some cases, it may be desirable also to add an oxidizing agent, such as nitrate or oxygen.

Next, it is necessary to determine the differential chromatographic separation of the nutrients to be injected and then rank the nutrients in order of their increasing retentiveness or their QFR. Those nutrients which are retained the most (e.g. phosphorus) are injected first (Figure 2A), followed by the injection of brine to mobilize the phosphorus, which undergoes continuous adsorption and desorption (Figure 2B). Then, the next most retained nutrient is injected (e.g. carbon). Since it is retained less than phosphorus, it will eventually combine with the phosphorus distal from the face of the wellbore (Figure 2C). Assuming that nitrogen was already present in the reservoir, a complete growth medium now exists deep in the reservoir. Now, a biological plug can form at depth and divert the flow of brine into the stratum of lower permeability (Figure 2D). Most of the recovered oil should come from lower permeability zones in the approximate area of the plug; however, some flow may invade areas proximinal to this plug. Due to the larger target of oil-in-place at this distal point in the reservoir vs. near the wellbore, higher incremental oil recoveries should be obtained.

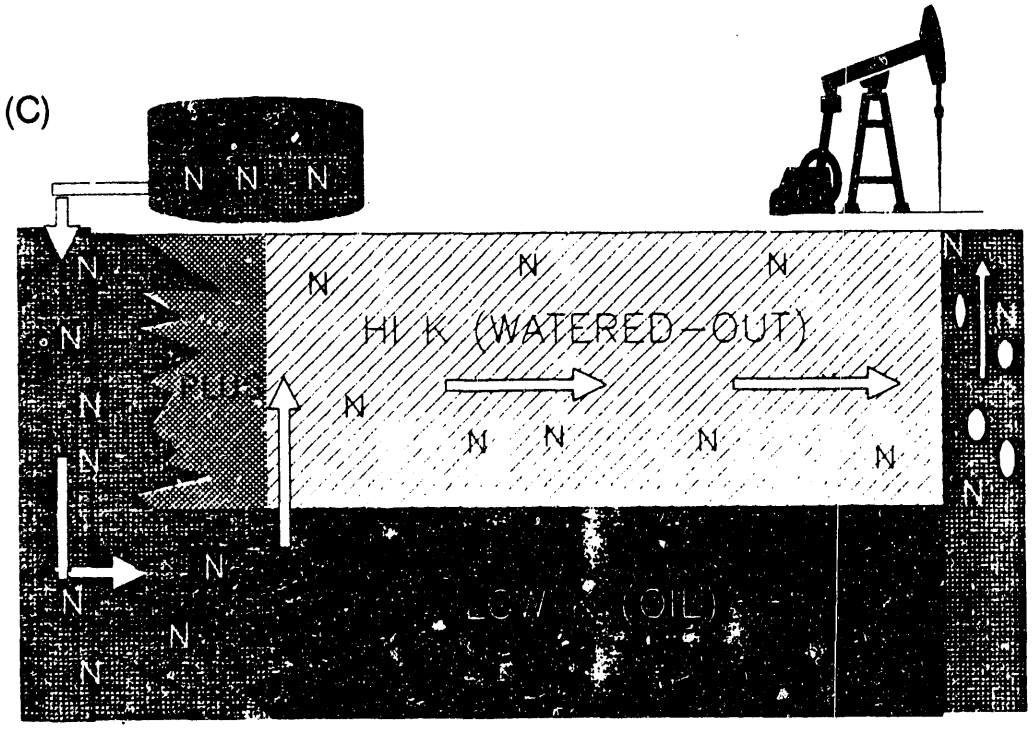


Figure 1C. See Figure 1 for details.

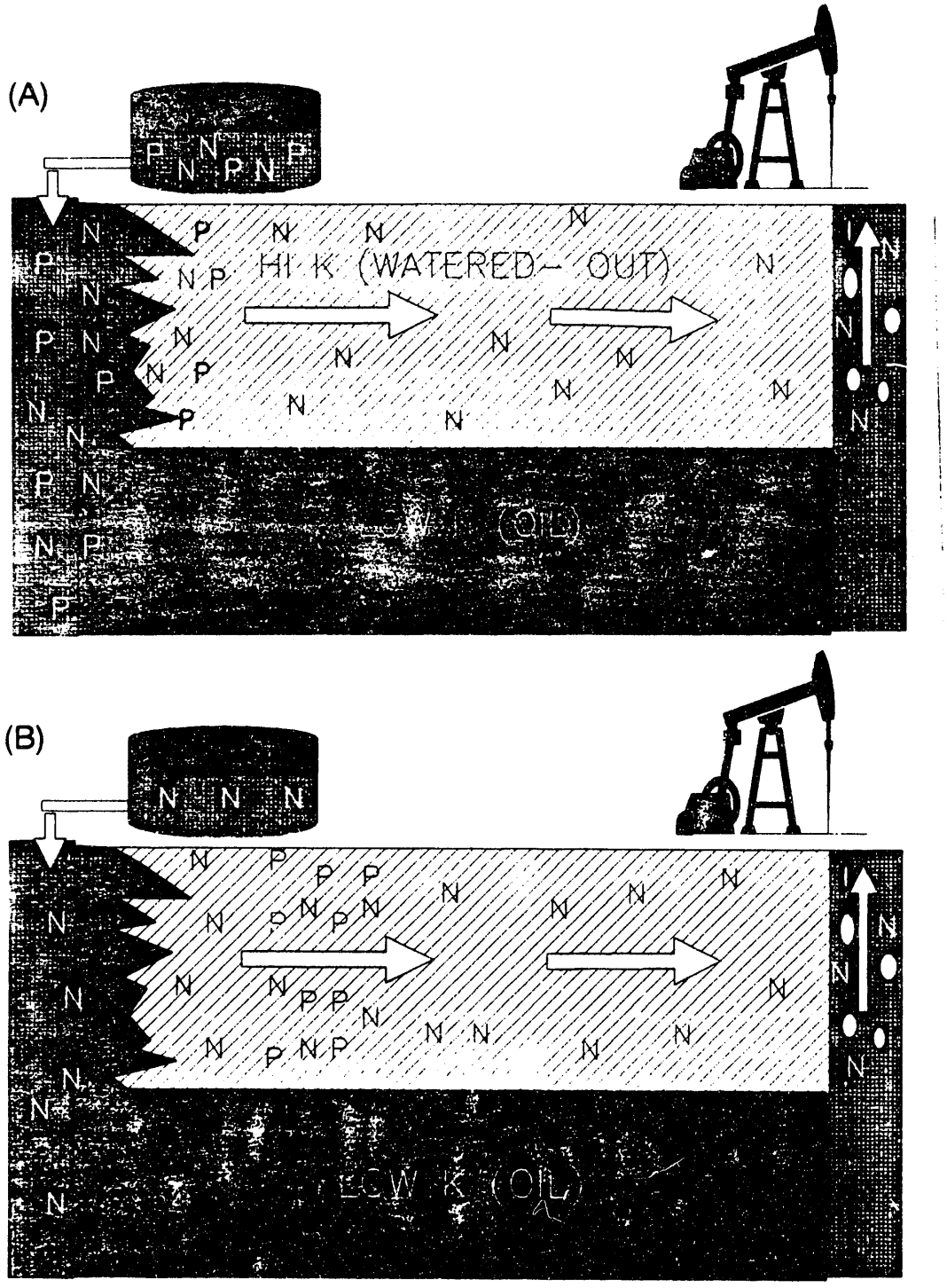


Figure 2. Injection of incomplete growth medium (nutrient control method) into a reservoir to stimulate in-depth plugging. (A) Phosphorus source(P), largest relative QFR, injected first into Hi K layer already equilibrated with a nitrogen source(N); (B) water injected to propagate the phosphorus slug in-depth; (C) carbon source(C), lowest relative QFR, injected last into Hi K layer; (D) carbon source combines with phosphorus and nitrogen in-depth to form a complete growth medium stimulating biomass buildup and plug formation. Subsequent water is diverted to Low K layer to recover oil.

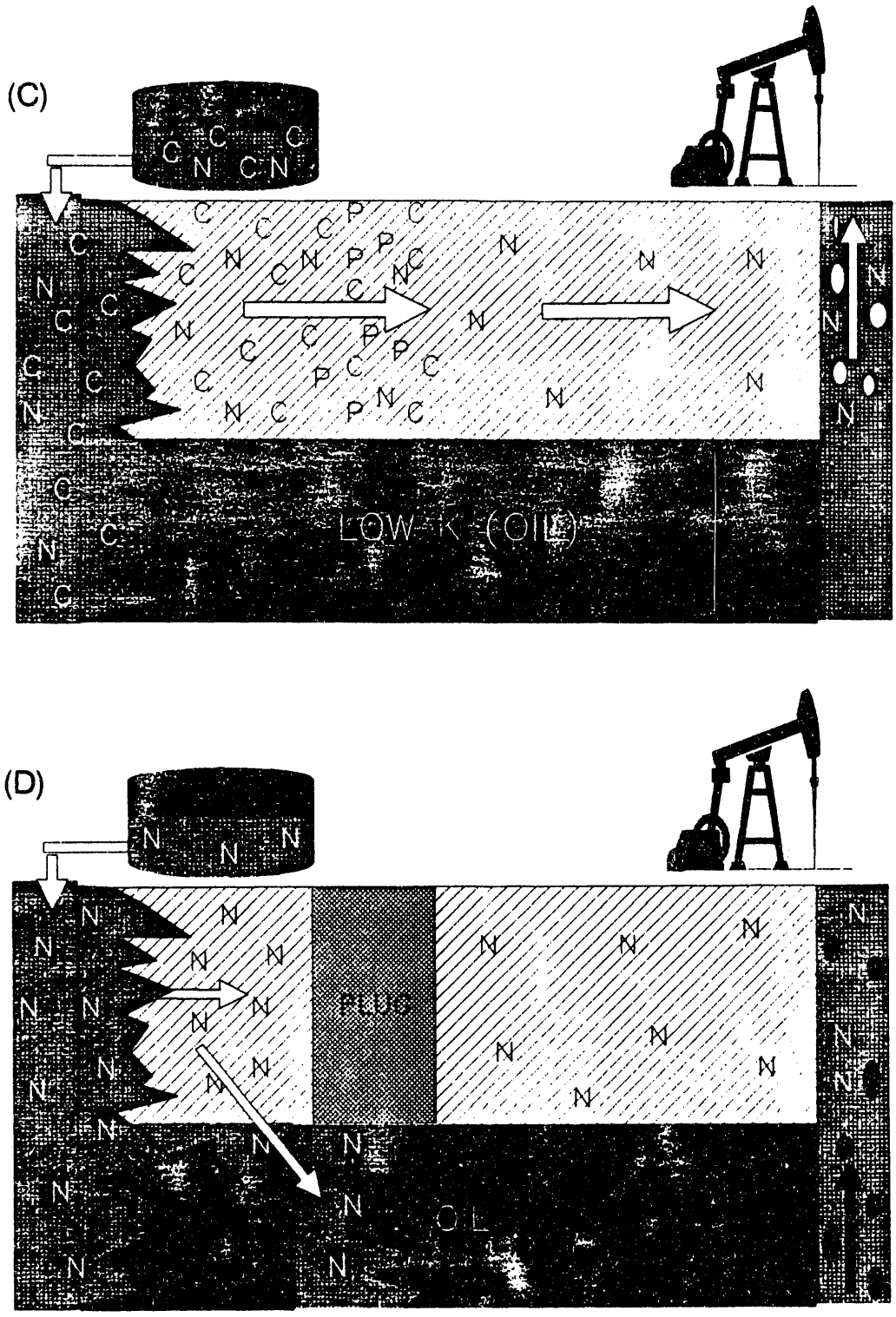


Figure 2C and D. See Figure 2 for details.

3. METHODS

3.1. Quantitative formation retainability (QFR)

Breakthrough curve analysis: Core plugs, 2.54 diameter x 7.6 cm long, were drilled from a 10.2 cm diameter core obtained from the North Burbank Field, Osage County, Oklahoma. Core plugs were drilled with a hollow core bit along the bedding plane. Cores were cleaned in a Soxhlet extractor using alternating toluene and methanol washes until all oil was removed. Taped Ryton end-plates were cemented to the ends of the cores with epoxy after they had been squared off with a diamond blade saw. A small space (approx. 0.01mm) was left between the ends of the core and the end plates to allow radial flow across the face of the core. Cores were then covered with epoxy along the sides and allowed to dry. The cores were vacuum-saturated with filtered brine collected from a North Burbank tank battery. This brine was collected in bottles containing oxygen-free gas (85% nitrogen, 10% hydrogen and 5% carbon dioxide) and transported back to the laboratory the same day. The brine was filtered via a 0.22 um cellulose acetate membrane filter into a sterile container housed inside a Coy anaerobic hood.

Cores were then injected with Burbank crude oil which had been cleaned by filtration and centrifugation, until irreducible water saturation was reached. More filtered brine was injected at a velocity of 21 ft/d until no more residual oil was displaced. Then, the inlet end of the core was fitted with 1/8 inch nylon tubing and the outlet end with 1/16 inch tubing. The core was placed inside an incubator (45° C) which was housed inside a Coy anaerobic glove box. The core was mounted vertically with the outlet side up. Filtered North Burbank brine was circulated through the core at a near reservoir velocity of approximately 1 ft/d with a Gilson Minipuls peristaltic pump for at least 24 hours to equilibrate the brine to the temperature and conditions inside the glove box.

A known amount of a carbon or phosphorus source was dissolved in filtered North Burbank brine, and then this solution (20 - 25 pore volumes) was pumped through the core. Effluent fractions were collected in pre-weighed glass test tubes (16 X 100mm). Then, the adsorbate was desorbed by injecting 5 to 10 pore volumes of filtered Tract 5 brine at the same velocity. The effluent fractions for both the adsorption and desorption phases of the test were weighed and the volume in each tube calculated by the difference in weight (i.e. full tube minus empty tube) divided by the density of the brine. The concentration of carbon or phosphorus for each tube was determined by inductively coupled plasma for phosphorus and glucose oxidase (Sigma Chemical Company, St. Louis) for carbohydrate-carbon. The results were plotted as the concentration in the effluent versus the pore volumes injected. These results were then entered into the linear, one-dimensional, time-dependent, adsorption-desorption model to generate sorption isotherm parameters.

4. RESULTS AND DISCUSSION

4.1. Process steps

1). **Determine limiting nutrients:** The first step is to determine which nutrient(s) is(are) deficient in the injection brine to attain the desired effect. For example, if permeability must be reduced to improve sweep efficiency of a reservoir, types and sufficient amounts of nutrients should be selected to stimulate the generation of large amounts of cells and polymer (i.e. soluble or insoluble). Any nutrient selected could provide more than one of the limiting nutrients; however, no one nutrient should provide a sufficient amount of all the

required nutrients. Otherwise, a complete nutrient medium would be injected, defeating the aim of this process. Likewise, instead of a nutrient it could be determined whether the proper microorganism(s) is(are) the limiting parameter required to attain the desired effect.

The method by which the limiting nutrient or microbe is determined is through enrichment culture or chemical analysis of the water. In the former case, water from the injector to be treated is sampled and nutrients are systematically screened to determine which are missing. The desired effect depends on the type of recovery mechanism anticipated under the field conditions. In the latter method, a chemical analysis of the water for known limiting nutrients together with experience determines which nutrient(s) is(are) limiting. Certainly the latter method requires a great deal of experience or good fortune since such small amounts of some nutrients (e.g. sulfur, iron, or vitamins) may be required that it would be difficult to determine if they are limiting for growth without experimentation.

Once the limiting nutrient(s) is(are) determined the process can be optimized by screening other potential sources of limiting nutrients. Furthermore, the optimal concentration of each nutrient must be determined.

2). **Determine QFRs of limiting nutrients**: Once the limiting nutrient(s) have been identified, it is necessary to determine their quantitative formation retainability. This is most easily accomplished by breakthrough curve analysis of the selected nutrients (see methods). It is suggested an actual core from

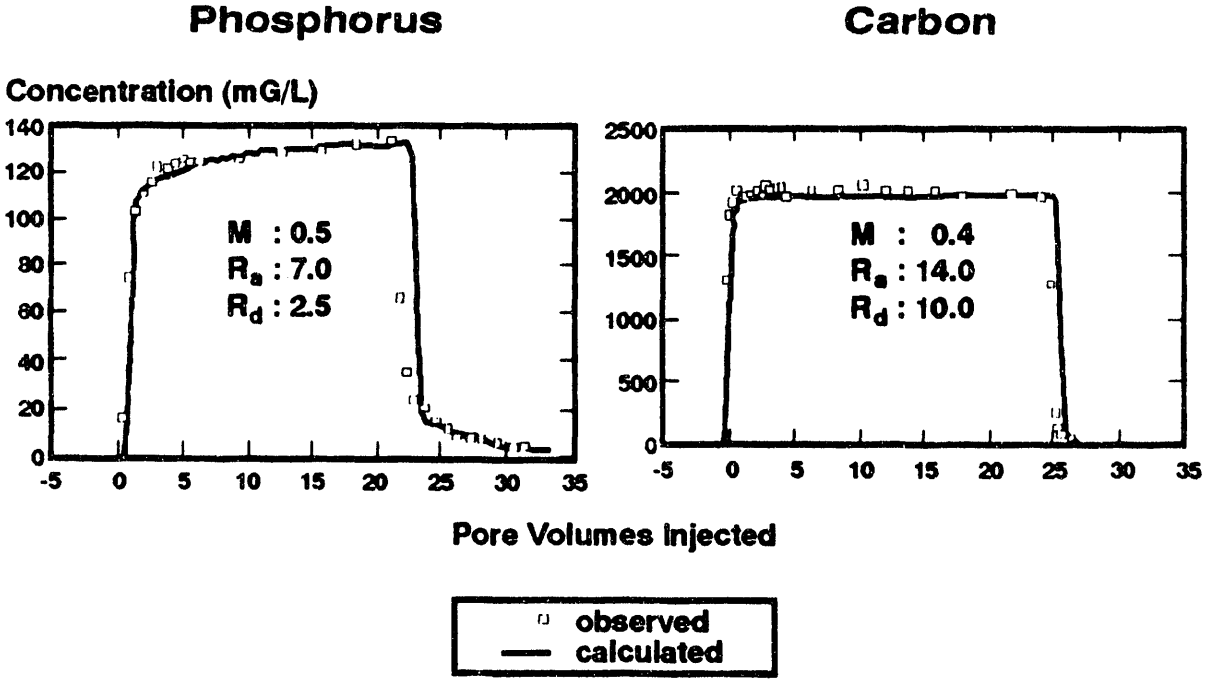


Figure 3. Breakthrough curve analysis of carbon and phosphorus sources for determination of QFR. Concentration of phosphorus (A) and carbon (B) in core effluents. (□) observed values; (-) calculated curve as determined by the one dimensional linear model. M = isotherm parameter, R_a = adsorption rate coefficient, and R_d = desorption rate coefficient.

the reservoir is analyzed under as many in-situ environmental conditions (e.g. pH, temperature, pressure, and salinity) as possible. Also the cores should be analyzed in the presence of oil to account for any nutrient retention due to phase separation. In addition, replicates should be run to account for variability in the results.

The results of the breakthrough curve analysis should show the concentration of the nutrients in the core effluent versus pore volumes injected. These results will provide partial input for a time-dependent adsorption/desorption model based on the Freundlich isotherm equation:

$$c_s = b\, c^M \qquad (1)$$

where b and M are isotherm parameters, c_s is concentration of absorbate on the surface, and c is concentration of absorbate in solution. The derivative with time of equation 1 is:

$$\frac{dc_s}{dt} = R_a - R_d c_s \qquad (2)$$

where R_a and R_d are the adsorption and desorption rate coefficients, respectively.

A computer program for a one-dimensional, linear or a two-dimensional radial model, designed by Carl Holloway, Phillips Petroleum Co., was used to simulate the coreflood results. The model assumed: 1) a single phase flow; 2) pressure and velocity distribution in steady state; 3) compressibility is ignored; 4) homogeneous adsorption in the core; and 5) the Freundlich isotherm describes the appropriate adsorption behavior. Input for the model includes: 1) core geometrical data; 2) isotherm parameters; 3) rate and duration of adsorption and desorption steps; and 4) run and plot control specifications.

Figure 3 is an example of the output produced by this linear model. Both observed and calculated (computer model) data for the concentration of limiting nutrients in the effluent (e.g. phosphorus and carbon) are plotted versus pore volumes injected. A best match of the calculated curve to the observed data is achieved by systematically varying values for the three input parameters: M, R_a, and R_d. The resulting parameters of M, R_a, and R_d are used as input for the two-dimensional version of the same model and the output of this model is used to design the injection protocol (see below).

3). **Design injection protocol**: After the adsorption/desorption parameters were determined by history matching the linear coreflood results, the time-dependent, adsorption model was used in the two-dimensional radial mode to design the injection protocol for the MEOR pilot injection test. The radial model made the same assumptions on flow and adsorption as the 1-D linear model. The reservoir description for the 2-D model was developed by history matching the waterflood production history of the MEOR pilot area in the North Burbank Unit (Table 1). The results of designing the injection concentrations and sequence are given below: 1) sequence with no brine spacer between the injected nutrient slugs (Figure 4), and 2) a sequence with brine spacers between the nutrient slugs (Figure 5) so the nutrients propagate in depth before they are available for microbial growth and biomass generation. The flowing concentration profiles shown in the plots are for the highest permeability layer as this is the layer in which most of the plugging was designed to occur.

Figure 4 (A-E) shows the propagation of the carbon and phosphorus slugs as they move radially away from the wellbore. Due to its slower rate of propagation

through the reservoir, a 50 mg/L slug of phosphorus is injected first for ten days, followed by a 4,000 mg/L slug of the carbon source for an additional ten days. For this case no brine spacer was placed between the two slugs. Figures 4A through 4E give the subsequent flowing concentration profiles of carbon and phosphorus. Figure 4A shows that after 10 days, only phosphorus has entered the reservoir while Figure 4B shows that after 20 days carbon has also entered the formation and both nutrients are present in a radial area approximately 50 to 150 feet from the wellbore. Figure 4C shows that, after 30 days the carbon slug has

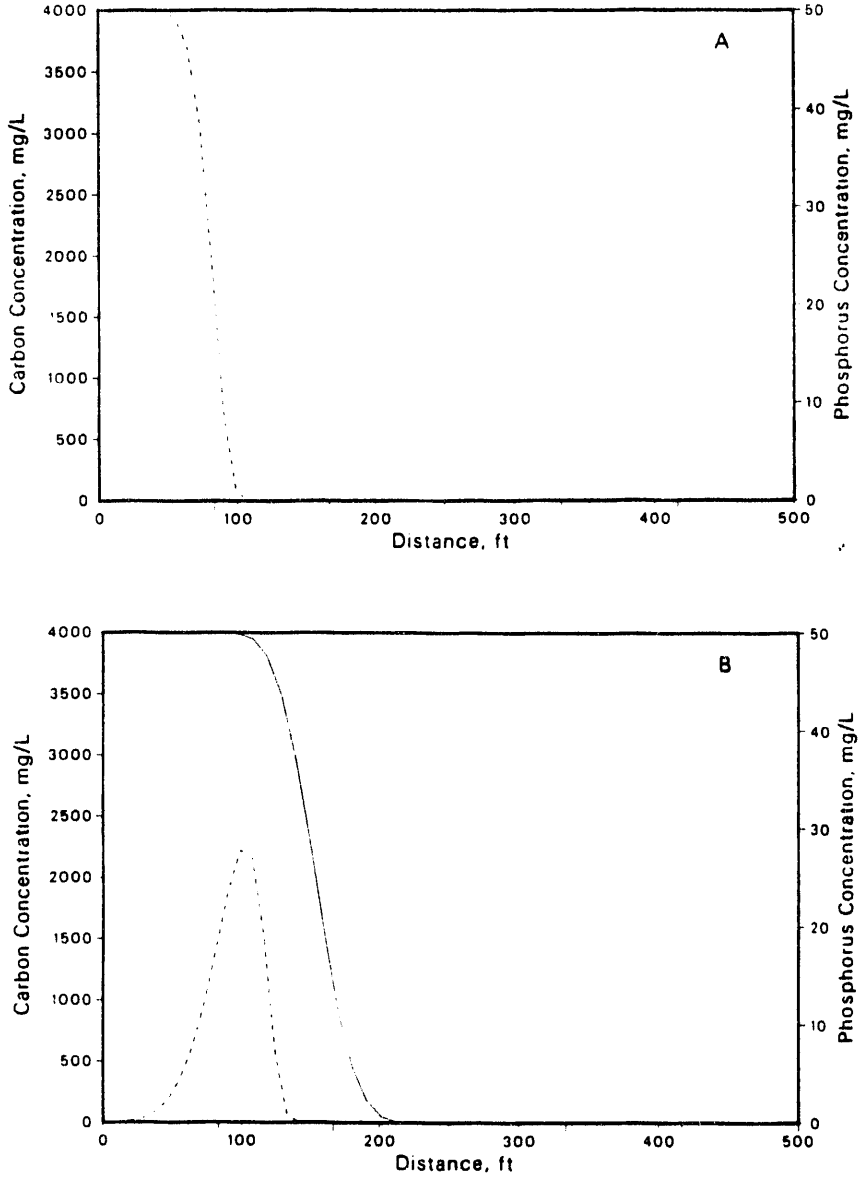


Figure 4. Output for the two-dimensional model where phosphorus (--) and carbon (-) are injected without a brine spacer. Flowing concentrations of phosphorus and carbon are shown for highest permeability layer only (see Table 1). The concentration vs. distance from the wellbore for the phosphorus and carbon slugs is shown at: A) 10 days, B) 20 days, C) 30 days, D) 50 days, and E) 70 days.

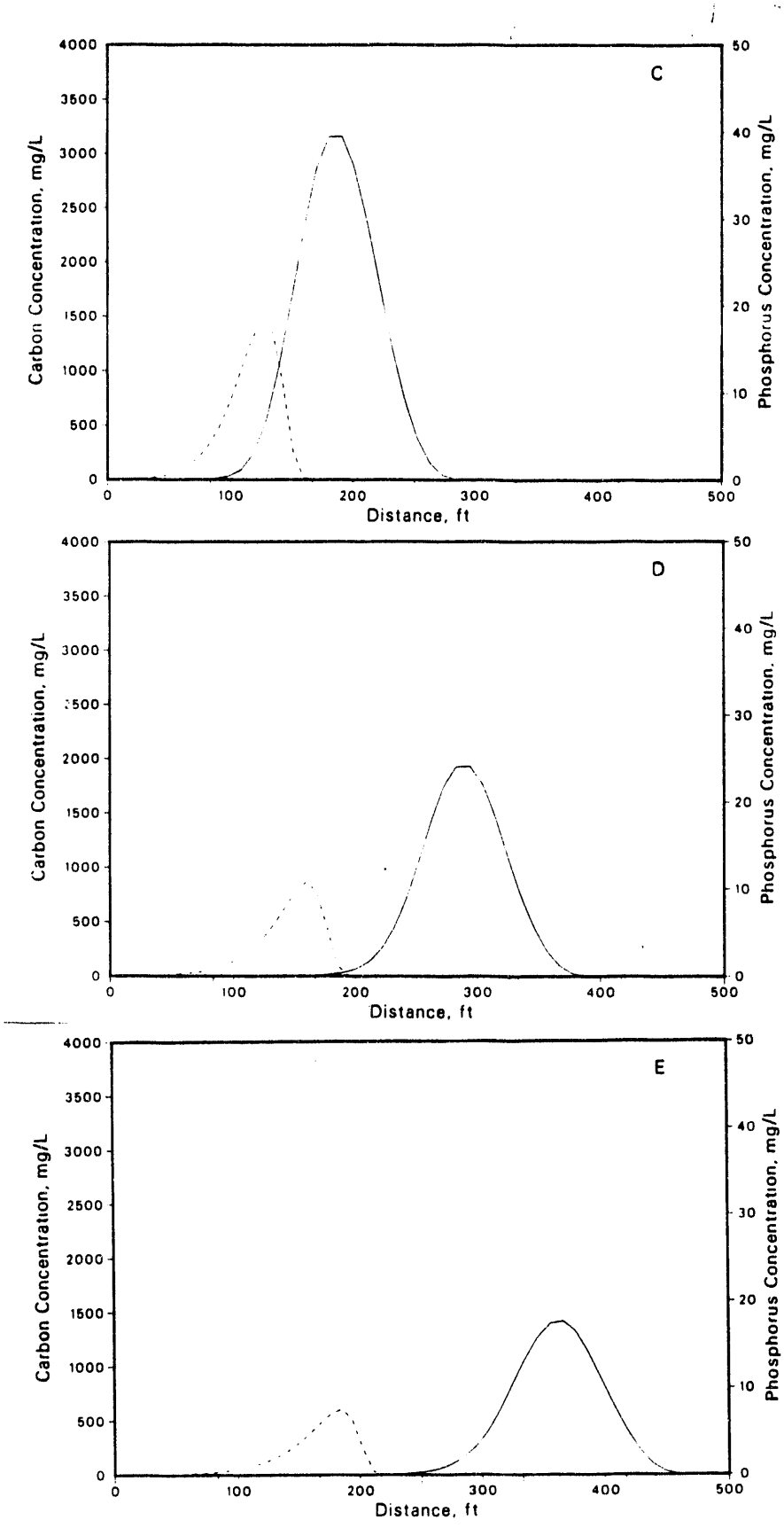


Figure 4C-E. See Figure 4 for details.

Table 1
2-D case - reservoir description

Layer	h,ft.	k_h, md.	k_v, md.	ϕ fraction
1	2.0	13.5	0.135	0.160
2	3.78	150.0	1.5	0.234
3	5.7	665.4	6.65	0.264
4	1.0	24.0	0.24	0.181
5	11.0	2.9	0.029	0.138
	23.48			

Average ϕ = 0.188

moved on past the phosphorus slug due to its lower retention in the reservoir, while Figures 4D and 4E show the separation with time between the carbon and phosphorus slugs. The flowing injection profiles shown in Figures 4A through 4E indicate that plugging due to biomass generation will occur primarily in the near wellbore region, with little plugging occurring distal to the wellbore due to the more rapid propagation of the carbon in the reservoir and consequent separation of the nutrient slugs.

Figure 5 (A-E) shows a similar case except that it was designed using brine spacers between the nutrient slugs. A 50 mg/L phosphorus slug was injected for ten days followed by 20 days of brine. The first carbon slug (4,000 mg/L) was then injected followed by ten days of brine and then another carbon slug for 5 days. The brine spacers allow the nutrients to penetrate deeply into the formation before being utilized for microbial growth and biopolymer generation. In addition, brine spacers allow dilution of metabolic end-products (e.g. acids and alcohols) that can inhibit microbial growth. Figure 5A shows that after 30 days, the phosphorus slug has penetrated almost 100 to 200 feet into the formation. After 35 days (Figure 5B), the first carbon slug is injected and begins to overtake the phosphorus slug. Figure 5C exhibits the flowing concentration profiles at 45 days; almost complete overlap of the carbon and phosphorus slugs occurs at a radial distance of 100 to 200 feet from the wellbore, which is where plugging due to biomass generation would be expected to occur. Figure 5D shows the injection of the second carbon slug after the first carbon slug has started to move on beyond the phosphorus slug, and Figure 5E shows the merging of the two carbon slugs as they move past the phosphorus slug at 70 days after injection. This time-dependent adsorption model does not take into account the consumption of the nutrients by the microbes, but it gives a qualitative indication of the propagation of the nutrients slugs in the formation. The figures as given above only show the flowing concentration profiles for the highest permeability layer; the same plots can also be generated for the other four layers in the reservoir model.

5. CONCLUSIONS

1. A nutrient control process was developed to achieve MEOR in-depth through the injection of nutrients limiting to growth and metabolism. Nutrients are injected individually and, therefore, do not comprise a complete growth medium until they combine deep into the reservoir.

2. Limiting nutrients are added in order of decreasing quantitative formation retainability (QFR); that is, nutrients that are retained the most by the formation are injected first, and those that are retained the least are injected last.

3. Methods have been described to identify limiting nutrients and QFR.

4. A one-dimensional linear model and a two-dimensional radial model, based on time-dependent adsorption/desorption of nutrients, is used to model laboratory and reservoir data to design a field injection protocol.

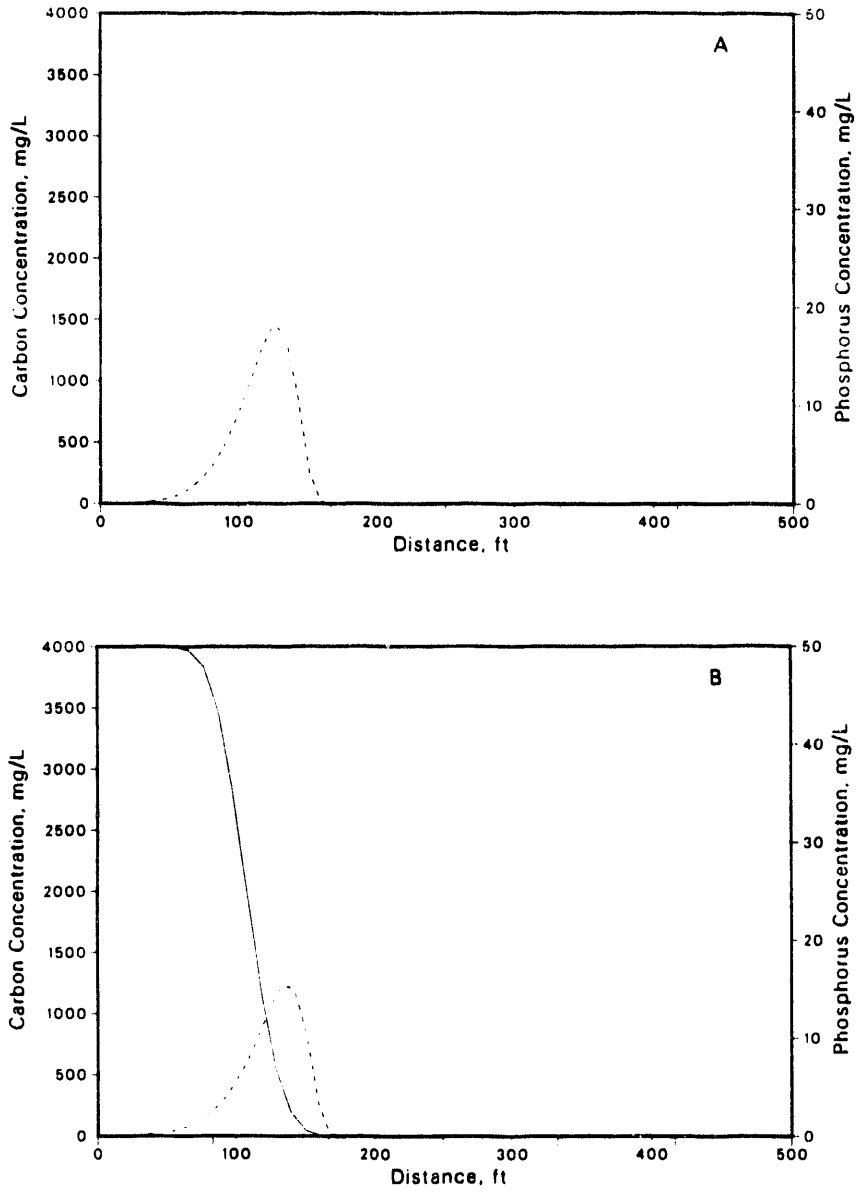


Figure 5. Output for the two-dimensional model where phosphorus (--) and carbon (-) are injected with a 10 day brine spacer. The concentration vs. distance from the wellbore for the carbon and phosphorus slugs is shown at: A) 30 days, B) 35 days, C) 45 days, D) 50 days, and E) 70 days. See Figure 4 for additional information.

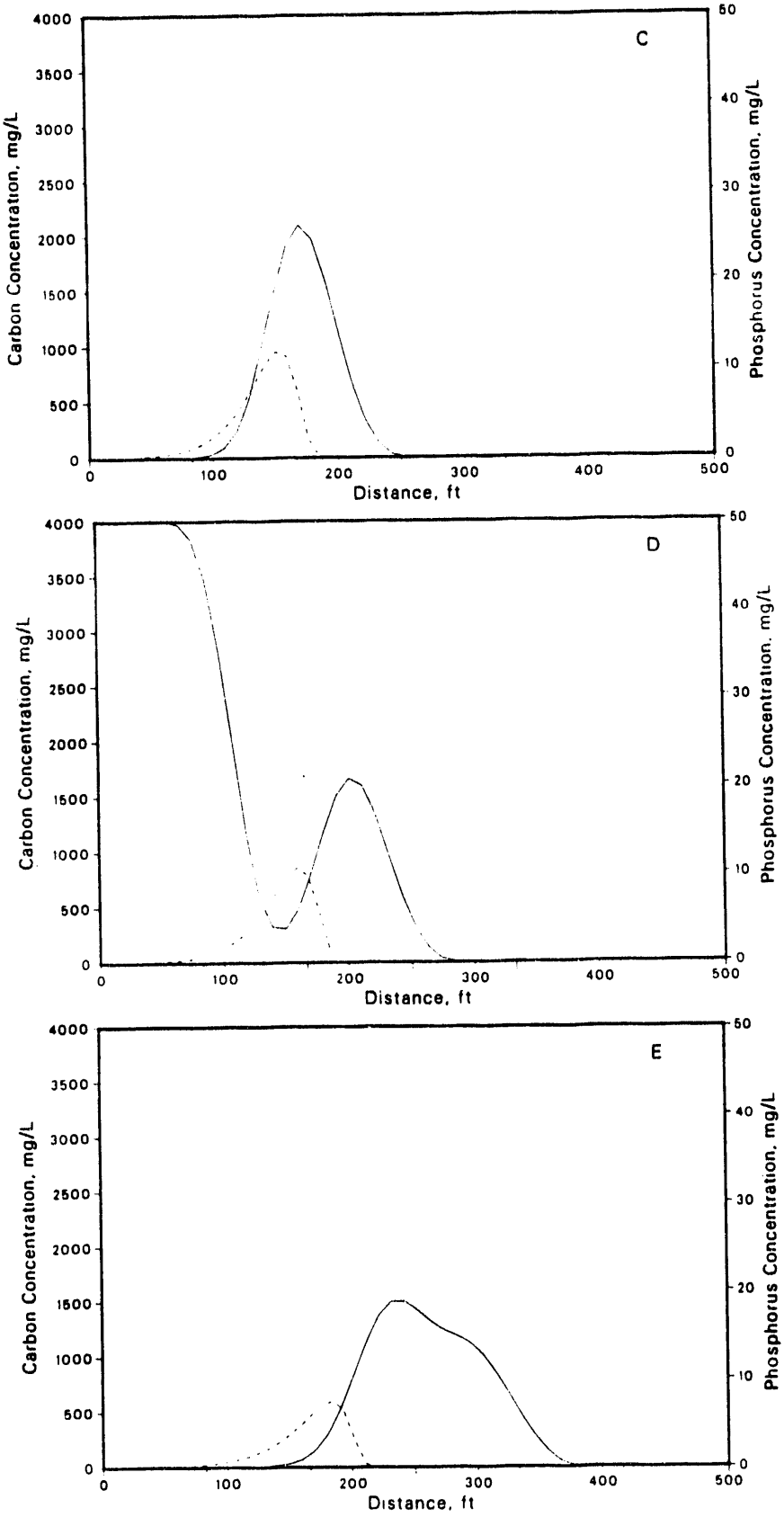


Figure 5 C-E. See Figure 5 for details.

6. ACKNOWLEDGEMENTS

The authors acknowledge the help of Carl Holloway who wrote the one- and two-dimensional computer models. Technical assistance was provided by Jim Stevens. Allen Rainer and Sheryl Baughman assisted with figures and illustrations. The authors appreciate the support of Phillips Petroleum Company, Bartlesville, OK, for allowing this work to be presented.

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Characteristics of Enriched Cultures and their Application to MEOR Field Tests*

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Abstract

Three enriched cultures (5GA, 26A, and 6A) selected from 86 environmental samples could grow anaerobically in the presence of crude oil at 45°C and ferment molasses to gasses, and organic acids. The oil displacement efficiencies of the cultures were evaluated in laboratory scale model experiments. Oil recovery was increased by about 11 percent of the original reserves by culture 5GA. This culture was mixed with Daqing strains and used as inoculum for EOR field tests at Daqing Oilfield with satisfactory results.

The enriched culture was composed of at least three species belonging to the genus *Bacteroides*. The importance of the role of these isolates in EOR was confirmed by their persistence and behavior in the fluids produced from the microbially treated reservoir.

1. INTRODUCTION

In situ MEOR technologies are based on the concept that petroleum reservoirs may be transformed into huge bioreactors, where oil displacing agents are produced by stimulating natural reservoir microbiota [1,2] or by injecting allochthonous bacteria into the oil formation [3-5]. Up to now, hundreds of MEOR trials have been performed throughout the world using a variety of bacterial inocula [6-7]. Mixed or enriched cultures mainly were used; however, the use of pure cultures is reported. The efficiency of the inoculum in releasing oil plays a very important role in the success of field programs.

The present paper describes the characteristics of enriched cultures isolated from environmental and petroleum reservoir samples and briefly reports on their application to a single well biostimulation at Daqing Oilfield.

2. MATERIALS AND METHODS

2.1. Samples

Samples were taken from sludges of a bean-curd plant, a waste water treatment tower at Shanghai Second Chemical Fibre Factory, and from oil-contaminated soil or water at various locations, including Daqing Petroleum Refinery, to collect gas- and acid-producing cultures.

Water samples for microbiological analyses were collected from sampling valves directly into sterilized flasks.

2.2. Media, cultivation and identification

Molasses broth (4% molasses, pH 7.0-7.5) was used to isolate anaerobic gas- and acid-producing cultures, unless otherwise stated. Microorganisms possessing vitality in the oil reservoir must be able to resist the toxicity of oil. To select for anaerobic gas- and acid-producing cultures adapted to the reservoir

*In cooperation with the working group of Daqing Oilfield.

conditions, 0.2g or 0.2 ml samples from each source was inoculated into tubes with deep molasses broth and crude oil from Daqing. Tubes with growth were subcultured into fresh media and reincubated to remove transient bacteria. In this way, only organisms able to grow and metabolize in the presence of oil and capable of resisting oil toxicity remained present and active.

Media for bacterial identification is described in Holdeman et al. [8]. All cultures were incubated at 45°C.

2.3. Assays

The enumeration of microorganisms was carried out by the MPN method with five tubes at each dilution. The growth curves were developed spectrophotometerically at 660 nm.

Analyses of gases, organic acids, and alcohols were performed by gas chromatography using a GC-7AG gas chromatograph (Shimadzu) or SC-3A gas chromatograph (Sichuan). Surface tension was determined with a surface tensiometer St-1 (Shimadzu).

3. RESULTS

3.1. Selection of enriched cultures

The characteristics of the reservoir in the area selected for MEOR stimulation are presented in Table 1. These conditions are not extreme for microbial growth, proliferation, and metabolism.

Nineteen enriched cultures, which could grow and ferment molasses to gases and organic acids, were obtained from eighty-six samples. Characteristics of these cultures are shown in Table 2. Overall the pH of the media dropped from 7.0-7.5 to 6.0-5.5, and surface tension from 60 to 55-50 mN/m. H_2S was produced during the initial transfer period by most cultures.

Figure 1 shows the time course of gas production by the three cultures (5GA, 6A, 26A) chosen for further study on the basis of quantity of gas produced. Figure 2 shows their typical gas production. After repeated subculture into pre-reduced molasses broth, H_2S was not produced.

3.2. Effects of some factors on metabolite production

(A) Temperature. Culture 5GA was incubated at different temperatures and gas production was measured. Results showed that the gas production at 60°C was higher than that at 45°C (Figure 3); in contrast total organic acids produced at 45°C was higher than that at 60°C (Table 3).

The broad temperature range of growth (28°-67°C) is favorable for the application of this strain to reservoir trials.

(B) Additives. The viability of microorganisms in crude oils and oil reservoirs can be enhanced by the addition of suitable additives [1,2,5,9]. Table 4 shows that the addition of calcium carbonate, oil sand, and scum into molasses broth had a positive effect on the metabolite production by 5GA and 26A. The addition of scum gave the most 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 oil sand from Daqing. The log phase of 5GA extended from the first day to the 5th day after the inoculation (Figure 4). Of the three additives, the presence of calcium carbonate had the most effect on gas production. From this result, it could be concluded that the carbonate content of an oil-bearing formation is an important factor in MEOR.

Table 1
Reservoir characteristics at MEOR test well site

Depth	1151- 1171 m
Temperature	45°C
Pressure	6.5 - 14 MPa
Permeability	400-920 x 10^{-3} m^2
Oil viscosity (50°C)	58.9 MPa.s
Co-produced water content	11-92 %
Salinity of formation water	7000 mg/L
pH of formation water	8.0

Table 2
Gas-producing enrichment cultures in the presence of Daqing crude oil at 45°C

Culture	Gas (ml)	H_2S	pH	Surface tension (mN/m)
0			7.0-7.5	60
5GA*	28	+	5.5-6.0	51
6A***	30	+	5.5-6.0	52
10A	18	+	5.5-6.0	52
13	20	+	5.5-6.0	53
19	21	+	5.5-6.0	52
20A	25	+	5.5-6.0	52
22B	21	+	5.5-6.0	54
26A**	25	+	5.5-6.0	54
27	9	+		55
28	2	+		55
29	1.5	+		50
H1	14	±		54
H2	17	±		52
H3	10	±		53
H4	6	±		54
D2-1	13	+		52
MA	19	+		55
MB	9	+		55
MC	8	+		55

*5GA: from sludge of a bean-curb plant
**26A: from oil-contaminated soil of Daqing Petroleum Refinery
***6A: from bottom sludge of a waste water treatment tower at the Shanghai Second Chemical Fibre Factory.

3.3. Displacement efficiency of the enriched cultures

The displacement efficiencies of three cultures were evaluated in laboratory scale model experiments. Oil recovery in the study with culture 5GA was increased by about 11% in total, which equates to 34% of the residual reserves. The other cultures increased recovery by about 7% (approximately 20% of residual oil) (Table 5).

Table 3
Effect of temperatures on culture 5GA

	Gaseous phase			Liquid phase				
	Total	content %		Organic acid mg/l				
(°C)	(ml)	H_2	CO_2	Total	C_2	C_3	C_4	C_5
30	6.0	6.6	8.6	1604	-	649	402	553
45	34.0	24.1	44.1	4004	1476	255	2176	87
60	39.5	21.5	42.9	3631	1140	151	2285	55

3.4. Isolation and identification of culture 5GA

The favorable results shown in Section 3.3 indicate that culture 5GA is a suitable culture for the single-well biostimulation tests in Daqing Oilfield. Culture 5GA was isolated and purified for characterization and identification. Three bacterial strains (5G-100, 5G-101, and 5G-102) were obtained by the roll tube method. Their morphological, physiological, and biochemical properties are as follows.

(A) Strain 5G-100. The organism is an obligate anaerobe, Gram-negative, non-motile, non-sporulating rod, 0.6-0.8 x 2-7 mm (Figure 5A). It does not grow in peptone yeast extract (PY) medium, but grows well in peptone yeast extract glucose (PYG) broth and molasses broth. Glucose is fermented to gas, acetate, butyrate, lactate, and minor amounts of valerate. The G+C-content of DNA is 39.3 mol% (Tm). It produces H_2S. Gelatin is not liquefied. Litmus milk is acid curded, coagulated, and reduced.

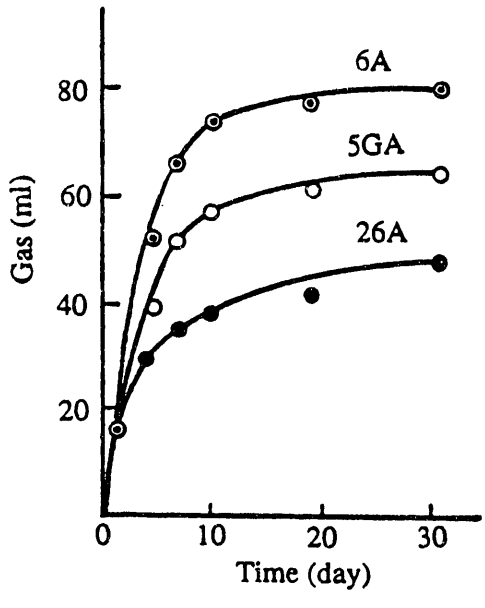


Figure 1. Time course of gas production.

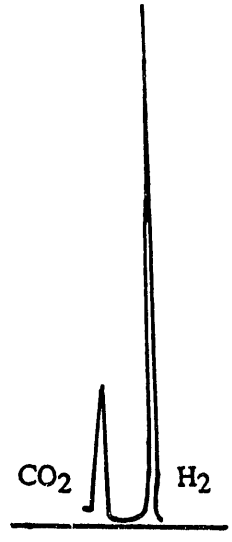


Figure 2. Gas chromatogram of gases produced by culture 5GA.

Table 4
Metabolites of the enriched cultures

Culture	Medium	Gaseous phase				Liquid phase								
		Amount*		Content %			Organic Acid mg/l				Alcohols mg/l			pH
		ml	H_2	CO_2	H_2S	Amount	C_2	C_3	C_4	C_5	Amount	C_2	C_3	
	Molasses	27	29.3	32.4	-	3845	2066	24	1755	-	-	-	-	5.7
5GA	Molasses + $CaCO_3$	30	31.4	34.2	-	4526	2234	21	1907	364	170	158	12	6.2
	Molasses + oil sand	47	35.9	30.6	-	5608	1931	19	3484	173	-	-	-	5.4
	Molasses + scum	125	31.2	37.8	+	12607	4151	53	8392	11	108	108	-	5.4
	Molasses		38.9	8.1	-	833	642	20	160	11	270	142	128	6.0
26A	Molasses + $CaCO_3$	10	35.9	12.6	-	272	170	65	37	-	225	161	64	6.7
	Molasses + oil sand	11	42.2	13.5	-	367	236	38	93	-	231	231	-	6.1
	Molasses + scum	33	42.2	36.9	+	3300	1656	-	1644	-	141	141	-	5.0

* ml from 15 ml medium in the first 5 days

Table 5
MEOR in laboratory model experiments

	Displacement efficiency				MEOR (4 months)				
	Water-free Period		Final						
Test	Oil (ml)	Recovery (%)	Oil (ml)	Recovery (%)	Culture	Oil (ml)	Recovery (%)	Recovery of residual oil (%)	Gas production (ml)
3	245.5	55.8	42.5	9.66	26A	32.0	7.30	20.1	12040
4	188.7	46.59	75.0	18.52	6A	29.0	7.20	21.0	4750
5	243.5	52.37	72.0	15.48	5GA	50.5	11.12	34.6	6500

In molasses broth with or without oil sand, scum, or $CaCO_3$, the fermentation products are H_2, CO_2, acetate, butyrate, propionate, valerate, and ethanol (Table 6). The growth curve (Figure 6) shows that the stationary phase is achieved after 5 days growth. Its time course of gas production was similar to that of the enriched culture 5GA.

(B) Strain 5G-101. The organism is a Gram-negative, motile by peritrichous flagella, non-sporulating rod, 0.6-0.8 x 2-6 mm (Figure 7), obligate anaerobe. The strain grows poorly in PY broth, but grows well in PYG broth and molasses broth. Its fermentation products from glucose are acetate and butyrate. H_2S is produced. Gelatin is not liquefied. Litmus milk is acid curded, coagulated and reduced. The mol% G + C of the DNA is 45.6 (Tm).

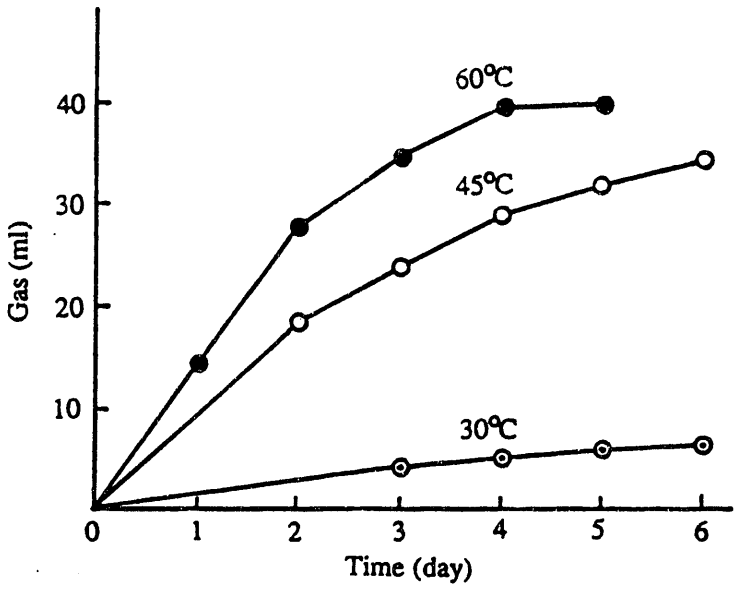


Figure 3. Effect of temperatures on gas production.

In molasses and molasses with oil sand or scum, its fermentation products include short fatty acids (Figure 8), and ethanol, while the products in molasses broth with $CaCO_3$ are acetate, ethanol, and butyrate; however, propionate and valetate were not produced (Table 6). The data in Table 6 and Figure 9 show that the amount of H_2 produced in four different media are almost the same, but CO_2 production varies.

Its time course of growth and gas production (Figure 10) indicated that the log phase occurred between the 4th and 5th day after inoculation. Gas production in molasses broth or molasses broth with scum was not increased or increased very slowly after 10-15 days of incubation, while it was continuously enhanced in the presence of $CaCO_3$ or oil sand.

Strains 5G-101, 5G-100, and 5G-102, all yielded H_2S in the molasses + scum medium. Formation of H_2S may result from the abundance of protein present in the scum.

(C) Strain 5G-102. This strain is an obligate anaerobe, Gram-negative, motile by peritrichous flagella (Figure 11), non-sporulating rod, 0.8-0.9 x 2-6 mm. The strain grows and produces gas and acids in PY, PYG, and molasses broth. Its fermentation products from glucose include acetate, lactate, butyrate, and valerate. H_2S is produced. Gelatin not liquefied. Litmus milk is acid curded, coagulated, and reduced. The mol% G+C of the DNA is 45.1 (Tm).

In molasses broth or in molasses broth with oil sand, scum or $CaCO_3$, the fermentation products are H_2, CO_2, short chain fatty acids, and ethanol. In the four supplemented media, the production of H_2 was the same, while CO_2 production differed. Highest CO_2 production occurred in molasses with scum, whilst the lowest CO_2 production occurred in molasses broth (Table 6).

The growth and gas production curves (Figure 12) show that the growth achieved stationary phase after 5-6 days of incubation. After 10-15 days, no further increase in gas production was observed in molasses broth and molasses broth with scum, while it still increased in the molasses broth with $CaCO_3$ or oil sands.

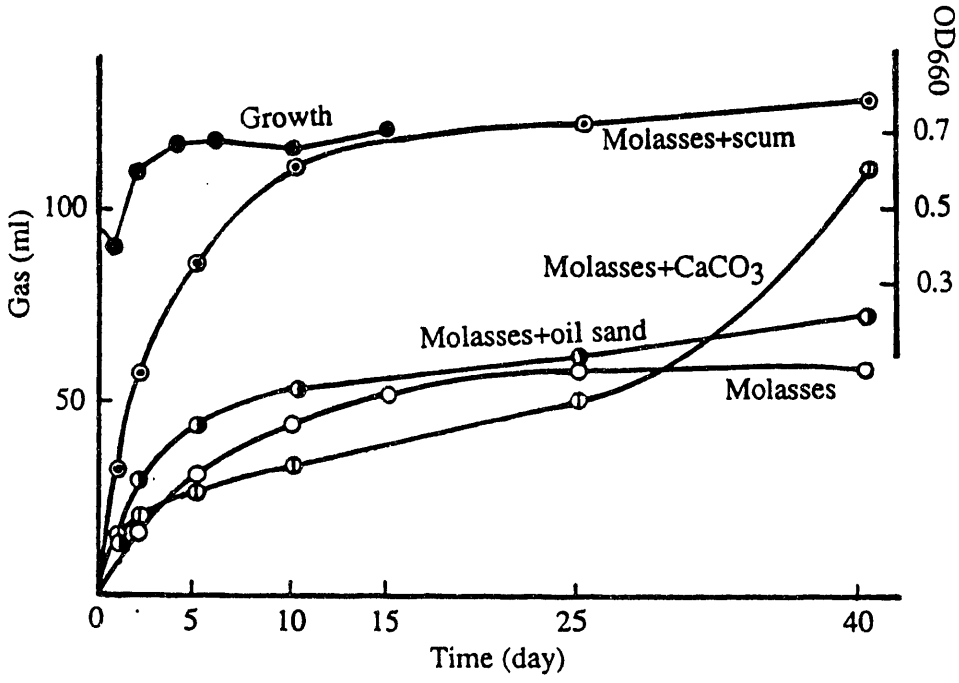


Figure 4. Time course of gas production by the culture 5G-A in different media.

Table 6
Metabolites of the anaerobic strains isolated from culture 5GA

Strain	Medium	Gaseous phase				Liquid phase								pH
		Amount	Content %			Organic acid mg/l					Alcohols mg/l			
		ml	H_2	CO_2	H_2S	Amount	C_2	C_3	C_4	C_5	Amount	C_2	C_4	
5G-100	Molasses	21	31.5	20.7	-	2880	1306	14	1300	260	167	167	-	5.6
	Molasses+$CaCO_3$	33	32.7	37.8	-	4467	2111	77	2279	-	862	862	-	6.5
	Molasses+oil sand	42	36.3	31.5	-	6159	2175	12	3619	353	85	85	-	5.4
	Molasses+scum	74	35.7	34.2	+	9078	5655	42	3357	24	176	176	-	5.3
5G-101	Molasses	27	30.4	21.6	-	2288	891	22	1336	39	77	77	-	5.2
	Molasses+$CaCO_3$	38	30.4	27.0	-	1926	1026	-	900	-	374	173	201	6.3
	Molasses+oil sand	40	33.4	26.1	-	2393	705	4	1676	8	102	102	-	5.4
	Molasses+scum	99	33.3	40.5	+	6448	2000	17	4426	5	110	110	-	5.2
5G-102	Molasses	20	42.8	24.3	-	1774	737	57	917	63	23	23	-	5.4
	Molasses+$CaCO_3$	30	42.8	31.5	-	1754	810	56	864	24	186	95	91	6.5
	Molasses+oil sand	41	42.8	30.6	-	2904	822	12	2021	49	40	40	40	5.3
	Molasses + scum	66	42.6	36.0	+	4286	1516	29	2680	61	65	65	65	5.1



Figure 5. Electron micrograph of the strain 5G-100 (A) x 8000; (B) from the well E6-J22 x 1200.

Based on their major morphological and physiological properties, all the three strains (5G-100, 5G-101, and 5G-102) belong to the genus *Bacteroides* [10], but they are different from all species described previously in this genus. Thus, enriched culture 5GA is composed at least of three species, belonging to the genus *Bacteroides*. This genus has not been reported in past references on MEOR.

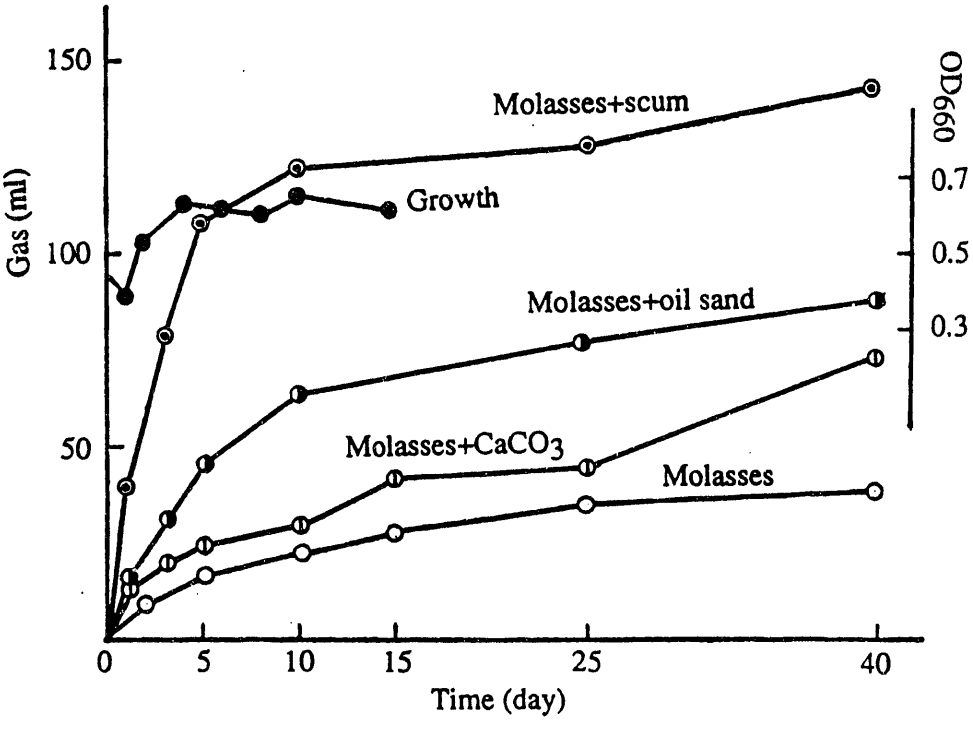


Figure 6. Time course of gas production by the strain 5G-100 in different media.

3.5. Oil field tests

On the basis of the properties of culture 5GA and the evaluation of results from laboratory model experiments, this culture mixed with Daqing strains was applied as inoculum in two wells at Daqing Oilfield. Detailed information on these tests is described by Zhang and Zhang [11] in this volume.

Briefly, well E6-J22 was injected with 548 t of water containing 3.2% (w/v) raw sugar and 36.4m^3 of inoculum and shut in. After 40 days, the well was placed back in operation.

Well E5-J18 was injected with 502 t of water containing 3.2% raw sugar (w/v) and 36.4 m^3 of inoculum and shut in. After 40 days, the well was reopened.

Results obtained from the two wells during seven months of production are:

Oil production increased	> 1800 t
CO_2 output increased	> 50000 m^3.

Microbiological analyses of the co-produced water before MEOR treatment indicated that the anaerobic gas-producing bacteria proposed for inoculation were absent. After the treatment, the numbers of gas-producing bacteria in the coproduced water were 4.0 x 10^2-2.5 x 10^6/ml (Table 7). Organic acids in the co-produced water (Table 8) were similar to those produced by the gas-producing anaerobes (Tables 4 and 7). Both components of the gas phase in enriched culture 5GA (Table 4) and in gas-producing cultures from the co-produced water (Table 7) were identical.

4. DISCUSSION AND CONCLUSIONS

It is well known that allochtonous bacteria injected into petroleum reservoirs compete with the indigenous microflora. The success of MEOR trials depends on the predominance and persistence of the injected bacteria in the reservoir ecosystem

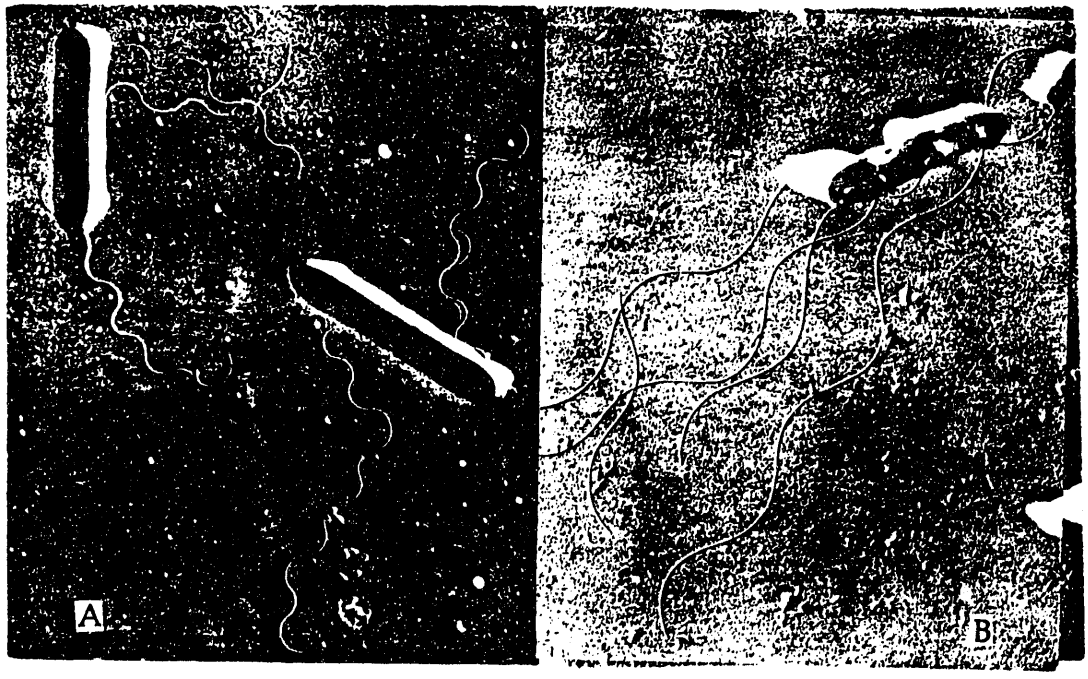


Figure 7. Electron micrograph of the strain 5G-101(A) x 8000; (B) from the well E5-J18 x 8000.

Table 7
Content of the gas- and acid-producing anaerobes and their metabolites in the co-produced water after the microbial flooding treatment

Sample	Gas- and acid-producing anaerobe/m.	Gas%		Organic acid mg/l					
		H_2	CO_2	Total	C_2	C_3	C_4	C_5	C_6
		Well E6-J22, reopened on August 5, 1990							
Wellhole	$0.5x10^4$	20.3	12.3	5159	2507	1302	775	-	575
05.08.90	$1.6x10^3$	35.0	49.0	7432	6593	113	449	-	227
06.08.90	$2.5x10^6$	6.9	25.0	192	192	-	-	-	-
11.08.90	$0.5x10^3$			1398	667	315	325	91	-
		Well E5-J18, reopened on September 20, 1990							
30.09.90	$2.5x10^3$	23.5	13.1	1624	730	-	894	-	-
06.10.90	$0.4x10^3$	15.9	40.5	2489	1243	-	1246	-	-
22.10.90	$4.5x10^3$	25.3	13.1	2901	1172	181	1544	4	-

Table 8
Organic acids in the co-produced water after microbial biostimulation

Sample	Organic acid mg/l					
	Total	C_2	C_3	C_4	C_5	C_6
	Well E6-J22, reopened on August 5, 1990					
Wellhole	681	299	144	143	52	43
05.08.90	978	456	149	217	99	57
06.08.90	278	278	-	-	-	-
11.08.90	1294	543	364	387	-	-
16.08.90	1503	630	368	505	-	-
21.08.90	1389	598	365	426	-	-
11.09.90	248	101	31	77	28	11
20.09.90	207	122	-	64	21	-
	Well E5-J18, reopened on September 20, 1990					
Wellhole	90	46	-	30	14	-
26.09.90	7317	2225	926	4141	25	-
30.09.90	1341	432	245	664	-	-
06.10.90	147	72	20	55	-	-
11.10.90	494	189	71	213	21	-
20.10.90	10088	3497	1331	4545	715	-

through natural selection processes. It is necessary for MEOR technologies to detect and 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, in past MEOR trials, information concerning the isolation and detection of bacteria injected into the tested reservoirs often is not available. For this reason, the injected anaerobic gas- and acid-producing bacteria were isolated from post-treatment samples of coproduced water to support chromatographic analyses for their metabolites as outlined above.

On morphological and physiological criteria, strains 5G-100 (Figure 5B) and 5G-101 (Figure 7B) remained present and active in both the tested wells. These results support the hypothesis that the enriched culture 5GA played an important role in EOR.

The satisfactory results obtained from the single-well biostimulation tests at Daqing Oilfield gave a new impetus to the development of *in situ* MEOR technologies in China. Preparation for further field trials is progressing.

The predominance of injected bacteria in the natural selection processes as seen in the Daqing field tests is a major premise for the success of MEOR. The development of methods for monitoring ecological interactions would make an important contribution to our theoretical and practical knowledge of MEOR processes.

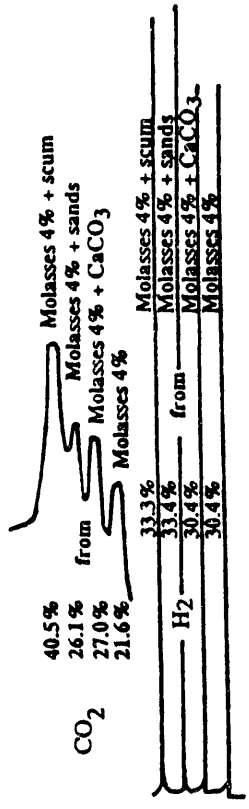


Figure 8. Gas chromatogram of the fermentation products from molasses 4% medium by the strain 5G-101 under anaerobic condition.

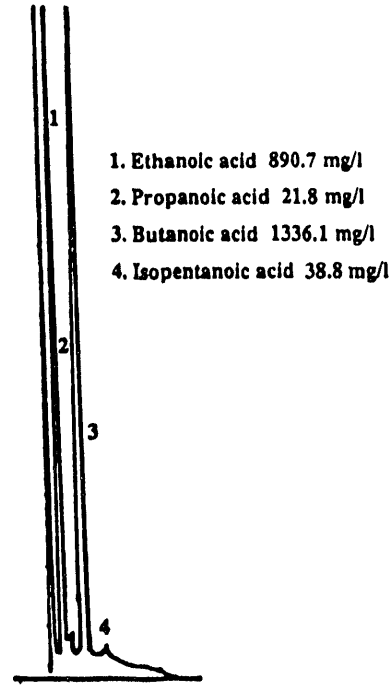


Figure 9. Gas chromatogram of gases produced by the bacterial strain 5G-101 in several media under anaerobic condition.

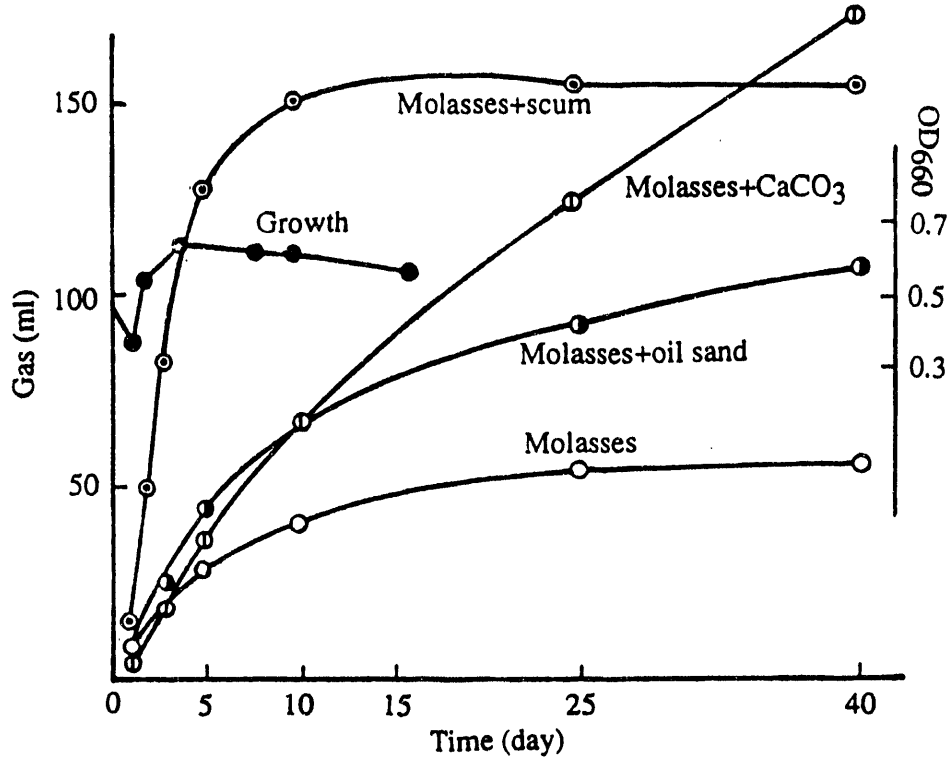


Figure 10. Time course of gas production by the strain 5G-101 in different media.

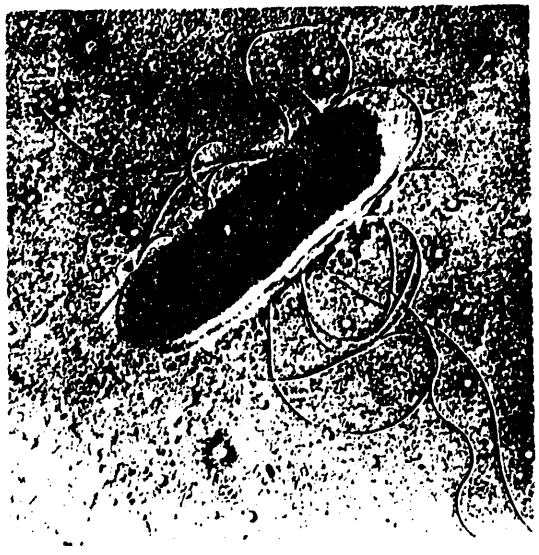


Figure 11. Electron micrograph of the strain 5G-102 x 1500.

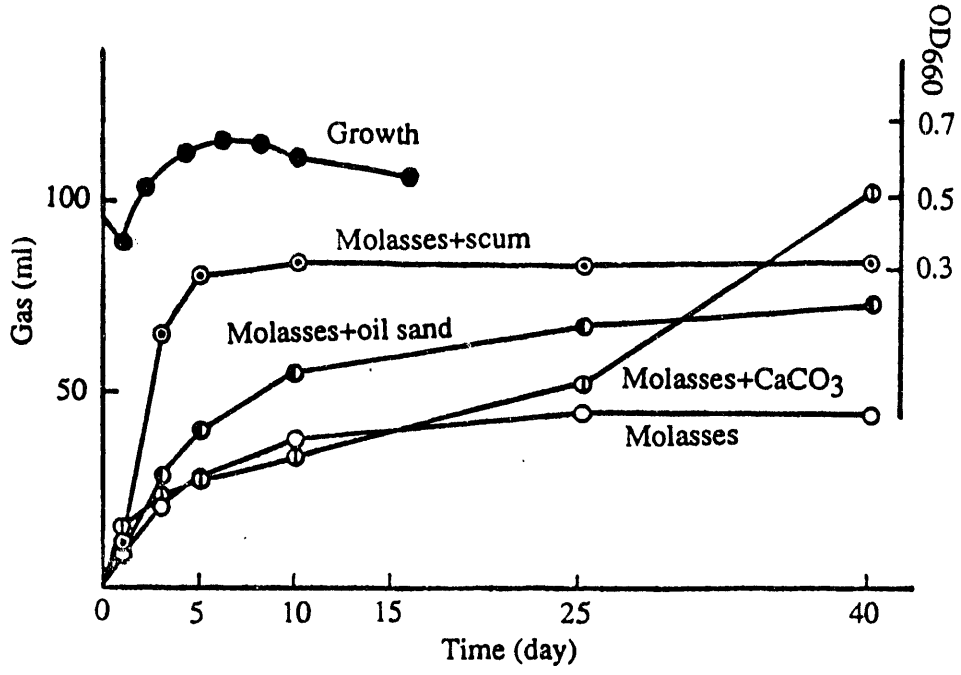


Figure 12. Time course of gas production by the strain 5G-102 in different media.

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On-site Bioaugmentation Treatment of Petroleum Tank Bottom Wastes: A Case Study

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Abstract

Weathered crude oil tank bottom waste, and waste-impacted soil was treated on-site using a commercial bioaugmentation system in land farm-type treatment cells to reduce hydrocarbon contamination. Approximately 10,000 yds^3 of weathered sludge was discovered concentrated in a bermed pit near a former above-ground tank battery in rural Kansas. Laboratory tests of biocompatibility and growth indicated that bioremediation was an option for treatment at this site. Permission was granted by State authorities to bioremediate the hydrocarbon wastes on-site.

Former pits were converted to bermed treatment cells by excavating to a clay or bedrock base. In each cell, six inches of concentrated sludge, three inches of chopped hay, and six inches of impacted soil were layered and homogenized by tilling. Blending of the soil and sludge reduced the hydrocarbon concentrations overall by approximately 50%. The prepared soil-sludge mixture was inoculated with a commercial mixed culture of naturally occurring hydrocarbon-degrading bacteria, inorganic nutrients, and growth factors. The treatment was applied by spraying and subsequently tilled into the soil. Treatment cells were tilled twice monthly. Microbiological, chemical, and environmental parameters were monitored in each cell, and nutrients were reapplied as required. Hydrocarbon concentrations were evaluated by U.S. Environmental Protection Agency (EPA) method 8020 for benzene, ethyl benzene, toluene, and xylene (BETX), and EPA method 8015 for total petroleum hydrocarbons (TPH).

TPH was reduced from starting concentrations of 39,000 to 140,000 mg/kg in the concentrated sludge to less than 100 mg/kg in each of the six treatment cells within six months. BETX was reduced from low starting levels to undetectable levels. No hydrocarbons were detected below the base of the treatment cells. The site was closed by State regulatory officials within 12 months of the start of treatment.

The combined bioaugmentation-land farming technique was an appropriate technology for the on-site treatment of hydrocarbon sludge at this site. This technology shows substantial promise for broad application to hydrocarbon waste remediation in the oil industry.

1. INTRODUCTION

Hydrocarbons produced and transported in the upstream oil industry often become misplaced by operational leakage and normal waste generation. Misplaced hydrocarbons in soil and groundwater are recognized today as pollutants that must be cleaned up.

During the last ten years, bioremediation, a process that uses microorganisms to transform harmful substances to non-toxic compounds, has become an important

tool for environmental restoration [1]. The bioremediation of hydrocarbon-contaminated soil and groundwater is a technology that relies on many of the same principles as does the microbial enhancement of oil recovery [2]. In this paper, we report how mixed cultures of naturally occurring microorganisms were applied and managed to remediate weathered hydrocarbon sludge generated by crude-oil tank farm operations.

2. SITE INFORMATION

The site is located on a former crude-oil tank farm in rural Montgomery County, Kansas. None of the physical infrastructure of the tank farm remains at the site. During construction of a regional highway in the 1940s, sludge was encountered in the right-of-way and was pushed into surface pits in the bermed tank-farm holding area. The site has been abandoned for approximately 40 years. In 1991, the new owners of the site decided that the sludge should be cleaned up. Transport of the sludge material away from the site would require handling and shipment as a hazardous waste. Laboratory tests of biocompatibility and growth indicated that bioremediation was a viable option for treating this site. A clean-up program of on-site sludge preparation and bioremediation was proposed to, and accepted by, State environmental authorities.

The site is located within a low-lying area approximately 790 feet above sea level. During periods of heavy rainfall, the bermed area in which the sludge was deposited collects and holds water. Drainage is generally to the north and west.

The suite of soils encountered at the site are classified as the Bates-Collinsville complex. The surface soil consists of a layer of light brown loam, approximately 8-14 inches thick. Subsoils vary between fine sandy loam and dark clay. There is a clay-rich undersoil beneath the bermed area at a depth of 4-6 feet. This layer appears to act as an aquitard, slowing or preventing the infiltration of surface water into the water table at 13 feet.

The average depth to groundwater, which occurs within the unconsolidated sediments above bedrock, is 13 feet. The chemical quality of the water is poor because of naturally high salinity. The local groundwater gradient is to the north and northwest. Wells developed in this sediment yield water at a rate of 5 to 100 gallons per minute, indicating that the soil has good permeability and porosity. Local residents obtain their drinking water from a municipal supply and do not rely on groundwater. Some local groundwater is pumped for livestock and irrigation.

Bedrock is usually encountered at depths of between 15 and 20 feet below surface and most likely belongs to a member of the Stanton Limestone formation. This formation ranges between 70-130 feet thick, and consists of interbedded shale and fine-grained dense carbonates.

3. WASTE CHARACTERISTICS

Hydrocarbon sludges were discovered on and in the shallow subsurface soils. An environmental site assessment found that TPH in the sludge ranged from 39,000 to 140,000 mg/kg, or 4-14% of total volume [3]. Surface soils contained 400 mg/kg TPH. Toluene, benzene, and xylene were not detected in most samples, but ethyl benzene was detected in sludge samples at concentrations ranging from 1,500 to 12,000 mg/kg. Low levels of BETX fraction hydrocarbons are normal in heavily weathered oil sludges.

Trace amounts of polyaromatic hydrocarbons (PAHs) were detected in both the sludges and soils and included napthalene, phenanthrene, floranthrene, pyrene, and benzo(a)anthracene. Metals in the soil included chromium, lead, vanadium, and zinc at levels up to 150 mg/kg, with a combined average of 20 mg/kg, only slightly above normal background for soil in this area.

4. METHODS

The approach to the bioremediation of this site was to optimize environmental and chemical conditions for microbial growth in the sludge/soil waste. To accelerate the rate of natural degradation and increase the completeness of degradation across the spectrum of hydrocarbons at the site, cultures of non-indigenous hydrocarbon-degrading bacteria and inorganic nutrients were added.

To bioremediate the sludge most efficiently, it was decided to amend the sludge to increase permeability, porosity, retain soil moisture, and provide access for microbes to the hydrocarbon contaminant. Five former diked tank locations and the original sludge disposal pit were excavated to below contamination levels into the clay undersoil. Berms were reinforced with uncontaminated dirt from the excavations. Concentrated sludge was deposited in a layer 6-8 inches deep across the bottom of each treatment cell. Five cells were loaded with approximately 1,200 yds^3 each, and one cell received approximately 4,000 yds^3. A layer of chopped hay was put on top of the sludge, and an additional 8-inch layer of lightly contaminated soil was added on top of the hay. The layered waste was homogenized by tilling. Blending of the soil, hay, and sludge reduced its overall TPH concentration by approximately 50%. After blending, the porosity of the homogenized waste averaged 23%.

5. TREATMENT

The materials that comprised the bioaugmentation system consisted of a mixed culture of naturally occurring hydrocarbon-degrading bacteria, a mixture of inorganic nutrients, chemicals, and local water. Each strain of bacteria in the mixed culture was isolated, identified, and reviewed for human pathogenicity; none are recognized human pathogens [4]. The mixed culture also has proven non-pathogenic and non-toxic for a variety of mammalian and aquatic organisms [4].

A slurry of bacteria culture, inorganic nutrients, chemicals, and water was prepared on site, and spread over the surface to achieve initial concentrations of 4×10^{12} bacteria/yd^3, and a carbon to nitrogen ratio of 10:1. The surface then was tilled to increase the distribution of bacteria throughout the homogenized waste material, and to facilitate bacterial transport into the subsurface by fluid infiltration. Altogether, 3160 barrels of this slurry were applied as an initial treatment during one day across the six cells.

6. MAINTENANCE

Each treatment cell was tilled every two weeks for two months, and then once again six weeks later. More of the water and nutrient mixture (a total of 2000 bbls) was added to each cell two months after the initial inoculation. Samples were collected from each of the treatment cells and analyzed for TPH, soil moisture, pH, and occasionally, for the total population of hydrocarbon-degrading

Table 1
Concentration of BTEX in mg/kg in composite samples by EPA method 8020. Values of 0.01 or less are shown by a dash

		Cell Number					
		1	2	3	4	5	6
9/18/91	benzene	-	-	-	-	-	-
	toluene	-	-	-	-	0.02	-
	ethyl benzene	-	0.02	-	-	0.03	-
	M & P xylene	-	0.08	0.02	0.02	0.14	6
	O xylene	-	-	-	-	0.03	-
10/24/91	benzene	-	-	-	-	-	-
	toluene	-	0.03	0.03	-	0.03	-
	ethyl benzene	-	-	-	0.03	0.08	-
	M & P xylene	-	-	0.03	0.01	-	0.02
	O xylene	0.03	-	0.07	0.03	-	0.03
12/20/91	benzene	-	-	-	-	-	-
	toluene	-	-	-	-	0.12	-
	ethyl benzene	-	-	-	-	0.17	-
	M & P xylene	-	0.03	-	0.03	0.42	-
	O xylene	-	-	-	-	0.07	-

bacteria. As a result of soil sampling, limited areas of high hydrocarbon concentration were identified and re-treated with the complete bioaugmentation system seven months after initial inoculation.

7. MONITORING AND SAMPLING

To determine the overall trends in biodegradation activity, many discrete samples from each treatment cell were collected, ranging across the extent of the cell and from surface to the bottom of the waste, and combined in composite samples. To estimate the final concentrations of BETX and TPH for State closure of the site, discrete samples were collected from each treatment cell.

Three collections were made over the first three months of the project . Samples were collected with a decontaminated stainless steel spoon and shovel. For each cell, ten grab samples were homogenized in decontaminated stainless-steel vessels into a composite sample, transferred to laboratory-cleaned glass jars and sealed with Teflon lids. Samples were immediately stored on ice for transport to the laboratory. All the analytical work was performed by State qualified independent laboratories. EPA method 8020 was used for the analysis of BTEX and method 8015 Modified was used to quantify TPH.

8. RESULTS

Over six months, the concentrations of BETX from both composite and discrete samples fell from their low initial levels to non-detectable levels in each treatment cell (Table 1).

Table 2
Concentration of TPH in mg/kg by EPA method 8015. Modified composite samples

	9/18/91	10/24/91	12/20/91
Cell 1	630	90	<1.0
Cell 2	1200	540	<1.0
Cell 3	830	130	<1.0
Cell 4	460	280	<1.0
Cell 5	380	180	<1.0
Cell 6	910	470	10

Table 3
Concentration of TPH in mg/kg by EPA method 8015 modified individual confirmation samples for site closure*

	Cell Number					
	1	2	3	4	5	6
2/2/92	7.0	9.0	<1.00	5.0	<1.00	<1.00
	<1.00	8.3	<1.00	19.0	<1.00	12.00
	78.0	3.0	8.0	<1.00	<1.00	<1.00
					<1.00	

*Cell 5 was analyzed later and the concentrations were the following: 60 mg/kg on 3/4/92; <1.00 mg/kg on 3/14/92; and <1.00 mg/kg on 5/6/92.

TPH concentrations derived from the composite samples decreased from 39,000-140,000 mg/kg in the original sludge to less than 100 mg/kg in each of the treatment cells (Table 2). Discrete samples from each cell showed TPH concentrations ranging from less than 1 to 83 mg/kg, and averaging 8.4 mg/kg (Table 3).

9. CONCLUSIONS

The combined bioaugmentation-waste conditioning technique was an appropriate technology for the on-site treatment of hydrocarbon sludge at this site. The owner of the site was notified by State authorities within 12 months of the start of remediation work that no further clean-up actions were required at the site. This technology shows substantial promise for a broad application to hydrocarbon-waste remediation in the oil industry.

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Six Years of Paraffin Control and Enhanced Oil Recovery with the Microbial Product, Para-Bac™

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Abstract

The history of the first commercial microbial culture product for controlling paraffin accumulation in oil field production systems is described. This product is composed of a consortium of different, naturally occurring marine microorganisms that are selectively isolated and adapted to reduce the precipitation of high molecular weight alkanes. First used in 1986 in the Austin Chalk formation, the Para-Bac™ product line began as a single product for paraffin control. Now in use throughout the major producing formations of North America, the product line currently consists of over twelve different products directed at enhanced oil recovery, mitigation of sulfate-reducing bacteria, as well as control of scale, corrosion, and paraffin. A typical treatment is described including testing and monitoring protocols. Case histories are presented describing the use of the product on individual wells and extended formation treatments. New applications include use in various types of lift systems and in hydrogen sulfide control.

1. INTRODUCTON

A rapidly growing market for microbial culture products has developed in the petroleum industry. Over the past six years, a variety of microbial culture products have been developed to treat various types of production problems in oil fields including paraffin, scale, and corrosion, and to enhance oil recovery. These products are being applied successfully throughout the major producing regions of North America and are enjoying increasing success overseas. Micro-Bac International, Inc. is a research and development company producing a variety of microbial culture products for use in the treatment of various environmental problems.

Since 1986, Micro-Bac International has been engaged in treating oil wells and various other aspects of petroleum production systems including injection wells, tanks, and flowlines. The microbial products used are all composed of naturally occurring (non-genetically engineered) non-pathogenic microorganisms that have been specifically selected and adapted for the function desired. The company has made a practice of expanding into new product areas as success has been attained with existing products.

2. MODE OF ACTION OF PARA-BAC™ PRODUCTS

A variety of effects are observed in oils treated with Para-Bac products (Table 1). These effects are not seen in all oils, but nevertheless, represent a consistent pattern.

The reductions in viscosity, pour points, and cloud point are most notably associated with significant and prolonged increases in production, measurable as

Table 1
Observed effects of Para-Bac™ treatment on oil (not seen in every type of oil)

Decreased viscosity
Reduced pour point
Elevation in API gravity
Elevation in transport fluid fraction
Reduced sulfur content (SRBs)

changes in the production decline curves. No negative effects from treatment have been noticed and marketability of the treated petroleum is unaffected. At least three mechanisms have been identified as being associated with the product's effect on the physical properties of crude oil; these include direct degradation of higher molecular weight alkanes (paraffins), production of fatty acids and other microbial metabolites which act as paraffin solvents and dispersants, and the production of biosurfactants.

3. COMMERCIAL APPLICATION OF THE PRODUCTS

The basis for the successful application of any commercial product is the benefit to the customer. The successful use of Para-Bac™ products results in a variety of financial advantages to the user, including reduced lease operating expenses, which result from reduced capital expenditures by increasing the lifetime of production equipment, and reducing the downtime in production. By reducing paraffin accumulations and other mechanical or hydraulic problems, production rates can be stabilized. Most importantly, treatments with Para-Bac™ products can be targeted to increase production. There are few limitations as far as the types of lifting systems that can be treated. The most common limitation that is found is whether the treatment is cost-effective for the operator, not whether the treatment is technically feasible. Wells that have been treated include pumping, flowing, hydraulic, plunger, and gaslift types.

4. DOCUMENTATION OF PRODUCT PERFORMANCE IN ENHANCED OIL RECOVERY

There is an extensive documentation of product efficacy in the form of numerous case histories collected over six years of product use. Some examples are shown in the production curves seen in Figures 1 through 5, which are taken from Society of Petroleum Engineers publications.

Tables 2 and 3 give examples of the significant savings and increased production that can be obtained through these treatments.

Savings of $348 per well/month were seen simply through a reduction in cost of chemicals, hot oil, lease oil, lease fuel use, wax cutting, electricity, maintenance, and hydrogen sulfide gas penalties. A dramatic enhancement of oil production can be seen with an increased income of $96,000,000 over the estimated production life of the lease. The benefits of microbial production use are summarized in Table 4.

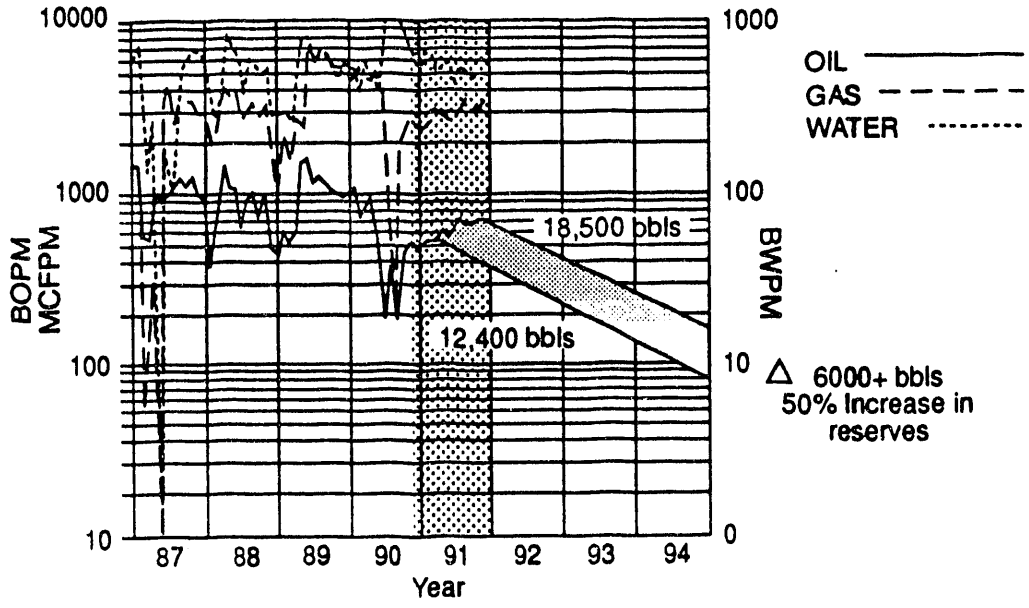


Figure 1. J & P Schroeder Decline Curves (from Reference 1).

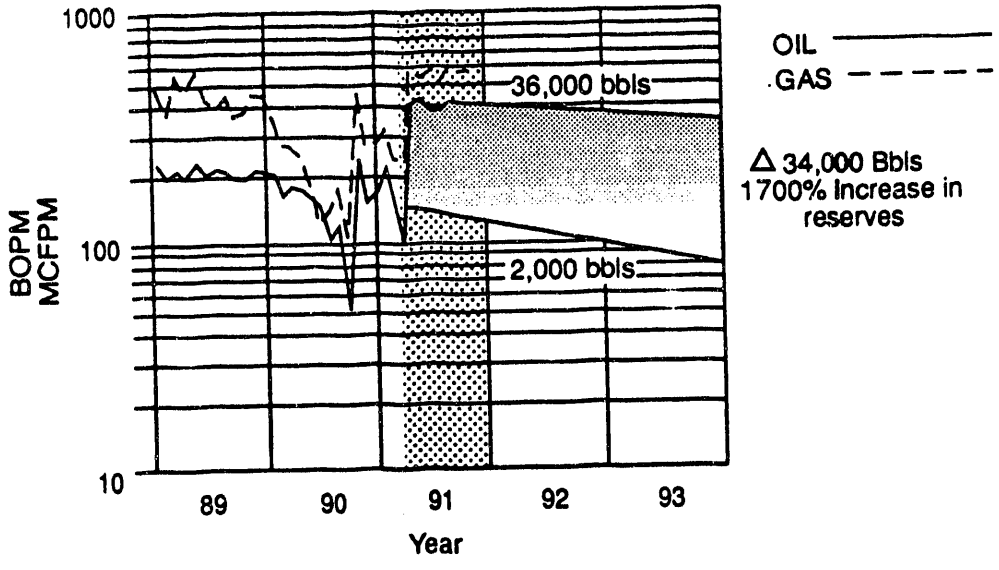


Figure 2. San Andres formation: Permian Basin Area —— 3 well pilot (from Reference 2).

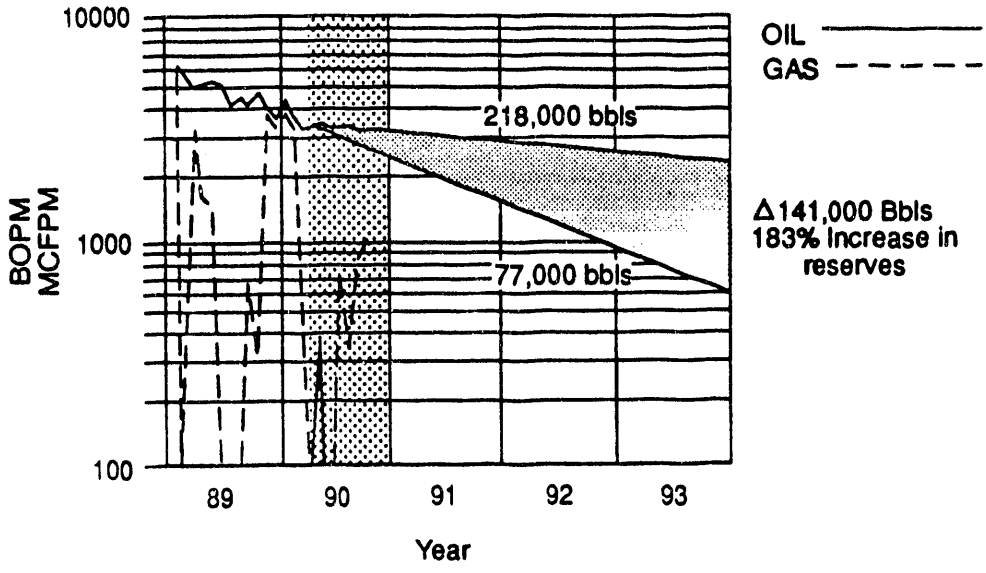


Figure 3. Mississippian formation: Permian Basin Area —— 1 well pilot (from Reference 2).

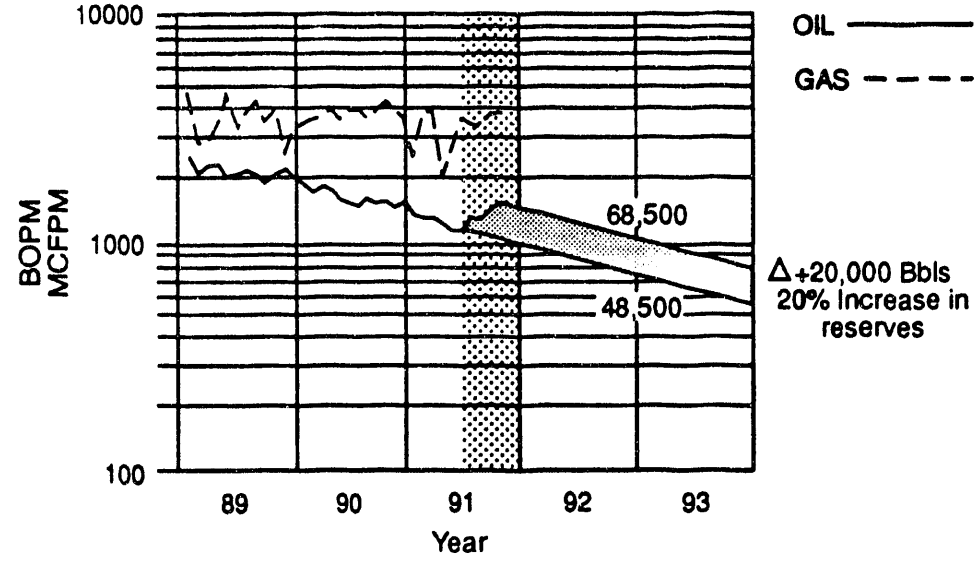


Figure 4. Canyon formation: Permian Basin area —— 3 well pilot (from Reference 2).

Table 2
Total microbial treating project - economic summary (from references).

Category	146 Well Avg., $/Well/Month Conventional	Microbes
Chemical	234	63
Hot Oil	65	47
Lease Fuel Use	2104	1684
Wax Cutting	47	41
Electricity	1114	1061
Chemical Pump Maint.	82	0
H_2S Gas Penalty	430	266
Microbes	0	566
Total	$4076	$3728
Cost Savings		$348/Well/Month

5. HYDROGEN SULFIDE CONTROL USING MICRO-BAC MICROBIAL PRODUCTS

Micro-Bac International continues to expand into all areas of petroleum production. Recent efforts have been devoted to controlling hydrogen-sulfide problems in gas wells through the use of bacterial culture products that specifically antagonize sulfate-reducing bacteria in a variety of ways. A case history of the efficacy of these products is shown in Figure 6. Hydrogen sulfide levels were reduced from 18 ppm to 7 ppm, which, in turn, resulted in a savings

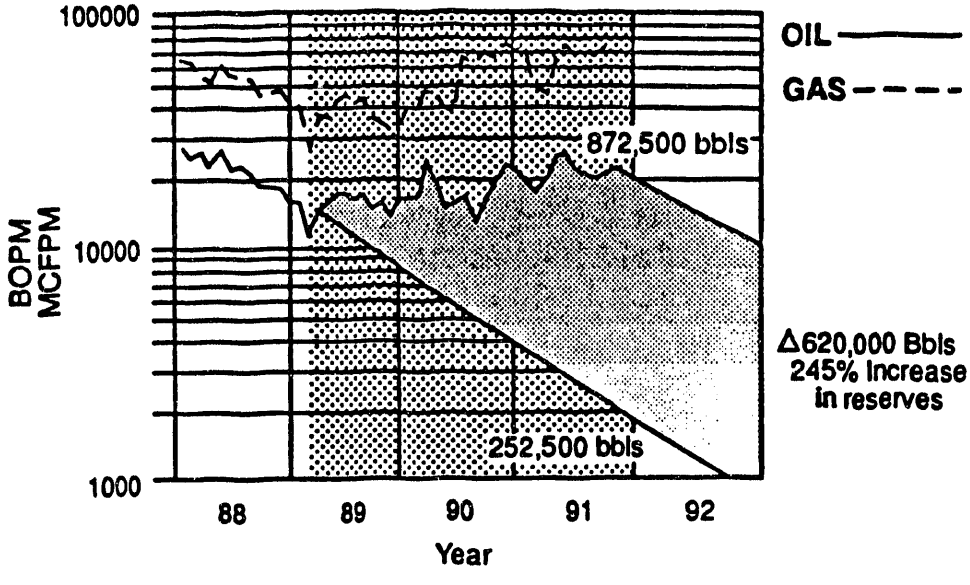


Figure 5. Wasatch formation: Uinta Basin area — 10 well composite (from Reference 2).

Table 3
Altamont/Bluebell Project [estimated reserves and economics (oil price - $19.50/bbl., gas price - $1.00/mcf)]*

	Conventionally Operated Well	MMEOR Operated Well	Microbial Enhancement
First 12 Months of Operations			
Production:			
Oil	12,800 bbls	16,000 bbls	3,200 bbls
Gas	48,800 mcf	60,000 mcf	11,200 mcf
Income:			
Oil	$249,600	$312,000	$ 62,400
Gas	$ 48,800	$ 60,000	$ 11,299
Operating Cost:	$ 48,900	$ 44,700	$ 4,200
Microbial Enhanced Income/Well -			$ 77,800
Microbial Enhanced Income for 146 Well -			$11,359,000
Life of Project			
Production:			
Oil	34,500 bbls	164,500 bbls	30,000 bbls
Gas	129,400 mcf	235,400 mcf	106,000 bbls
Income:			
Oil	$627,700	$1,258,000	$ 630,300
Gas	$129,400	$ 235,400	$ 106,000
Operating Cost:	$146,700**	$ 223,500***	$ (76,800)
Microbial Enhanced Income/Well -			$ 659,500
Microbial Enhanced Income for 146 Wells -			$ 96,287,000

* from Reference 3
** Producing Life - 3 yrs.
***Producing Life - 5 yrs.

Table 4
Advantages of microbial treating program compared to conventional operations (from Reference 3)

1. Increases profits.
2. Reduces frequency of hot oil treatment (minimizes killing wells with hot fluids causing high melting-point paraffins to be pumped into producing zones).
3. Reduces loss in production due to hot oil treatments.
4. Reduces heating requirements (minimizes use of expensive lease fuel).
5. Removes chronic deposits of paraffin-containing scale and iron sulfide. These deposits are the result of chemical/high heat operations and occur in flowlines, annulus, tubing, treaters, and heating coils in oil storage tanks.
6. Reduces tubing pressure in hydraulic-pumped wells (reduces the work required to produce, resulting in less wear and tear on pump parts.)
7. Reduces the temperature of the power water resulting in cooler pumping temperatures (increases the efficiency of triplex and less wear and tear on pump parts).
8. Reduces the carryover of oil into power water and SWD tanks.
9. Eliminates chemical use (removes toxic and combustible liquids from well and lease sites).
10. Enhances the quality of the oil produced (resulting in higher gravity and lower viscosity).
11. Reduces the temperature of oil in storage tanks (less weathering and boil off).
12. Reduces scaling tendencies of produced water.
13. Reduces gauger labor (chemical-pump maintenance).

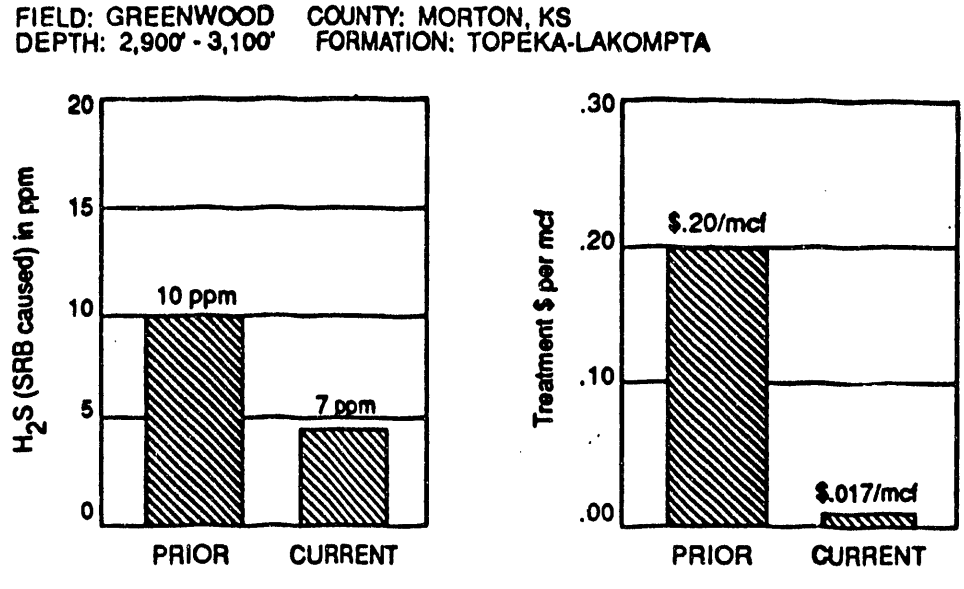


Figure 6. Case history of H_2S and iron sulfide scale.

to the customer of $0.20 per mcf for the prior treatment method (biocides) to $0.05 per mcf with the use of Micro-Bac International products.

Successful commercial applications of microbial culture products in the petroleum industry is a reality. The use of such products has become an established alternative for the petroleum industry, and its future will be one of continuing expansion into all areas of the petroleum industry.

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Causes and Control of Microbially Induced Souring

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Abstract

Petroleum reservoirs are harsh environments, but are not devoid of life. Rather, they are functional ecosystems with diverse, and metabolically active microorganisms. Thus, there is the potential for microbial sulfide production in the reservoir when environmental conditions permit. Some oil field operations may change the environmental conditions in the reservoir and inadvertently stimulate sulfide production. Waterflooding with brines high in sulfate can supply the oxidized sulfur needed for microbial sulfide production. Many oil field chemicals can be metabolized by the anaerobic populations present in the reservoir, and thus, stimulate sulfide production. One approach to control microbially induced souring is to shift the electron-accepting reaction from sulfate reduction to nitrate reduction. The ability of a sulfide-resistant strain of *Thiobacillus denitrificans* (strain F) to reduce sulfide levels was tested in an experimental system using cores and formation water from a gas storage facility. The addition of nitrate alone (40 mM) decreased concentrations of effluent sulfide from 170 μM to 110 μM. After inoculation with strain F, effluent sulfide concentrations decreased to between 3 and 20 μM. A large scale test of the efficacy of nitrate treatment in controlling souring was conducted in the Southeast Vassar Vertz Sand Unit, Payne Co., OK. The injection of 2.6 metric tonnes of ammonium nitrate resulted in a 56% decrease in sulfide concentrations in coproduced brine from three adjacent wells. These studies indicated that nitrate additions may be effective in controlling souring during actual field operations.

1. INTRODUCTION

Hydrogen sulfide is a toxic and corrosive gas that greatly increases the cost of exploitation of oil and natural gas. Many reservoirs have high sulfide levels that result from physiochemical processes occurring in the reservoir such as thermal decomposition of organic sulfur compounds, and thermochemical sulfate reduction [1, 2]. In other reservoirs, the onset of sulfide production is often associated with waterflooding [3-5] or other exploitation practices [6]. The possible mechanisms responsible for hydrogen sulfide production during waterflooding include the nonoxidative dissolution of pyrite, and microbial sulfide production [1,4]. In many reservoirs with moderate temperatures (<80°C), microbial sulfate reduction is believed to be the primary cause of souring. Because microbial processes are influenced by the exploitation process, it should be possible to develop strategies to reduce or prevent microbially mediated souring. The ability to inhibit these microbial processes requires a thorough understanding of the ecology and physiology of the relevant microbial processes

involved. Our understanding of the physiology of sulfate-reducing bacteria [7] and the ecology of subsurface processes [8] has increased remarkably. The focus of this paper will be to use this information to better understand the causes and control of microbially mediated souring.

Specialized drilling and sampling procedures have been developed for collecting uncontaminated aquifer material from subsurface reservoirs down to a depth of 300 meters [9]. Using these specialized procedures, it was clearly shown that aseptically obtained aquifer material contains active and diverse microbial populations that are not the result of the contamination of the sample during its collection [8-11]. Some aquifer samples had relatively high concentrations of microorganisms, ranging from 10^5 to 10^7 cells per gram of material, close to many surface soil microbial concentrations. Subsurface samples often contained metabolically diverse populations of microorganisms, including aerobes, anaerobes and facultative organisms with metabolic properties ranging from the capacity to metabolize a variety of organic pollutants to methane production and sulfate reduction [8-11].

The fact that microorganisms have been found in aseptically obtained aquifer material at depths down to 300 m suggests that microorganisms may be found at even greater depths. Indeed, many studies suggest that deep terrestrial subsurface reservoirs contain active and diverse populations of microorganisms. Olson et al. [12] found sulfate-reducing and methanogenic bacteria in stratal waters from wells 1800 m deep. Isotopic studies by Dockins et al. [13] showed that the hydrogen sulfide produced in groundwaters from depths of 10 to 260 m was of bacterial origin. Although it is difficult to obtain core material from deep reservoirs, ZoBell [14] and Kuznetsov et al. [15] obtained uncontaminated consolidated core samples, which contained microorganisms with physiological properties that suggested that they were indeed indigenous to the formation. An extensive compilation of reports on the occurrence of sulfate-reducing bacteria in subsurface environments is given by Iverson and Olson [16].

The perception that the soil acts as a filter to remove bacteria and other particles from the percolating rain water and groundwater and, thus, isolating subsurface environments from surface microorganisms is no longer valid [17]. Bacteria can, and do, move considerable distances in aquifers and saturated soils. Jack et al. [18] reported that a Leuconostoc strain injected into an unconsolidated petroleum reservoir appeared within a day in a production well over a kilometer away. The ability of bacteria to penetrate and propagate in deeper, less permeable petroleum reservoirs was demonstrated [19]. Microbial penetration through nutrient-saturated consolidated and unconsolidated porous materials also can occur at relatively rapid rates in the absence of fluid flow [17].

From the above discussion, we must conclude that, even though deep subsurface reservoirs are harsh environments, they are not devoid of life, but rather they are active, functional ecosystems. The terrestrial subsurface is not isolated from the rest of the biosphere. Since recharge of subsurface aquifers does occur, the exchange of nutrients and even organisms with the surface also must occur. Thus, the control or prevention of microbially induced problems such as souring and corrosion, will only occur after approaches have been developed to control the respective *in situ* activity and not just to remove or destroy the injected organisms. Prudent exploitation of oil and gas will only occur after realizing that the factors that affect microbial activity also are the important factors that affect the quality and recovery of these vital resources.

2. ECOLOGY OF SULFATE-REDUCING BACTERIA

In anaerobic environments, organic matter is degraded by the concerted action of several metabolic groups of bacteria [20]. In marine environments, estuaries, and petroleum reservoirs where sulfate is in high concentration, the complete oxidation of organic matter to carbon dioxide is coupled to the reduction of sulfate to sulfide [7, 21, 22]. Fermentative bacteria, such as *Clostridia*, hydrolyze complex polymeric compounds, such as polysaccharides and proteins, and ferment the hydrolysis products mainly to acetate, propionate, butyrate, H_2, and carbon dioxide. Acetate (equation 1) and hydrogen (equation 2) are probably the most important electron donors for sulfate reduction:

$$CH_3COO^- + SO_4^{=} \rightarrow 2\ HCO_3^- + HS^- \quad (1)$$

$$4\ H_2 + SO_2^{=} + H^+ \rightarrow HS^- + 4\ H_2O \quad (2)$$

Sulfate-reducing bacteria are a metabolically diverse group of bacteria that gain energy for growth by coupling the oxidation of organic compounds or H_2 to the reduction of sulfate [7, 21]. As a group, sulfate-reducing bacteria can use a wide range of organic compounds as electron donors, including several alcohols; fatty acids from formate to stearate; organic acids such as lactate and succinate; aromatic compounds, such as benzoate, phenol, p-cresol, and phenylacetate; heterocyclic compounds, such as nicotinic acid and indole; and alicyclic compounds, such as cyclohexane carboxylate. Hydrogen also serves as an electron donor for many species and hydrogen-using sulfate reducers can participate in interspecies hydrogen transfer reactions by using hydrogen produced by fermentative bacteria. In addition to using hydrogen, some sulfate reducers, in the absence of sulfate, can also produce hydrogen from organic molecules when grown in association with hydrogen-using methanogens [23, 24].

Most sulfate reducers use sulfate as the terminal electron acceptor, reducing it to sulfide. However, sulfite, thiosulfate, and tetrathionate also can be used as electron acceptors. Some species, such as *Desulfuromonas acetooxidans*, cannot use sulfate, but instead reduce sulfur to sulfide. Nitrate can be used as an electron acceptor by a few strains of sulfate reducers. Although oxygen inhibits the growth of the strictly anaerobic sulfate-reducing bacteria, some sulfate reducers can grow in the presence of oxygen and use it as an electron acceptor to produce ATP.

The anaerobic oxidation of methane or higher saturated hydrocarbons by sulfate-reducing bacteria or other anaerobes has been a controversial subject for many years. The oxidation of these hydrocarbons is thermodynamically favorable (equation 3) [7], but the mechanism by which these compounds could be oxidized in the absence of oxygen is unknown.

$$CH_4 + SO_4^{=} \rightarrow HCO^{3}- + HS^- + H_2O \quad \Delta G^{\circ\prime} = -16.6\ kJ \quad (3)$$

Geochemical studies of methane profiles in marine sediments and radioisotopic studies with sediments suggest that anaerobic methane oxidation may occur [7]. However, no species of sulfate reducers is known to use methane. The microbial transformation of petroleum in reservoirs was shown to be the result of the interaction between aerobic hydrocarbon-degrading bacteria that produced alcohols and fatty acids that could then be used as substrates by sulfate reducers [5]. However, Aeckersberg et al. [25] recently isolated a sulfate-reducing bacterium that can completely oxidize C_{12} to C_{20} alkanes to carbon dioxide with the stoichiometric reduction of sulfate to sulfide. This suggests that hydrocarbons

can be used directly by sulfate reducers. However, the occurrence and importance of anaerobic hydrocarbon degradation is not known.

3. CAUSES OF SOURING

Because of their diverse metabolic properties and widespread occurrence, sulfate-reducing bacteria 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 [26]. 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, the process should be referred to as microbial sulfide production. It also is important to note that methods to detect or control souring based solely on detecting or controlling sulfate-reducing bacteria may not be effective in actual field situations.

As discussed above, it is likely that sulfide-producing microbial populations already exist in many petroleum reservoirs. However, it is possible that these populations are introduced into the formation as a consequence of drilling or other practices. Beeman and Suflita [27] did not detect sulfate-reducing bacteria or other anaerobes in the aseptically sampled interior portions of a core from a discovery well in Kansas, but these organisms were detected in the drilling fluid and portions of the core that were contaminated by the drilling fluid. Once introduced into the formation, sulfate-reducing bacteria may be able to penetrate throughout the reservoir. ZoBell [14] estimated a rate of movement of *Desulfovibrio* species of 0.5 to 13 meters per year, which is fast enough to allow their dissemination throughout a significant portion of the reservoir during the normal operational life of the reservoir. However, we found that the penetration of sulfate reducers is much slower. In carbon-limited medium, *Desulfovibrio desulfuricans* and an acetate-using, sulfate-reducing enrichment penetrated Berea sandstone cores at rates of about 0.03 centimeter per day [28]. Somewhat faster rates (0.16 centimeter per day) were observed when higher carbon concentrations were used (M. Rozmin and M.J. McInerney, unpublished data). These slower rates suggest that sulfate-reducing activity may be localized near the injection wells.

In addition to active microorganisms, an oxidized sulfur species, suitable organic electron donors, and inorganic nutrients such as nitrogen and phosphorous, must all be present for microbial sulfide production to occur. It is possible that components of petroleum can serve as the organic electron donor [25]. In addition, several studies have shown that many oil field brines contain short-chain fatty acid anions, such as acetate and propionate [3, 29, 30], and aromatic compounds, such as toluene and phenols [29], that can be used by many sulfate-reducing bacteria. Thus, carbon availability may not be the limiting factor for sulfide production in many reservoirs. Most petroleum reservoir brines contain sulfate, but nitrogen and phosphorous sources may be limiting [31]. These limiting compounds may be inadvertently introduced into the formation during waterflooding operations. In some cases, the connate water has little or no sulfate, and introducing a sulfate-containing flood water leads to sulfide production. Such is the case for North Sea and offshore Alaska waterflooding operations where sulfide production began after the injection of seawater [3, 4]. In the Southeast Vassar Vertz Sand Unit, high sulfate brine from another aquifer was used as the water source of the waterflood [32]. Brines from the waterflooded regions of the reservoir contained sulfate, sulfide, and sulfate-reducing bacteria. Brines from portions of the reservoir, which had not been waterflooded in 1987, such as well 1A-7, did not have sulfate, and had less than detectable

Table 1
The effect of waterflooding on sulfide levels in well 1A-7

Sampling date	Sulfate (mg/l)	Sulfide (mg/l)	Sulfate reducers (MPN[a]/ml)
June 1987	<1	<0.1	<1
July 1989	330	16	2

MPN, most probable number

levels of sulfide, and sulfate-reducing bacteria (Table 1). Two years later, concomitant with the arrival of the waterflood waters as indicated by the presence of sulfate, sulfide and sulfate-reducing bacteria also were detected in the brine from this well. Thus, adding the limiting nutrient (in this case, sulfate) stimulated the growth and metabolism of sulfate-reducing bacteria. The cost effectiveness of using a water source high in sulfate should be reconsidered in light of its potential to stimulate microbial sulfide production.

In addition to supplying limiting nutrients, the injection of brine into a formation may increase the water activity of the formation and thereby, stimulate microbial growth and activity. This may be particularly important in formations that are predominantly oil-wet. Little or no work has been done on the influence of water activity on microbial activity in oil reservoirs.

Several chemicals used in oil field operations can be metabolized by sulfate-reducing or other anaerobic bacteria [6]. Mobility control agents, such as xanthan gum and polyacrylamide, can be degraded by the mixed microbial population to potential substrates for sulfate reducers. Antioxidants, such as sulfite and bisulfite, and corrosion inhibitors, such as sulfones and polyorganosulfones, also can be reduced to sulfide. Microbial inhibitors, such as benzoic acid, acetic acid, or ethylene glycol, can serve as substrates for certain anaerobic bacteria. Thus, rather than inhibiting the growth of sulfate reducers, adding these compounds may actually stimulate their growth.

4. CONTROL OF SOURING

The detrimental activities of sulfide-producing bacteria can be controlled by the effective use of appropriate biocides [33]. Because sulfate-reducing bacteria are associated with other anaerobic bacteria in polysaccharide-containing biofilms which coat the surfaces of pipes and other facilities, these bacteria are in a somewhat protected environment which water-soluble biocides may not effectively penetrate. Thus, it is important to select a biocide that can partition into the biofilm.

The use of biocides is most successful in controlling unwanted activities in surface facilities. However, the use of biocides to sterilize the injection water, or to kill sulfate reducers in the formation, is often difficult and expensive. Our approach is to manipulate the ecology of the reservoir by changing the terminal electron-accepting process from sulfate reduction to nitrate reduction [28, 34]. Thus, even if sulfate reducers are present in the reservoir, the accumulation of the unwanted product of their metabolism, sulfide, is prevented. Nitrate-using bacteria should be able to more readily use common electron donors, such as organic acids rather than sulfate-reducing bacteria. In

Table 2
The effects of nitrate addition and *T. denitrificans*, strain F, inoculation on sulfide concentrations in a porous rock biofilm

Treatment (μM)	Sulfide	Cell Concentrations (cells/ml)		
		SRB[a]	APB[a]	Strain F
None	160	10^5	10^5	<10
Nitrate	110	10^5	10^7	<10
Nitrate + strain F	3 to 16	10^7	10^7	10^7

SRB, sulfate-reducing bacteria; APB, acid-producing bacteria

addition, reducing nitrate can produce nitrogen oxides, which are strong oxidizing agents that increase the redox potential of the environment and inhibit the growth of sulfate reducers [34]. Some nitrate-using bacteria, such as *Thiobacillus denitrificans*, oxidize sulfide to sulfate by reducing nitrate to nitrogen gas (equation 4).

$$5\ HS^- + 8\ NO_3^- + 3\ H^+ \rightarrow 5\ SO_4^{=} + 4\ N_2 + 4\ H_2O \quad (4)$$

In the presence of nitrate, these bacteria would prevent the accumulation of sulfide.

Sublette and Sylvester [35, 36] showed that sulfide concentrations as low as 100 to 200 μM inhibit the growth of the wild-type strain of *T. denitrificans* (ATCC 23642) on thiosulfate. Complete inhibition was observed at initial sulfide concentrations of 1 mM. However, a sulfide- and glutaraldehyde-resistant strain (strain F) of *T. denitrificans* has been isolated by enrichment from cultures of the wild-type [37]. This strain is tolerant of inorganic sulfide concentrations in excess of 1000 μM and glutaraldehyde concentrations of 25 to 40 ppm, concentrations which are lethal to the wild type. *T. denitrificans*, strain F, has been successfully grown with the sulfate-reducing bacteria both in liquid culture and through Berea sandstone cores without the accumulation of sulfide [28]. Because *T. denitrificans*, strain F, is a chemoautotrophic bacterium, no additional organic nutrients are needed to support its growth. This will limit the growth of any indigenous organism present which might require organic nutrients.

The effectiveness of *T. denitrificans*, strain F, in reducing sulfide concentrations in a porous rock biofilm system was studied using cores and formation water from a gas storage facility (Table 2) [38]. The addition of nitrate (40 mM) alone decreased the effluent sulfide concentration from 160 to 110 μM. After the core system was inoculated with strain F, the effluent sulfide concentration decreased to less than 20 μM. Large concentrations of autotrophic, thiosulfate-using, nitrate-reducing bacteria, presumed to be strain F, were detected in the core system for several weeks after inoculation. Concomitant with the reduction in sulfide was a decrease in the concentrations of effluent nitrate, and an increase in the concentration of effluent sulfate. The increase in concentrations of effluent sulfate (1 to 3 mM) was much larger than expected

Table 3
Effect of nitrate addition on sulfide concentrations in brine samples collected from the Southeast Vassar Vertz Sand Unit

Well	Before		After 45 days	
	Nitrate (mg/l)	Sulfide (mg/l)	Nitrate (mg/l)	Sulfide (mg/l)
5-1	<1	35	9	26
5-2	<1	65	2	21
7-1	<1	60	4	23

from the oxidation of sulfide in the formation water (160 μM). This suggested that sulfur-containing compounds had accumulated in the core, which were being oxidized by strain F.

Before starting a field pilot to show the effectiveness of a microbial process to selectively plug high permeability zones and improve sweep efficiency [39], we tested the effectiveness of adding nitrate to control brine sulfide levels. The test was done in the Southeast Vassar Vertz Sand Unit located in Payne County, OK. The formation is a hypersaline environment with brine salinities ranging from 15 to 19%. These brines contain diverse populations of microorganisms, including various kinds of anaerobic halophilic, heterotrophic bacteria, denitrifiers, sulfate reducers, and even methanogens [32, 40, 41]. Other characteristics of the reservoir and injection protocols are described in Knapp et al. [39]. An increase in sulfide levels from <10 mg/l to over 70 mg/l occurred in some brine samples about three months before the start of the test, which probably was a consequence of doubling the rate of brine injection. Forty-five days after the injection of 2.6 metric tonnes of ammonium nitrate into the formation, sulfide levels decreased 40 to 60% in three adjacent production wells, and nitrate was detected in the brine samples (Table 3).

5. CONCLUSIONS

The production of sulfide in petroleum resevoirs is not limited to the activity of sulfate-reducing bacteria. Other organisms, such as *S. putrificaciens*, can use other sulfur compounds for sulfide production. In anaerobic environments, the degradation of organic compounds often involves the concerted action of several different metabolic groups of bacteria. Thus, compounds that are not directly used by sulfate-reducing bacteria can be degraded by other anaerobic microorganisms to potential substrates for sulfate-reducing bacteria. Analysis of organic components in oil field brines and the unequivocal demonstration that some sulfate reducers can use aliphatic hydrocarbons as carbon electron donors for sulfate reduction indicates that carbon availability may not be the limiting factor for sulfide production in many petroleum reservoirs. Thus, the use of flood waters with high concentrations of sulfur oxyanions, phosphates, or nitrogenous materials should be avoided. The use of many anti-oxidants and anti-corrosion chemicals should be reevaluated because many of these compounds are known to be metabolized by anaerobic populations in reservoirs.

Control of biogenic sulfide production can be achieved by a better understanding of the ecology of anaerobic environments. If the cause of souring is microbiological in origin, then the control of any one of the following factors should limit sulfide production:

1. Microbial biomass and activity through water filtration and biocide treatment.
2. The sources and availability of electron donors and acceptors. The use of flood waters high in sulfate and chemical additives that can be readily metabolized by anaerobic microorganisms should be avoided. Alternately, shifting the electron-accepting status of formation water to nitrate reduction may be an effective remediation strategy.
3. Water activity in gas fields and pipelines to limit microbial growth and activity. However, little information is available on the effectiveness of this approach.

Better understanding of the microbiology of petroleum reservoirs will lead to the development of new exploitation practices that avoid the stimulation of unwanted microbial activities. This will ultimately be a more cost effective approach than using expensive remediation strategies once souring has occurred, and more likely to maximize recovery and extend the operational life of the reservoir.

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Additional Oil Production During Field Trials in Russia

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Abstract

In field experiments, we tested an original technology to enhance oil recovery based on the activation of the stratal microflora of flooded oil fields. The technology includes the injection of aerated fresh water with added mineral salts. The activity of microflora (aerobic and anaerobic) increased sharply in the near-bottom zone of the injection well. The microbiological processes occurred in two stages. The first stage includes the activation of aerobic oxidation of organic matter in the oil, which leads to the formation of oil displacement agents, such as organic acids, surfactants, polysaccharides, and carbonic acid. During the second, anaerobic stage gases, methane, and carbonic acid were formed. The field tests were carried out in three pilot tests of different waterflooded areas of the Romashkino field. The additional oil that was recovered reached a total of 41.08 t.t, that was 32.9% of the total pilot production. The enhancement of oil recovery correlated with the rate of methanogenesis. The carbon isotope composition of the carbonates of stratal waters and the concentration of one of the main degradation products (acetate) were changed.

1. INTRODUCTION

The existing techniques of oil-field exploitation allow us to extract not more than a half of the geological reserves. Further, the extraction coefficient has shown a tendency to decrease in several regions due to additional exploitation of fields with viscous oil and to the complicated geophysical conditions. The decrease in oil production in the oil fields as a rule is caused by an increase in flooding. Thus, to maintain oil production at a high level, it is necessary either to exploit new fields, often situated in regions that are difficult to access, or to develop new techniques to enhance oil recovery. Considering the undeniable fact that the natural oil supply is limited and cannot be restored, we conclude that the creation of highly effective methods to enhance oil recovery is a matter of great importance in maintaining a high level of oil production for the coming decades.

Various microbiological technologies can affect the oil strata and so enhance oil recovery [1]. We have elaborated a technology based on the activation of the stratal microflora in waterflooded oil fields. The method involves first the activation of hydrocarbon-oxidizing and methane-producing bacteria. Studies conducted over many years in the oil fields of the USSR have shown that it is possible to influence the bacterial community of the oil strata. The injection into the strata of additional aerated water containing phosphorous and nitrogen mineral salts considerably stimulated the microflora of the area. Stimulation was highest in the near-bottom zone of the injection well. The main target of

this effort was the activation of microbial metabolitic synthesis, enhancing oil recovery from the oil reservoir. We emphasize that the microbial metabolites were formed by the stratal microflora during partial oxidation of the residual oil. This feature is the main distinction between our technology and other MEOR technologies that use injections of organic substrates and/or microorganisms grown in a bioreactor [2,3].

Field trials of our technology in the oil fields of Bashkortostan, Tatarstan, and Azerbaijan have given positive results. This paper deals with experiments carried out in three areas of the Romashkino oil field; the experiments included the following three steps:

1. Preliminary examination of an experimental area to determine the composition and numbers of the stratal microflora, and the background hydrochemical and physicochemical parameters of the oil-bearing strata.
2. Determination of the influence of the trial on the microflora of the near-bottom zone of the injection well to test the possibility of targeted activation.
3. Determination of the influence of the trial on the strata by periodical activation of stratal microflora with further control of the microbiological, hydrochemical and physico-chemical environment. Collection and processing of the data on the enhancement of oil recovery.

2. OBJECTIVES AND METHODS OF INVESTIGATION

The field experiments were conducted in the Romashkino oil field, located in the Tatarstan. Table 1 shows some properties of the oil-bearing rocks of this oil field.

The samples of stratal liquids were taken from production and injection wells in sterile vessels and gassed with N_2 without contact with air. Chemical and physicochemical analysis of the injection and formation waters were made during one day of sampling as described earlier [5]. The microorganisms were enumerated by serial dilutions on different media [6]. The intensity of bacterial methanogenesis and sulfate reduction was estimated using the radioisotopes $NaH^{14}CO_3$, $^{14}CH_3COONa$, and $Na_2{}^{35}SO_4$. The radioactivity of released methane and H_2S were registered on a scintillator [3].

The carbon isotopic composition of methane and carbonates obtained from stratal liquids was measured with a mass spectrometer CH-7 ("Varian-Mat") by the double-beam compensation method, using CO_2 as a working gas. Methane was purified from its gaseous homologs on molecular sieves. Methane purity was monitored by gas chromatography [3]. The content of acetate in stratal waters was measured by ion chromatography with a "Biotronic" ion chromatograph. 5.10^{-4}M paraminobenzoic acid solution was used as an eluent. The sample was first desalinated and, if necessary, concentrated by vacuum distillation.

3. RESULTS AND DISCUSSION

The Romashkino oil field is one of the largest in Europe. Three experimental areas were chosen in different parts of this oil field. The first (Sarmanovskaya area) has three injection and six production wells. The second pilot is located in the Zay-Karatay area and includes one injection and two production wells. The

Table 1
Some properties of oil-bearing rocks and oil of the Romashkino oil field [4]

Depth of rocks	1500-1700 m
Lithology	Sandstones and silty sandstones
Geological age	Upper Devonian
Average porosity	21.8%
Average permeability	500 millidarcies
Type of oil	High sulfurous paraffin
Relative density of oil	0.871-0.876
Temperature of brine	30-40°C

third pilot, from the Aznakaevskaya area, includes two injection and five production wells.

The main experimental data were obtained in the Sarmanovskaya pilot experiment (Figure 1). Three injection wells were used for activation: 12139, 12140, and 12141. In the same area, there are the following six production wells: 12107, 12108, 12109, 12158, 12159, and 12160.

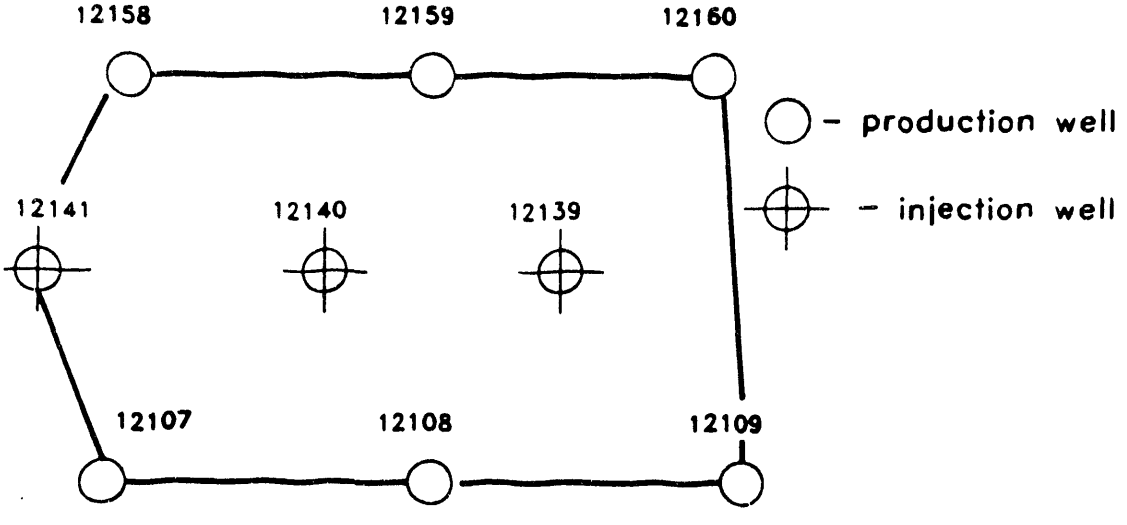


Figure 1. Scheme of the experimental wells in the Sarmanovaskaya area.

Table 2
Chemical composition of stratal waters of the Romashkinskoe oil field

Well Number	Salinity g/l	pH	Eh, mv	Content, mg/l			
				HCO_3^-	SO_4^{2-}	CH_3COO^-	Ca^{2+}
12108	2.5	7.0	+230	494	12	1.4	800
12107	8.1	7.4	- 15	299	15	1.0	500
12158	21.5	6.7	+ 5	287	34	0.9	2600
12159	32.7	6.9	+ 40	311	39	0.8	1500
12160	40.0	7.0	- 10	220	34	2.2	1780

The productive horizons of the oil field are represented by fine-grained sandstones and coarse-grained Devonian silts (D_o). Quartz is the main terrigenous fragmentary material forming the reservoir rocks. Porosity averages 20-22%. The formation depth is 1500-1700 m. The temperature was below 30-40°C. The oil of this field is of the high sulfurous and paraffin type, with a specific density of 0.862-0.871 g/cm^3. In the experimental area, relic Devonian brines were found below the D_o stratal. Therefore, the chemical composition of the waters sampled from the production wells reflects the interaction of the injected surface water with the reservoir rocks. The stratal waters of this field are of the sodium chloride type, and, as a rule, their pH value is higher than 7.0. The mineralization level varied from 2.5 to 40 g/l and Eh from -15 to +230 mv. The stratal waters contained neither oxygen, nor hydrogen sulfide (Table 2).

The Romashkinskoye oil field has been exploited using intracontour waterflooding to maintain a high pressure in the reservoir. Fresh surface waters, as well as those with a different extent of mineralization (waste water) remaining after oil separation, have been used for waterflooding. At the experimental area, waters with mineralization up to 0.5-0.7 g/l and a low sulfate content (40-60 g/l) containing all groups of microorganisms under investigation were injected into the oil-bearing strata. Together with the aerobic microflora, such strict anaerobes as the sulfate-reducing and methanogenic bacteria have constantly penetrated into the reservoir. Microorganisms were introduced into the reservoir with the injected water. A bacterial community, degrading the organic matter of residual oil, formed in the near-bottom zone. This community includes microorganisms which oxidize oil, hexadecane and methane, as well as the saprophytes and oligocarbophiles which also belong to this microbial group. The anaerobic microflora was represented by the methanogenic and sulphate-reducing microorganisms.

Bacterial processes of sulfate reduction and methanogenesis take place in the near-bottom zone. As far as the injected water moved along the strata, the rate of sulfate reduction decreased from 7.8 to 0.5 mkg.S^{-2}.l^{-1}.day^{-1} whereas that of methanogenesis increased from 200 to 1200 nl CH_4.l^{-1}.day^{-1}. The amount of methane produced from the acetate methyl group was equal to 56-89%.

Microbiological analysis of samples taken from the production wells also showed that the stratal waters contained both aerobic and anaerobic microflora (Table 3). There were thousands of saprophytic and oligocarbophilic cells in 1 ml of water; some samples contained up to 10^4 oligocarbophilic cells. The numbers of methane-, hexadecane-, and oil-oxidizing bacteria varied from tens to hundreds of cells in 1 ml. Methanogens were present in all samples and their

Table 3
Number of bacteria (cell/ml) in stratal waters of the Sarmanovskaya area (Romashkino oil field)

Bacteria	Well					
	12140*	12107	12108	12158	12159	12160
Oil-oxidizing	25000	60	60	250	250	25
CH_4-oxidizing	100	1	3	3	60	3
Oligocarbophiles	25000	2400	15000	14000	20000	3700
Saprophytes	25000	80	5000	120	1700	60
Methanogens	1000	25	250	1	25	1
Sulfate-reducing bacteria	1000	1	1	1	1000	100

*Injection well

number reached tens, sometimes hundreds of cells in 1 ml of water. The rate of methanogenesis was 83-123 nl $CH_4.l^{-1}.day^{-1}$. The sulfate-reducing microorganisms were present in all water samples but their numbers were very low (below 1 in 1 ml).

The effect of aeration and the addition of mineral phosphorus and nitrogen salts on the microbial community, which inhabits the near-bottom zone of the injection well, was studied in two kinds of experiments. In the first, injection of an aerated salt solution was followed by daily injections into the strata of 250 m^3 of water over a month. In the second variant, the well was closed for 10 days on the day after reactivation. We found in both experiments that, compared with control measurements, the Eh values in the stratal waters increased as we moved away from the near-bottom zone. Trace amounts of oxygen of about 0.05 ml/l were found in the outflow water in both cases, but in the first experiment the outflow volume was 156 m^3, while in the second one, it was only 22 m^3.

The injection of an aerated solution of mineral salts caused an increase in the oil-oxidizing microflora. In some cases, the difference between the control and experimental data reached 4 orders of magnitude. The maximum value occurred rather far from the bottom, indicative of an increase of the near-bottom microbial biocenosis. The numbers of methanogens also increased, but only by 1-2 orders of magnitude. The deeper the strata from which the samples were taken, the higher was the methanogenic activity. The more reduced conditions were characterized by a higher level of methanogenesis (the first type of experiment). The trial also resulted in an increased number of hexadecane- and methane-oxidizing microorganisms. From these data we conclude that it is possible to activate the stratal microflora from the near bottom zone.

Pilot tests of the MEOR technology, based on activation of the stratal microflora, were carried out according to the following scheme. The process consisted of several cycles of activation of the injection wells. During each, an aerated mineral salts solution was injected into the strata and then fresh water was pumped in. Three injection wells were involved in the experiment. The oil production volume and the level of water content were checked regularly, with concurrent determination of microbiological properties and chemical composition of the stratal waters.

Table 4
Dynamics of microbial activity during field trials (the Sarmanovskaya area)

Well Number	1987	1988	1989	1990
		$\delta^{13}C_{HCO_3}$ ‰		
12158	-16.9	- 0.1	- 0.3	-11.4
12159	-13.9	-11.6	- 7.6	-15.6
12160	-13.2	-10.6	-	-
12107	-13.6	-14.3	-14.5	-13.1
	Acetate in stratal water (mg/l)			
12158	0.9	1.2	11.0	2.5
12159	0.8	3.4	10.2	1.3
12160	2.2	2.4	-	-
12107	1.0	1.8	13.6	5.4
	Rate of bacterial methanogenesis (nl CH_4/l.day)			
12158	121	441	4441	1670
12159	123	535	1464	145
12160	85	433	-	-
12107	83	1107	656	1610

The results showed that the maximum changes occurred in the intensity of bacterial methanogenesis, in acetate content and in the carbon isotope composition of carbonates in the stratal waters.

Table 4 summarizes these results. The data show the existence of intensive microbiological processes along the whole thickness of the oil-bearing strata. Between 1987 and 1989, the intensity of bacterial methanogenesis increased 8-37 times. The carbon of dissolved carbonates became heavier.

The ^{13}C content was maximum in samples with the highest methanogenesis (wells 12158 and 12159). The depletion of ^{12}C in residual carbonate by bacterial methanogenesis (CO_2 reduction to CH_4) is well known.

The content of acetate, one of the main products of bacterial oil degradation, increased considerably (generally, by one order of magnitude). This increase can be considered the result of activation of the aerobic processes of microbial oil degradation.

Thus, we conclude that there is rather intensive activation of microbiological processes in the oil-bearing strata as seen from the following parameters:

1. The increase in acetate content is, first of all, characteristic of the activation of microbial oil destruction in the zone of penetration of the injected molecular oxygen.

2. The increase in the intensity of methanogenesis results from secondary microbiological processes in the anaerobic zone of the oil-bearing strata.

Table 5
Main pilot parameters for MEOR (to July 1, 1992)

Area	Activation date	Initial water cut, %	Cumulative additional oil, T.T.	Total oil production, T.T.	Water cut %	Additional share, %
Sarmanovskaya	08-11.87	85.0	14.30	33.08	87.1	43.2
Zay-Karatayskaya	06-11.88	40.2	4.40	43.37	39.4	10.0
Azbajaevsjaya	06-09.88	95.5	22.38	48.33	94.7	46.3
		Total	41.8	124.78	-	32.9

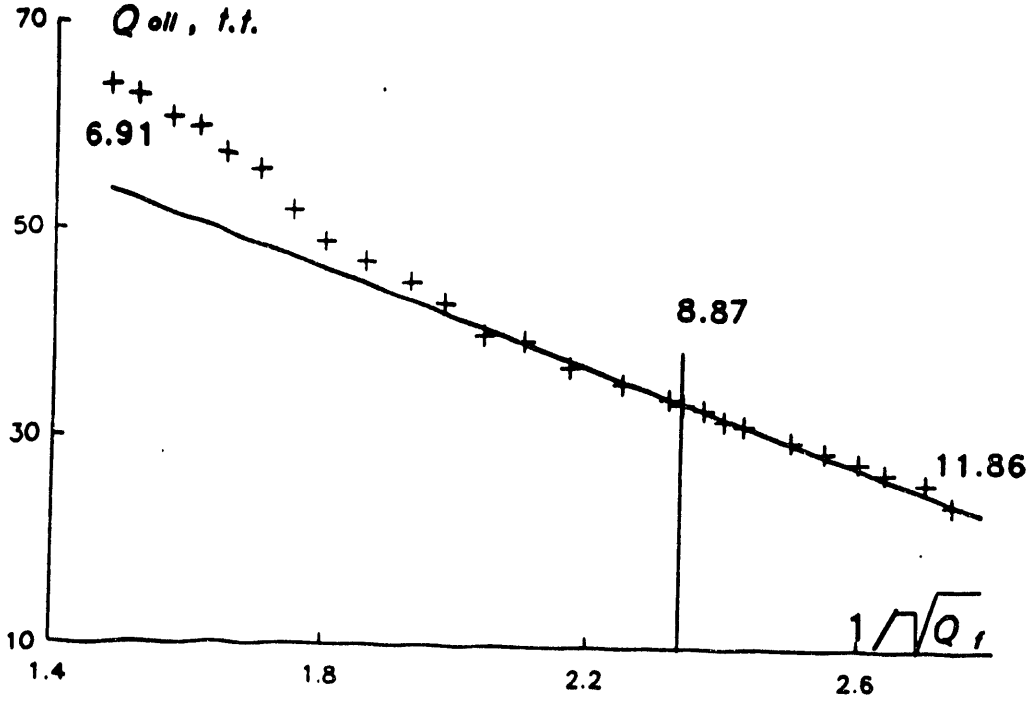


Figure 2. Pilot results of MEOR (Sarmanovskaya area).

3. The fractionation of the stable carbon isotopes in the dissolved carbonate is an integrating index of current microbiological processes. In some cases, the carbonate carbon was heavier as a result of anaerobic methane production. In other cases, it was lighter due to aerobic destruction of organic components in the oil.

The results of field trials in 1990 showed that the activity of microbiological processes in the oil-bearing strata had begun to decrease.

Two pilot tests (in the Sarmanovskaya and Zay-Karatayskaya areas) were stopped due to technological requirements in the first half of 1991 (Figures 2 and 3).

Preliminary results from the Aznakaevskaya area were obtained till July 1, 1992. The process parameters were determined on the basis of different decline curves (Table 5), thus:

1. The total additional production of oil was 41080 t.
2. The MEOR share of the total oil production since the beginning of the experiment until July 1, 1992 was 32.9%.

A good correlation between the activation of microbiological processes and the enhancement of oil recovery was also observed, making the pilot MEOR application profitable.

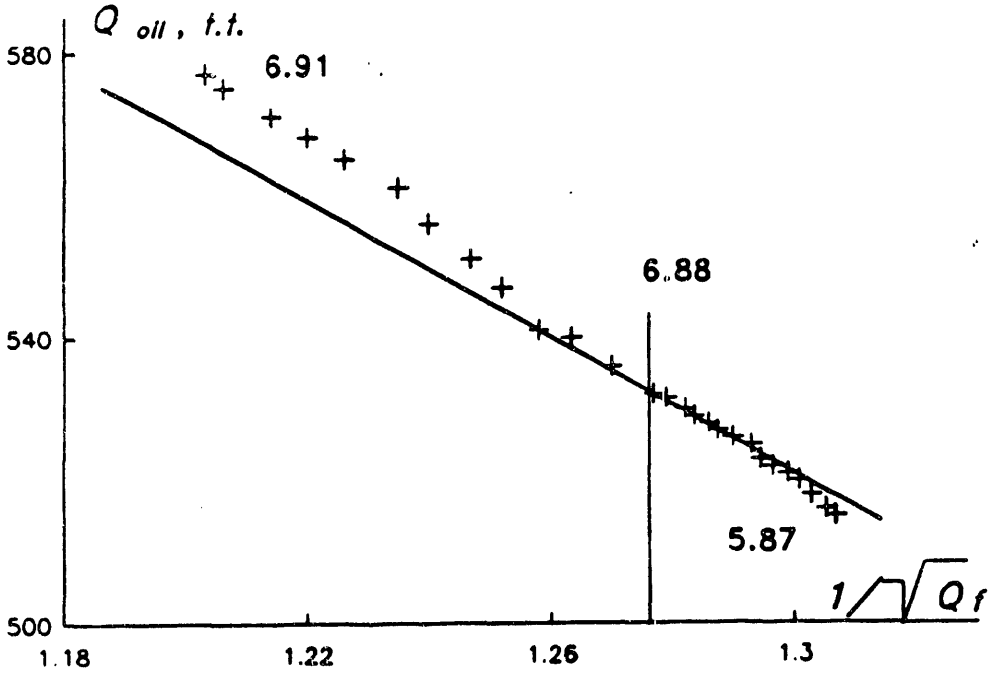


Figure 3. Pilot results of MEOR (Zay-Karatayskaya area).

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Isolation of Thermophilic Bacteria from a Venezuelan Oil Field

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Abstract

A pool of thermophilic bacteria was enriched from the formation waters of a Venezuelan oil field. The reservoir, located at Maracaibo Lake, has a temperature of 60-80°C and a pressure of 1,200-1,500 psi.

Brine samples were amended with different industrial wastes as a carbon source, NH_4NO_3, and traces of peptone. Culture media were incubated anaerobically at 80°C. The best rate of growth and metabolic activity were obtained when maltose-rich waste was used as a carbon source. Alcohols, short chain fatty acids, and gases were the main fermentative products in this medium. Hydrogen sulfide was not detected during growth.

The ability to use fermentable sugars and to grow under reservoir pressure at extremely high temperatures make this pool of bacteria a good candidate for MEOR in the Venezuelan reservoir.

1. INTRODUCTION

Microbial enhanced oil recovery involves the use of microorganisms and their metabolic products to increase oil recovery. However, to succeed, the microorganisms must grow and metabolize under the conditions of pressure and temperature in the reservoir.

In Venezuela, most of the reservoirs under EOR processes have extreme physical conditions, with temperatures of 60-90°C and pressures of 800-2,000 psi. The reservoir under study is C5-VLA8, located at the northwest of Maracaibo Lake. It has a temperature of 80°C and a pressure of 1,200 psi. This harsh environment seems to be unsuitable for microbial growth. However, it was reported that extreme thermophilic bacteria have been isolated from deep-sea, hydrothermal vents with high temperatures and pressures [1,2]. Therefore, it seemed likely that thermophilic-barotolerant bacteria would be present in the C5-VLA8 reservoir.

Another important factor for MEOR is the nutrient supply. Molasses seems to be the most popular nutrient to stimulate microbial growth and metabolism. Other substrates, such as industrial wastes from food processing plants, can be used as nutrients due to their high content of carbohydrates, protein, and nitrogen [3]. Also, they are very economical because prices are low. However, most of these industrial wastes contain elements that are toxic to bacteria; therefore, they must be tested carefully.

The purpose of the present study was to determine whether microorganisms are present in the C5-VLA8 reservoir, culture them under reservoir conditions, and evaluate different industrial wastes to determine the best nutrient for bacterial growth and metabolism.

2. MATERIALS AND METHODS

2.1. Sampling procedure

Brine samples were taken from several wells in the C5-VLA8 reservoir. The samples were collected under anaerobic conditions at the well-head of the producing wells in sterile evacuated bottles. Bacteriological and chemical analyses were performed a few hours later.

2.2 Culture media

Industrial wastes were used as nutrients for microbial enrichment. The total sugar content [4] and total protein content [5] were analyzed in each waste. For the culture media, the wastes were used at 0.5% (w/v), based on the final concentration of sugar. Ammonium nitrate or peptone, at 0.3% (w/v) and 0.05 % (w/v), respectively, were used as a nitrogen source. All media contained cysteine-Na_2S as a reducing agent, and resazurin as a redox indicator. The final pH was 7.2.

2.3. Enrichment culture and isolation procedure

Brine samples were amended with industrial wastes and nitrogen sources to stimulate the growth of indigenous microorganisms. All enrichments were set up under anaerobic conditions in stoppered 50 ml serum bottles [6] incubated at 80°C and at atmospheric pressure. Some samples were incubated under pressure as shown in Figure 1. The pressure cylinders are the piston type, made of stainless steel. The inoculated culture media were pressurized to 1,200 psi with sterlized water using a flow constant pump. During sampling, the inoculated culture was

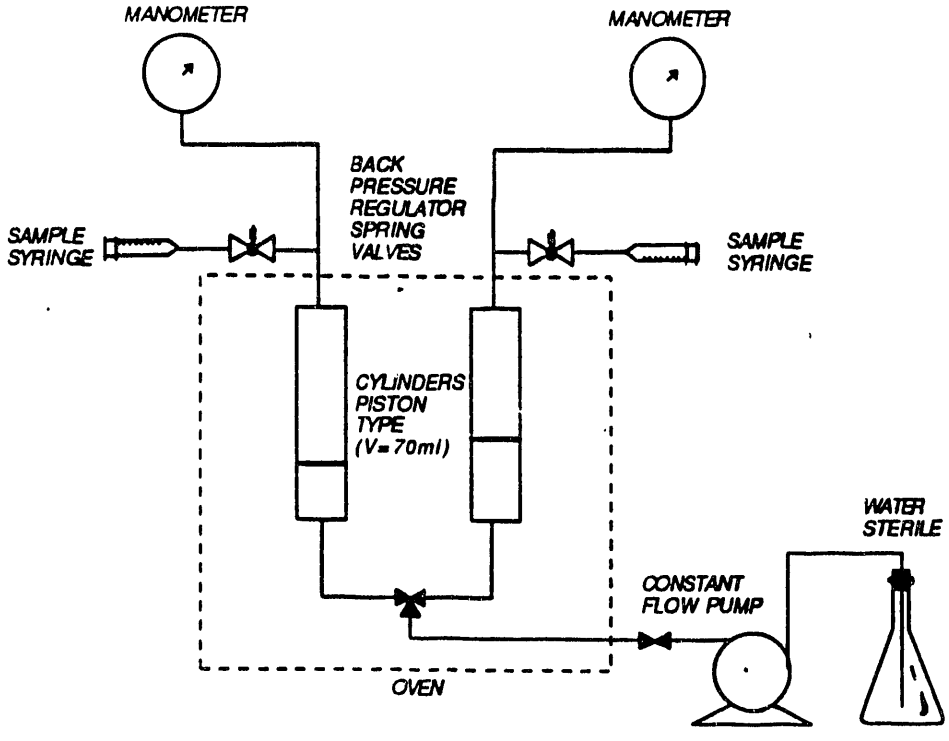


Figure 1. System used to incubate bacterial cultures at high pressures and temperatures.

Table 1
Content of major ions in the brine samples

Ions	Concentration (ppm)
Na^+	2665
Ca^{++}	19
Mg^{++}	10
Cl^-	2856
$SO_4^=$	85
$CO_3^=$	132
$HCO_3^=$	1922
Total Dissolved Solids	7689 (0.77 %)

extruded with a syringe by pressurizing the piston of the cylinder until the system pressure exceeded the setting of the back pressure regulator. The same procedure was followed for the other cylinder.

Dominant species in the enrichment culture were isolated by serially diluting the cultures and plating them on media of the same composition as that used for enrichment, but hardened with Gelrite Gellan Gum (Kelco Division of Merck and Co., San Diego, Ca). Plates were incubated in anaerobic jars at 80°C. Pure cultures were obtained by successive transfers on solid medium.

2.4. Growth and metabolic products analyses

To determine the best industrial waste to stimulate the indigenous microorganisms, growth in five different media was followed by measuring the increase in absorbance at 660 nm in a HP8452A Diode Array Spectrophometer.

Metabolic products, such as volatile acids and alcohols, were analyzed with a VARIAN 3400 Gas Chromatograph at 120°C for volatile acids and 80°C for alcohols using a Carbopack glass column. To analyse the gases produced by bacterial metabolism, samples were taken from the gas phase and injected in a CARLET 500 Gas Chromatograph at 240°C using a series of stainless-steel columns packed with Porapak Q and Molecular Sieve 5Å.

3. RESULTS AND DISCUSSION

3.1. Description of the reservoir and characteristics of the oil and brine

The C5-VLA8 field is located at the northwest of Maracaibo Lake, in northwestern Venezuela. It is a sandstone formation with a temperature of 60-80°C and pressure of 1,200 psi. The depth is 6,000 feet and the porosity and permeability are 25% and 450 mD, respectively. The crude oil is a light type, with a gravity of 28°-30° API. Gas chromatographic analyses of the paraffinic fraction reveals that there is no biodegradation. The brine has a total dissolved solids content of 0.77% and a pH of 7.1. Table 1 shows the content of major ions in the brine. The brines have low salt concentration, as seen by the NaCl values. Carbonates are important anions in the water. However, low levels of divalent cations were detected, indicating the softness of the brine.

Table 2
Chemical analysis of industrial waste

Waste	Total sugar (%)	Total protein (%)	Main sugar
A	48	16	sucrose
B	55	7	glucose
C	81	unidentified	glucose
D	10	0.6	unidentified
E	14	0.85	maltose

3.2. Chemical analyses of the industrial wastes

Five different industrial wastes from food processing plants were tested to determine the best nutrient to stimulate the growth of the indigenous microorganisms. However, it is important to know the chemical composition of such wastes before using them to formulate the culture media. Therefore, total sugar and protein as well as the main sugar present in each waste were analyzed; the results are listed in Table 2. Wastes B and C have the highest sugar content, with glucose as a main sugar, followed by wastes A and E having sucrose and maltose as the main sugar, respectively. Waste D has the lowest sugar and protein content, and the main sugar could not be identified. All these wastes seemed to be suitable for microbial fermentation because they contain sufficient sugar and protein to support bacterial growth and metabolism.

3.3. Isolation, growth, and metabolic products of enrichment

Due to the high temperature of this reservoir, it was important to determine if there were indigenous microorganisms present that were useful for oil recovery.

The use of industrial wastes as nutrient caused an increase in absorbance at 660 nm as early as 2 days of incubation at 80°C. Figure 2 shows the bacterial growth in different industrial wastes. The best growth was found in the maltose-rich waste, followed by sucrose-rich waste. Little growth was obtained in the glucose-rich wastes (media B and C) even though they reached higher absorbance readings after culturing them in a defined mineral media with analytical grade glucose. This finding suggests that some nutrients in those wastes are present in low concentrations, and they are consumed quickly by the growing population, thus creating limiting conditions. No growth was obtained in medium D which indicates the presence of toxic substances.

Table 3 lists the fermentation products of the enrichment in each industrial waste. Organic acids, alcohols, and gases were detected in all media that stimulate growth; these products have been reported to be useful for MEOR.

Two different strains were isolated from the enrichments; their characteristics are listed in Table 4. Both strains are heterotrophic, rod-shaped with a terminal spheroid structure, obligate anaerobes, non-motile, and obligate thermophiles.

This study shows that the brine of C5-VLA8 contains microorganisms which can grow on maltose- and sucrose-rich industrial wastes. They grow anaerobically at the reservoir temperature of 80°C but not at temperatures lower than 55°C. Thus, they will not survive outside the reservoir and their accidental release to the environment will not cause a problem.

Table 3
Metabolites produced

Media	Metabolites
A	i-valeric acid, CO_2, H_2
B	i-valeric and acetic acids, ethanol, buthanol, CO_2, H_2
C	i-valeric and acetic acids, ethanol, buthanol, CO_2, H_2
D	None
E	i-valeric, butiric and acetic acids, ethanol, buthanol, CO_2, H_2

Although only two strains were isolated, there may be more strains in the reservoir that can be selected by other nutrient formulations. Amino acids, vitamins, and specific micronutrients are very important for growth of thermophilic bacteria [7,8], some of which may be missing in those industrial wastes. The microorganisms isolated are able to ferment sugars; as a consequence, they produce organic acids, alcohols, and gases that are useful for oil recovery. Hydrogen sulfide was not detected in any of these enrichments; however, this does not mean that sulfate-reducing bacteria (SRB) are absent.

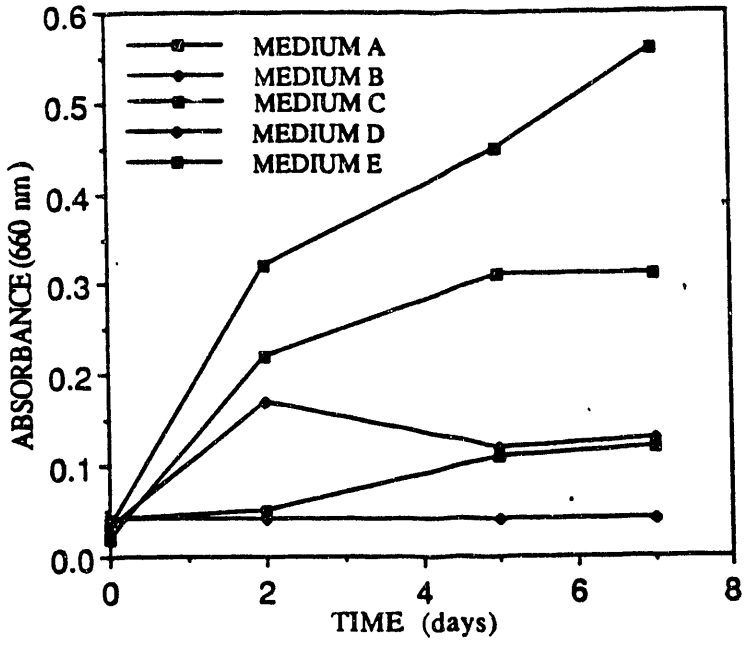


Figure 2. Bacterial growth in different industrial wastes.

Table 4
Characteristics of the isolates

Characteristic	Strain I-1	Strain I-2
Morphology	Rod	Rod
Motility	-	-
Carbohydrate fermented	+	+
Obligate anaerobic	+	+
H_2S produced	-	-
Temperature range	55-90°C	55-85°C

Perhaps, the sulfate concentration is low or nutrients are exhausted by the fermentative bacteria, preventing the growth of the SRB.

3.4. Growth under reservoir conditions

For microbial oil recovery to be successful, the microorganisms must function under reservoir conditions. Therefore, the enrichment also was cultured at 80°C and 1,200 psi (Table 5). It took about 2 weeks to see an increase in absorbance at 660 nm. Growth was very low, and O.D. readings did not increase beyond 0.19 during 6-weeks incubation. Therefore, pressure seems to be an important factor to be considered. It has been reported that the pH of fluids is lowered under pressure [9]. CO_2 solubility increases under hydrostatic pressure, which increases the acidity of the culture media by the formation of carbonic acids. This may be the reason why the bacterial cultures did not grow as well as those cultures at ambient pressure. CO_2 was detected as a metabolic product from these enrichments. In addition, microorganisms become more sensitive to other factors, such as high salinity, toxic metal ions and extreme pH when they are cultured at

Table 5
Growth at the reservoir conditions (80°C, 1200 psi) in Medium A (sucrose-rich)

Time (weeks)	Growth (absorbance at 660 nm)
	0.06
1	0.06
2	0.12
3	0.19

high temperature and pressure [10]. Sometimes, industrial waste have low levels of substances that may be toxic for bacteria growing under these conditions. Therefore, growth under pressure should be evaluated in defined culture media.

4. CONCLUSIONS

There are indigenous microorganisms in the reservoir able to grow at 80°C and 1,200 psi under anaerobic conditions.

Most of the industrial wastes tested can be used to stimulate bacterial growth and metabolism. The best growth was obtained with the maltose-rich waste.

Organic acids, alcohols, and gases, useful for oil recovery, were detected as end products of fermentation. No H_2S was detected in any culture media tested.

5. ACKNOWLEDGEMENT

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The Potential for MEOR from Carbonate Reservoirs: Literature Review and Recent Research

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Abstract

The potential for MEOR in carbonate petroleum reservoirs was assessed in a review of the relevant literature and further examined in the laboratory. This review showed that MEOR appeared to be applicable in about 40% of the carbonate reservoirs surveyed. Microbial acid, gas, and surfactant production are likely to be the more important mechanisms for MEOR from carbonates. A study of the microbiology of native produced fluids showed that a carbonate reservoir may contain a diverse microbial community; however, the number and activity of microorganisms present would probably be low, due to limiting nitrogen and phosphorous. Microorganisms could change the hydrogeologic properties of carbonate rock. The growth of an acid-producing strain through a carbonate rock core increased the permeability from <0.1 to 4.7 mD and the porosity from 11 to 16%. Laboratory study of MEOR from waterflooded limestone-packed cores showed that >40% of the remaining oil-in-place was recovered after three treatments with an acid-producing or a surfactant-producing microorganism, showing that both of these processes can increase oil recovery from carbonates. While further laboratory research is required, there is enough information to support controlled field trials of MEOR from carbonate reservoirs.

1. INTRODUCTION

Over half of the world's petroleum reserves is found in carbonate reservoirs [1], which are distributed worldwide and throughout almost all of the oil-producing areas of the United States [2,3]. However, most research in MEOR has been conducted in sandstone systems, primarily because these possess simpler fluid dynamic properties and are chemically more inert than carbonates. Most carbonate reservoirs have dual porosity flow mechanics [4]. The carbonate rock matrix, which contains most of the trapped petroleum, is often of low permeability. Fractures in the reservoir may contain only a small fraction of the total porosity but dominate reservoir drainage. This property of dual porosity is difficult to capture in the laboratory and to deal with in the field. The presence of high concentrations of divalent cations in brines from carbonates has also complicated the application of most EOR processes in these reservoirs, with the exception of carbon dioxide flooding [5].

The MEOR literature relevant to carbonate reservoirs was reviewed [6] and further research on MEOR from carbonates conducted [7]. This work shows that the prospects for MEOR from carbonate reservoirs should be considered favorable.

2. LITERATURE REVIEW AND FEASIBILITY OF MEOR FROM CARBONATE RESERVOIRS

The literature on MEOR from carbonate reservoirs was found to be sparse [6]. Several field trials in Europe have been described and increases in oil production reported, ranging from 60-200% [6]. In these, enough microbial acid and gas production occurred to lower the pH of produced brines by 1-2 units and significantly increase the carbon dioxide concentration of the produced gas; this showed that sufficient microbial activity can occur in a reservoir to change the properties of the produced fluids. Lazar and his colleagues have considered the presence of carbonates essential for successful MEOR in the field, primarily because of its ability to buffer microbial acid production and allow continued microbial metabolism in the reservoir [8]. The success of the field demonstration of MEOR in a sandstone reservoir in Union County, Arkansas, was attributed to microbial acid production, which reacted with carbonates in the reservoir matrix, leading to an improvement in reservoir fluid flow properties [9]. The observations made from these field trials, combined with the results from the relatively small amount of laboratory research on MEOR from carbonates, lead to the conclusion that the microbial production of acid, gas, and/or surfactant are the important mechanisms for MEOR from carbonate reservoirs [6].

The general feasibility of MEOR from carbonate reservoirs was estimated using data in Dwight's Energy data TOTL System, managed by the Natural Resources Information System at the University of Oklahoma [6]. The physical parameters that could limit MEOR from carbonates were examined. A lower limit of permeability for MEOR of 15 mD was selected based on the discussions by Updegraff [10] and the finding that microorganisms could penetrate carbonate rock with a permeability of <0.1 mD [11]. Other physical conditions permitting, this may not be a limiting permeability if the reservoir has undergone waterflooding. An upper limit of temperature for MEOR of 82°C was selected based on our understanding of the microbiology of hyperthermophilic (>90°C) environments and the report of a successful MEOR field trial in a carbonate reservoir with a temperature of 97°C [12]. An upper limit of salinity for MEOR of 25% was selected based on our understanding of the microbiology of hypersaline environments [13]. Using these criteria, 40% of the 19,297 carbonate reservoirs in the database found in 10 oil-producing states (AR, CO, KS, LA, MS, ND, NM, OK, TX, WY) could be considered candidates for MEOR [6].

3. MICROBIOLOGY OF CARBONATE RESERVOIRS

Most microbiologists have considered petroleum reservoirs as hostile environments devoid of life. This conception has changed as research on the microbiology of subsurface environments continues [6]. We studied the microbial composition of produced native water from four wells completed in a single zone of a carbonate formation using standard analytical procedures and most-probable-number methodology [14,15], similar to those of Bhupathiraju et al. [16]. The goal of this work was to determine if a microbial community existed in these reservoirs, which could be stimulated to support an *in situ* MEOR process.

Table 1 shows the basic properties of the wells and their produced waters. Phosphate, nitrate, nitrite, and ammonium were not detected in any of these produced waters.

Table 2 gives the basic microbial composition of these produced waters. The total number of microorganisms recovered from these samples was low, as expected, given the nutrient limitations found during the chemical analysis of these produced waters. However, the microbial compositions found are consistent with

Table 1
Characteristics of wells/produced waters sampled for microbial composition

Well	Formation	Depth (m)	T (°C)	TDS[a] (%)
Pharoah	Viola	1,800	55	22.3
Froug	Hunton	1,240	44	18.6
Mid Fitts	Hunton	1,040	45	14.5
Ward	Chester J	2,530	63	0.5

[a]Total dissolved solids.

Table 2
Microbial composition of produced waters

Well	Aerobic Heterotrophs	Anaerobic Heterotrophs	Molasses	Molasses-Nitrate	SRB
Pharoah	20	2	2	4	0.4
Froug	nd[a]	0.7	2	1	nd[a]
Mid Fitts	0.1	3	2	2	3
Ward	9	900	90	2	5

[a]Not detected; <0.01 viable cells per ml.

the hypothesis that there is a numerically small, yet metabolically diverse microbial community in these carbonate reservoirs which could be stimulated for MEOR.

4. EFFECT OF MICROORGANISMS ON RESERVOIR ROCK PORE STRUCTURE

The effect of microbial growth through cores of Bethany Falls limestone on this rock's porosity and permeability was examined [17] using an experimental system described by Jenneman et al. [18]. Growth of a solvent-producing species, *Clostridium acetobutylicum*, or a polymer-producing strain, SP018, had little effect on the effective porosity of these cores, but reduced the permeability of the cores, a result of biomass plugging. However, when a strain that carried out a vigorous acid fermentation of glucose, and which produces only a relatively small amount of biomass during fermentation, *Vibrio aspartigenicus*, was used to treat a limestone core with no measurable permeability, a dramatic result was observed. The permeability of this core was increased from <0.1 to 4.7 mD and its porosity increased from 11 to 16%. This result confirmed the finding of Myers and McCready [11] that bacteria could penetrate carbonate rock which had no detected permeability.

Table 3
Microbially enhanced oil recovery from unconsolidated limestone cores

Microorganism	Treatment	Cumulative Oil Recovery (%)
Vibrio aspartigenicus GSP-1	1	15
	2	33
	3	44
Bacillus licheniformis JF-2	1	18
	2	32
Control	1	2
	2	4
	3	7

5. MEOR FROM UNCONSOLIDATED CARBONATE CORES

MEOR from unconsolidated limestone cores packed with crushed and sized (20-50 mesh), oil-wet Viola limestone was investigated in the laboratory [19,20]. After these core systems were assembled, the cores were waterflooded with a 10% brine to a point of residual oil saturation, a glucose/yeast extract in brine medium was introduced into the core, and the cores were inoculated with *Vibrio aspartigenicus*, which produces about three carboxylic acids per glucose fermented, or with *Bacillus licheniformis* JF-2, a surfactant-producing species [21]. An uninoculated control core also was constructed. These cores were shut in for 14 days, then waterflooded with brine; any oil recovered was measured. Nutrients were re-introduced and the treatment repeated. Acidic end-products of metabolism and calcium also were measured from the core effluents; for every two carboxylic acids produced a calcium ion was released from the limestone in the core. The microbiology of the cores was confirmed after each treatment. Table 3 shows the oil recovery observed from these unconsolidated carbonate cores.

These results show the utility of both acid and surfactant production for MEOR from carbonate reservoirs. We think that this is an impressive demonstration of MEOR in a laboratory system.

6. FUTURE RESEARCH

Given the paucity of the literature on MEOR in carbonate systems, there is a clear need for further laboratory research. While significant progress has been made during the past few years, we still need to rigorously examine the mechanisms of MEOR from carbonates. The microbiology of carbonate reservoirs merits more research, with an accounting for the effects of pressure, high concentrations of divalent cations, and high concentrations of dissolved carbon dioxide on this microbiology. Most importantly, MEOR experiments in laboratory systems, which capture the dual porosity nature of many carbonate reservoirs, need to be done; this will likely require using a larger scale system than one or two inch diameter cores, given the properties of many carbonate rocks.

However, we have sufficient information and knowledge to conduct field trials of MEOR in carbonate reservoirs. It would appear that some approaches for MEOR from carbonates are relatively simplistic, especially the stimulation of *in situ* microbial acid production. Given the potential of MEOR from carbonates and the problem of well abandonment in the United States, the time to start this field work is now.

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Using Bacteria to Improve Oil Recovery from Arabian Fields

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Abstract

Large quantities of residual oil remain in Arab oil reservoirs after the primary recovery and waterflooding stages. Because of the large area of the Saudi oil fields, the amount of residual oil is enormous. According to the latest publications, the original oil in place in Saudi Arabia is about 700 billion barrels. Only around 250 billion barrels, 35% of the total oil in place, can be produced by conventional methods. More than 90 billion barrels, as much as twice the proven reserve of the United States and Canada combined, could be added to Saudi Arabia's proven reserve if only 20% out of the 450 billion barrels left in place was produced through methods for enhanced oil recovery. Any method that can recover a significant part of this residual oil would be of great importance and should be investigated. Microbial Enhanced Oil Recovery (MEOR) has been recognized as a potentially cost-effective method of recovery.

This paper investigates the applicability of MEOR for recovering more of the oil under the Arab oil fields. Based on the analysis of data from more than 300 formations in seven Arab countries (Saudi Arabia, Egypt, Kuwait, Qatar, United Arab Emirates, Iraq, and Syria), we studied the possibility of applying MEOR to the Arabian area. The basic parameters studied include the permeability of the formation, reservoir pressure and temperature, crude oil viscosity and API gravity, and connate-water saturation in the formation and its salinity. We found that the Saudi, Iraqi, and Egyptian oil fields are very good candidates for MEOR processes; Qatar, Kuwait, and Syria have some potential for MEOR. However, because of the conditions of its reservoirs, the oil fields of the United Arab Emirates have no potential for MEOR.

MEOR is expected to recover up to 30% of the residual oil under present conditions in the Arab reservoirs. However, the actual recovery can only be determined through laboratory and pilot tests under field conditions. A new technology should be developed so that MEOR can be applied successfully.

1. INTRODUCTION

Bacteria are the only microorganisms that have been proposed for use in enhanced oil-recovery processes. They are small, grow exponentially, and they produce metabolic compounds, such as gases, acids, surfactants, and polymers. Bacteria can tolerate harsh environments, such as high formation-water salinity, high pressure, and high temperature. In 1983, Bubela [1] found that the optimum metabolic temperature and rate of growth of rod-shaped bacteria increased with an increase in pressure. Moses and Springham [2] observed that bacteria are catalytically active at high pressure. Grula et al. [3] readily grew *Clostridium* sp. in salt concentrations up to 75000 ppm.

The earliest realization that bacteria are beneficial to the production of oil was made by Backman in 1926 [4]. ZoBell in 1946 presented a process for secondary oil recovery, using anaerobic, sulfate-reducing bacteria *in situ* [5]. Earlier, this investigator had used other types of bacteria to enhance oil recovery in laboratory tests [6].

In 1963, Kuznetsov et al. found that bacteria discovered in some oil reservoirs in the Soviet Union produced 2 gm of CO_2 per day per ton of rock [7]. Later, Synyukov et al. [8] employed microorganisms to aid in the recovery of oil.

Laboratory studies have been made with specific microorganisms either for the surface production of various compounds, or by injecting the cells into a reservoir for *in situ* metabolic production. Both methods enhance oil recovery. Grula et al. isolated salt-tolerant strains of some types of bacteria in the laboratory and then conducted field tests with them [9]. Donaldson and Grula [10] found that some species of bacteria produce emulsifiers in salt concentrations up to 75,000 ppm. Laboratory results by Torbati et al. [11] showed that the larger pores of Berea sandstone are plugged by bacteria, which reduced its permeability, thus increasing oil recovery by improving the mobility ratio. Other laboratory researches conducted by Bryant and Douglas [12] demonstrated the mechanisms of crude oil displacement by microorganisms.

Bryant et al. [13] reviewed many field applications of MEOR. Bryant [14] found that MEOR screening criteria fit 27% of U.S. oil reservoirs. Recently, MEOR field applications were presented in the proceedings of an international conference on MEOR edited by Donaldson [15]. Hitzman [16] also recently reviewed MEOR field testing.

2. MECHANISMS

Many species of microorganisms produce carbon dioxide and other gases, such as nitrogen (N_2), hydrogen (H_2), and methane (CH_4) that could improve oil recovery by increasing pressure and by reducing the viscosity of crude oil that improves the mobility ratio.

Because many types of microorganisms produce polymers, they have been used to plug high-permeability zones in petroleum-saturated sandstones to improve sweep efficiency and displace bypassed oil. However, these microorganisms also have been shown to reduce the permeability of rock [13,14,17-19]. Work in the Netherlands [20] involving selective plugging experiments with *Betacoccus dextranicus* reported a significant increase in oil production.

Recently, research in China generated novel microorganisms that produce polymers [21] and researchers at the University of Calgary, Canada have reported a methodology for using ultra microbacteria to selectively plug a subterranean formation [22].

The evaporation of volatile hydrocarbons and the destruction of paraffinic compounds by microorganisms generates large amounts of polynuclear aromatic compounds that degrade asphaltic material [23].

Microbes also produce low molecular-weight acids, primarily low molecular-weight fatty acids, that can improve permeability of limestone and sandstone rocks with carbonaceous cementation, and thus, improve oil recovery.

A potentially useful group of microorganisms produce alcohols and ketones. These compounds are typical co-surfactants that are used in microemulsion solutions for stabilizing and lowering interfacial tensions which promote emulsification.

Microorganisms produce bio-surfactants that can decrease surface and oil-water interfacial tensions as low as 5×10^{-3} dyne/cm which causes emulsification [24]. Several types of microorganisms that produce bio-surfactants have been identified and isolated [25-28].

Microbes have been shown to alter the wettability of glass micro-models in Berea sandstone [12]. In 1986, Kianipay et al. [29] found that *in-situ* microbial

growth mobilized residual oil by reversing wettability. Table 1 summarizes the different mechanisms of microbial-enhanced recovery.

2.1. Miscibility in oil reservoirs due to the production of natural gases and surfactants by bacteria

To mobilize the residual oil droplets, miscibility or a high pressure gradient is required. It is recognized that the efficiency of a given process to recover this amount of residual oil depends upon capillary and interfacial forces. The miscible process consists of displacing oil by injecting microorganisms that produce a solvent which is completely soluble in oil.

2.1.1. Natural gas

Figure 1 shows a phase diagram of a solvent/hydrocarbon system, assuming that the solvent (produced gas) consists entirely of the light component (G) [30]. Point O represents the composition of the crude oil, which must be rich in intermediate components. The displacement process is not first-contact miscible because the dilution path, OC_1, passes through the two-phase region.

The crude oil (O) and the gas produced (G) are not in thermodynamic equilibrium. Phase exchange takes place, and, as a result, generates the overall composition, M_1. The mixture splits into two phases, a gas G_1, and a liquid L_1, determined by the tie lines. The gas G_1 has a much higher mobility than L_1 and comes into contact with the newly formed residual oil of composition O. Phase exchange takes place to form mixture M_2, which splits into gas G_2 and liquid L_2; G_2 flows to form mixture M_3, and so the process goes on.

This process continues until the composition of the gas in contact with the oil becomes G_t (i.e., the gas phase will no longer form two phases on mixing with the crude). At this stage, the process has developed miscibility. The formation of the miscible bank is schematically shown in Figure 2; it is extremely stable. The design of a miscible process by injecting bacteria needs extensive laboratory studies and pilot tests. The parameters involved in the design encounter both the physical properties of crude oil viscosity and the characteristics of the reservoir, such as depth, temperature, pressure, and permeability.

2.1.2. Carbon dioxide

There is increasing interest in using carbon dioxide (CO_2) to displace oil from porous media. When carbon dioxide is produced from microorganisms injected into a reservoir, the gas dissolves in the crude oil and water under reservoir conditions and reduces the oil's viscosity, causing it to swell. In miscible carbon-dioxide flooding, liquid CO_2 mixes with oil, and through a mechanism of multicontact-phase equilibrium, eventually achieves miscibility, which increases the efficiency of recovery. Miscibility conditions are required to mobilize the trapped oil droplets.

Another principal effect of carbon dioxide is the action of carbonic acid, formed in solution with water [31]:

$$CO_2 + H_2O \leftrightarrow H_2CO_3$$

Its effect on calcareous rocks is to increase injectivity by partially dissolving the rock according to the following equations [31]:

$$H_2CO_3 + CaCO_3 \leftrightarrow Ca(HCO_3)_2$$

$$H_2CO_3 + MgCO_3 \leftrightarrow Mg(HCO_3)_2$$

Table 1
Mechanisms of enhanced oil recovery

Process	Type of microorganism used and displacement mechanism
Enhanced waterflooding	Microorganisms that produce low molecular-weight acids (improve formation permeability)
Improved oil recovery by gases	Microorganisms that produce gases such as CO_2, N_2, H_2, and CH_4 (improve mobility and miscibility)
Microbial permeability modification	Microorganisms that produce polymer and/or copious amounts of biomass (improve sweep efficiency)
Microbial polymer flooding	Microorganisms that produce polymers (improve mobility)
Microbial surfactant flooding	Microorganisms that produce surfactants and alcohols (improve miscibility and reduce capillary forces)

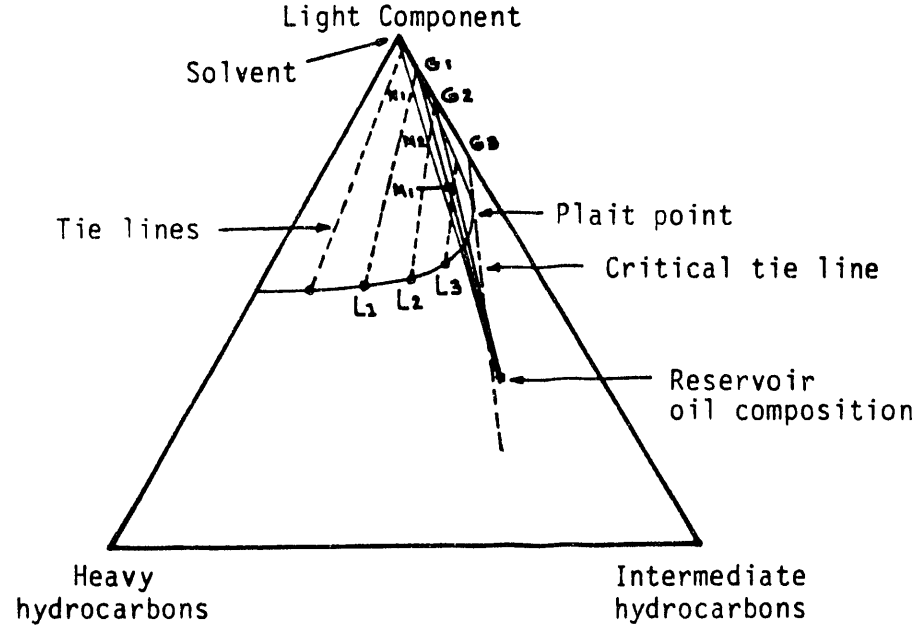


Figure 1. Schematic of the vaporizing gas drive process [30].

These biocarbonates formed are soluble in water, and so may increase the absolute permeability of the carbonate rock. Bubela [32] found that the porosity and permeability of calcite were increased by the presence of bacteria from 17% to 45% and from 2 darcy to 47 darcy, respectively, while those of the dolomite were increased from 24% to 27% and from 2 darcy to 14 darcy, respectively. These increases are due to the biological production of fatty acids and CO_2. Cementation resulting from the diagenesis of carbonates, due to an alteration of the CO_2 level in the interstitial fluids, decreased the permeability of the simulated sediments quite considerably.

The residual oil saturation obtained by producing CO_2 from bacteria is lower than that obtained by producing natural gases, which will improve the oil recovery factor.

2.1.3. Surfactants

Information on the phase equilibrium of the surfactant-oil-water systems is needed to formulate systems that exhibit small, two-phase regions. At low concentrations of surfactants, the contribution of phase equilibrium to oil recovery comes from the changes in phase volumes. Changes in the system's conditions, such as salinity, temperature, and the molecular weight of surfactant, tend to alter the phase behavior of these systems [33].

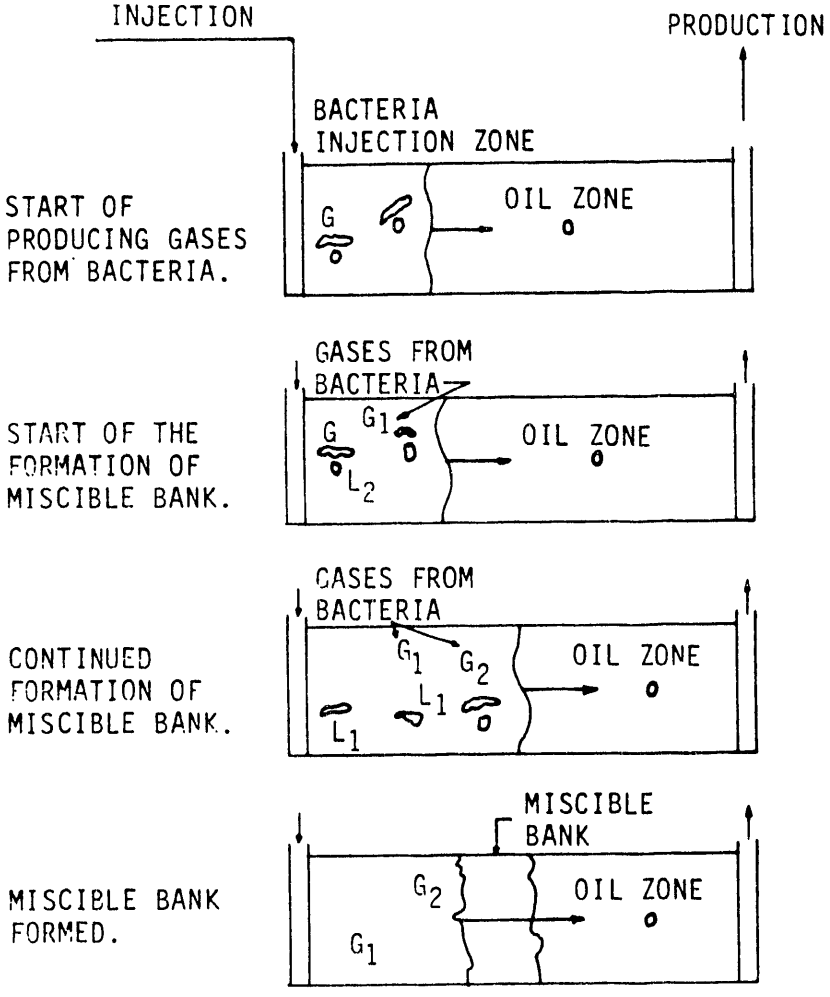


Figure 2. Formation of miscible bank by gases from bacteria.

Phase-diagram studies, under reservoir conditions, provide a powerful way to predict the formation of different phases in the reservoir as a function of composition. Figure 3 is a ternary diagram of a surfactant-crude-brine system. The binodal curve (dashed line) divides the diagram into a single-phase region above and a multiphase region below. Surfactant concentrations in excess of 15 percent are of no interest to tertiary oil recovery for economical considerations. Therefore, the composition of the injection for practical surfactant flooding is bounded from below by the binodal line, and from above by economic restrictions on the concentration of the slug. Based on the phase diagrams, some investigators [34] defined the displacement process by surfactant as a locally miscible displacement (along MY and MW in the phase diagram). If it is not locally miscible, it is an immiscible displacement (along YC). In other words, miscibility is a property of pairs of compositional points. Decomposition of the surfactant slug always implies associated changes in sulfonate concentration, viscosity, and interfacial properties. This behavior can be predicted using ternary diagrams.

2.2. Economics of MEOR

At the end of 1990, the world had 1015 billion barrels of proven reserves of crude oil. This amount is available through conventional production technology. The remaining oil in place, however, can reach up to three times as much, so, that at least 3000 billion barrels of oil are targeted for enhanced oil recovery technology. In Saudi Arabia alone, there will be more than 500 billion barrels of oil left in place after depleting its proved reserves by conventional recovery (amounting to 260 billion at the end of 1990). The ever-increasing world demand for oil calls for new recovery technology to economically produce the oil left in place. One of the emerging EOR technologies which holds promise as a cost-effective method for producing more oil is Microbial Enhanced Oil Recovery (MEOR).

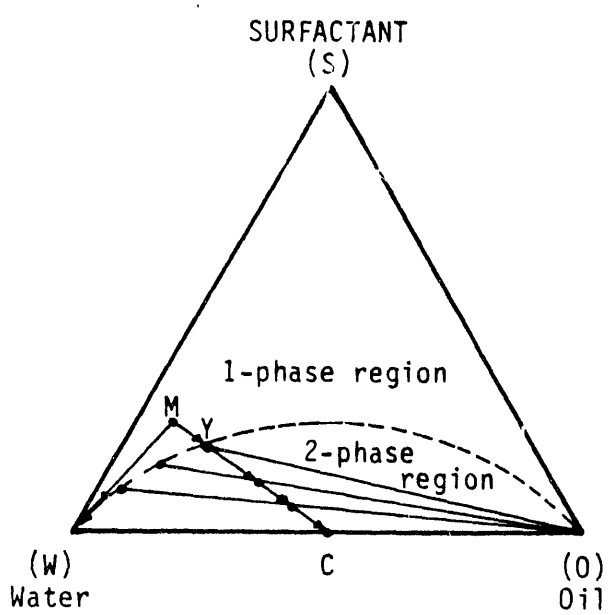


Figure 3. Surfactant-water-oil phase diagram.

2.2.1. Economic advantages of MEOR

Several publications have shown that MEOR is potentially a cost-effective technology for increasing oil production [13]. Many advantages of using MEOR can be cited; some of the most important are the following [13,35]:

1. The injected microorganisms and nutrients are inexpensive and easy to obtain and handle in the field.
2. MEOR is economically attractive for marginally producing fields.
3. The cost of the injected fluid is not dependent on oil prices.
4. The implementation of the process needs only minor modifications to existing field facilities, which reduces cost.
5. The method is easily applied with typical surface equipment for waterflooding.
6. MEOR is less expensive to install and more easily applied than any other EOR technique.
7. MEOR products are all biodegradable and will not accumulate in the environment.

2.3. Results of an improvement in recovery using MEOR

The following section summarizes some reports of improvements in recovery using MEOR.

The *Journal of Petroleum Technology* reported in its Technology Digest (September 1991) that tests at the Alton field in Queensland, Australia, showed that Biological Oil Stimulation (BOS) increased oil output by 70% [36]. The same study estimated that MEOR technology could unlock as much as 300 billion barrels of Australian oil left in place after conventional technology.

Injection of microorganisms and molasses improved the rate of oil production at the Mink Unit project by about 13% [37]. In the same application, Water-Oil Ratio (WOR) was reduced in producing wells by as much as 35%. Bryant et al. [12] showed that microorganisms and molasses nutrient can recover an average of 32% more of a light crude oil (Delaware-Childers) from Berea sandstone cores than after laboratory-simulated waterflood. Kianipey et al. [29] reported that the *in situ* growth metabolism of injected bacteria decreased residual oil saturation in the unconsolidated, thin reservoir flow cells by 9-24%. Another study [38] showed that a field core produced 28% more residual Mink crude oil than the waterflood process. Richard et al. [39] demonstrated that between 10 and 39% of the oil left behind in the cores after waterflood could be recovered.

3. SCREENING CRITERIA

The data from the Middle East oil fields provide the characteristics of oil reservoirs that can be used for MEOR field projects (see Table 2). Extensive research is going on today to develop new technologies in bio-technological processes that can be used under actual reservoir conditions of temperature, pressure, rock permeability, and water salinity.

The reservoirs of the Arabian Gulf area are characterized by the relatively high salinity of the formation waters that puts a serious limitation on the use of MEOR. Most screening criteria used a TDS upper limit of 100,000 ppm. Rock permeability ranges of 1-1000 md have been reported for MEOR field projects [14]. No MEOR field projects have been carried out where pressures and temperatures were too high for microbial growth. The usual biological limitation for temperature is about 160°F, and for pressure, it is about 20,000 psi. In Saudi reservoirs, the temperature and pressure ranges from 140 to 240°F and from 2000 to 5500 psi, respectively, which means that MEOR processes can be applied. The

Table 2
Screening criteria [14]

Parameter	Range suggested
Reservoir rock permeability	>75 md, unless highly fractured
Reservoir depth	<8000 ft
Crude oil type	>15° API; as yet, not enough information available for heavier crude oils
Reservoir temperature	<170°F
Salinity of reservoir formation	<10% sodium chloride; total TDS may be higher

permeability of the formation rock in Saudi oil reservoirs ranges from 100 to 3000 md, which is a wide range for MEOR application. Sayyouh and Al-Blehed [40,41] studied on the screening criteria for enhanced recovery of Saudi crude oils including thermal and non-thermal processes; however, MEOR was not included in the non-thermal methods.

4. FIELD DATA AND POSSIBLE APPLICATIONS

Table 3 summarizes the literature on field tests made using microorganisms.

The conditions in the oil fields in more than 300 petroleum formations in the Arab world were surveyed, and a summary for each country is presented in Table 4. Figure 4 shows the variations in rock permeability for six Arab countries. The average rock permeability ranges from less than 10 md to 3000 md, which includes permeabilities suitable for MEOR applications in Saudi Arabia, Qatar, Iraq, and in some Egyptian oil reservoirs.

The average depth for all reservoirs in the Arabian area ranges from 1000 to 12000 ft (Figure 5). The UAE is not suitable for MEOR due to the large average depth of the oil fields (i.e., from about 8000 to 12000 ft), as shown in Figure 5. However, Syria has a range of formation depth between 2000 to 6000 ft, which is suitable for many types and species of bacteria. Apparently this range is beneficial, because the optimum rate of growth of microorganisms is higher. Figure 5 also shows that Saudi, Egyptian, Kuwaiti, and Iraqi oil fields have some formation depths within the range of MEOR application.

Figure 6 shows the API gravity ranges of the different crude oils; all the API gravity of the Middle East crudes are within the range of MEOR applications, although some Iraqi crude oils are not.

Although the oil production in Saudi Arabia, Kuwait, Qatar, and UAE is essentially still in the primary and secondary phase, the production of oil in Egypt, Syria, and Iraq is mostly in the secondary phase because of the relatively old oil fields. The waterflooding of the older oil fields is becoming less productive. The oil left in place is trapped by capillary forces and needs more force to be displaced. The technology of microorganisms has improved so that it is possible to determine their nutrient requirements, metabolic products, such

Table 3
Some field applications on MEOR [16,20]

Location	Geology and lithology	Injected Bacteria	Response	Depth	Temperature °C	Permeability	Oil viscosity	Density or API
Romanian Field, Romania	Sand with a high content of marls and clays	*Bacillus* *Clostridium* *Pseudomonas* *Escherichia* *Arthrobacater* *Mycobacaterium* *Peptococcus* Bacteria accounts: $4x10^8$-$9x10^9$/ml	Bacteria produce gases and acids Increase in CO_2 in the produced water Increase in oil production: - Baicoi Reservoir: +2633 tons - Vata Reservoir: +32-1 tons (from 1977 to 1983) Increase in oil viscosity and density and light fractions increased in oil	336-1559m	27-55	100-1500 mD	6-53 cp	0.85-0.91 kg/dm^3

Salinity: 5-180 g/li

Table 3
Some field applications on MEOR (cont.)

Location	Geology and lithology	Injected Bacteria	Response	Depth	Temperature °C	Permeability	Oil viscosity	Density or API
Lioyd-minister Field Canada	Unconsolidated sand	*Leuconostoc mesenteroides* Bacteria accounts: 10^2-10^3/ml for aerobes and 10^4/ml for anaerobic 11.6 m^3 Molasses in 120 m^3 water	Bacteria produce acids and alcohols Decrease in pH and surface tension	650 m (5.4 m pay thickness)	21	1500 mD (porosity-30%)	heavy	API-15° 973.8 Kg/m^3 at 150°C

Salinity: 6% brine

Table 3
Some field applications on MEOR (cont.)

Location	Geology and lithology	Injected Bacteria	Response	Depth	Temperature °C	Permeability	Oil viscosity	Density or API
Lisbon Field, Union County Arkansas, U.S.A.	Nacatoch sand; a loosely unconsoilidated sand with 8% carbonate content	Beet molasses was added to injected water (4000 gal of 2% by wt.)	Bacteria produce gases (mainly CO_2 and H_2) and acids	1920 ft (30 ft net pay) thickness	90-105 (current 96°F)	5770 mD (porosity = 30.5%) (air permeability = 408 mD)	4.48 cp @100°F 8 cp @ 60°F	API=36° @ 60°
		Clostridium acetobutylicum suspensions	CO_2 content of the gas produced varied between 39-82% (air free) Methane CH_4 (80% increase) was produced (Some CO_2 and H_2 was converted to methane) Oil production increased from 0.6 to 2.1 BOPD in some wells Maximum increase in production rate was 250%					

Salinity: 42000 ppm

Table 3
Some field applications on MEOR (cont.)

Location	Geology and lithology	Injected Bacteria	Response	Depth	Temperature °C	Permeability	Oil viscosity	Density or API
Okmulgee Field, Oklahoma, U.S.A.	Sandstone (gas drive reservoir)	*Clostridium* (counts 10^7/ml)	Produced water contained ethanol, acetone, n-butanol, and CO_2	1750 ft	-	-	-	-
	(Gas composition: O_2, N_2, CH_4, CO_2, H_2S, and H_2).	Molasses (8.6%) in salt water (3% NaCl)	Oil viscosity increased, indicating oxidation and loss of dissolved CO_2 CO_2 considered important for increasing oil recovery					
Mineiuz-Rockdale Field, U.S.A. Micro-Bac Gulf Coast Inc., USA	Fine silty sandy shale - highly laminated from sand shale to shaley sand	-	Oil production increased from 1.5 to 4 BOPD	908-1022 ft	-	-	-	-

Table 3
Some field applications on MEOR (cont.)

Location	Geology and lithology	Injected Bacteria	Response	Depth	Temperature °F	Permeability	Oil viscosity	Density or API
Delaware, Childers, OK, U.S.A.	Sandstone	*Bacillus Clostridium* Nutrient = molasses 22 gal/ injection well	Bacteria produce gases (mainly CO_2), acids, and surfactants. Produced gas composition: Methane 60% CO_2 25.8% Butane 2.8% Pentane 2.6% Octane 3% About 19% increase in recovery	600 ft (porosity = 20%)	80	52 mD	7 cp	35°
West Germany Dutch I Dutch II	Limestone	-	-	4068 ft	150	10-50 mD	-	30.6°
USSR	Sandstone porosity = 20-23%	Bacteria in molasses (from 6 wells)	3% CO_2 produced Oil viscosity increased. Oil recovery = 37-40 m ton/d for 4 months	(1000-1200 m) 5577 ft	90	500-1000 mD	-	Heavy oil (asphalt)

Salinity: <0.02% - Delaware, Childers, OK
Salinity: 23% TDS - West Germany, Dutch I and Dutch II
Salinity: 0.02% TDS - USSR

Table 3
Some field applications on MEOR (cont.)

Location	Geology and lithology	Injected Bacteria	Response	Depth	Temperature °F	Permeability	Oil viscosity	Density or API
Australia	-	-	-	-	-	260 mD	-	-
Hungary	Sandstone and Limestone	Anaerobic thermopiles in molasses (from 10 wells)	Recovery - 126% yield from 12 wells	650-8061 ft	207	10-700 mD	-	-
Poland	Porosity - 13-25%	*Clostridium* in molasses (from 17 wells)	CO_2 produced increased Recovery - 20 - 200%	1325-3753 ft	-	-	-	-
Czechoslovakia	Porosity - 22-36%	*Desulfouibric Pseudomonas* in molasses.	Oil viscosity decreased Recovery - 50% (from 7 wells)	164-230 ft	-	3000-8100 mD	-	-

Table 4
Oil reservoir data for different Arab countries

	Saudi Arabia		Qatar		Kuwait		
Property of Reservoir	Northern Area	Southern Area	On-Shore	Off-Shore	Northern Area	Southern Area	Neutral Zone
Depth (ft)	4100-6800	5200-8000	5600-6600	7000	8300-8600	4800-10000	1100-1700
Lithology	Sandstone (Wasia)	Carbonate (Arab D)	Limestone	Limestone	Sandstone and carbonate	Carbonate, except Burgan	Sandstone and carbonate
Thickness (ft)	20-200	100-300	200-400	80-400	200-1400		100-250
Porosity (%)	20-29	14-22	18		18-24		20-35
Permeability (MD)	1000-3000	100-500	65-150	100-500			
Oil gravity (°API)	27-34	37-42	38	28-33	26-34	18-23	

Table 4
Oil reservoir data for different Arab countries (cont.)

	Iraq		UAE			Syria	Egypt
Property of Reservoir	Northern Area	Southern Area	On-Shore	Off-Shore	Dubai		
Depth (ft)	2800-6500	10000-11000	7500-7900	8500-9150	7500-12900	2000-6000	2105-11900
Lithology	Carbonate	Sandstone	U. Thamana Limestone	U. Thamana Limestone	Limestone	-	Sandstone and carbonate
Thickness (ft)	-	200-300	81-170	100	-	-	-
Porosity (%)	20	20-25	25-30	19-29	-	-	-
Permeability (MD)	200	400-1000	15-80	1.5-30	3-70	5.3-3000	-
Oil gravity (°API)	Shallow heavy oil 10 deeper light oil 34-42		41-44	37-39	30-50	-	20-38

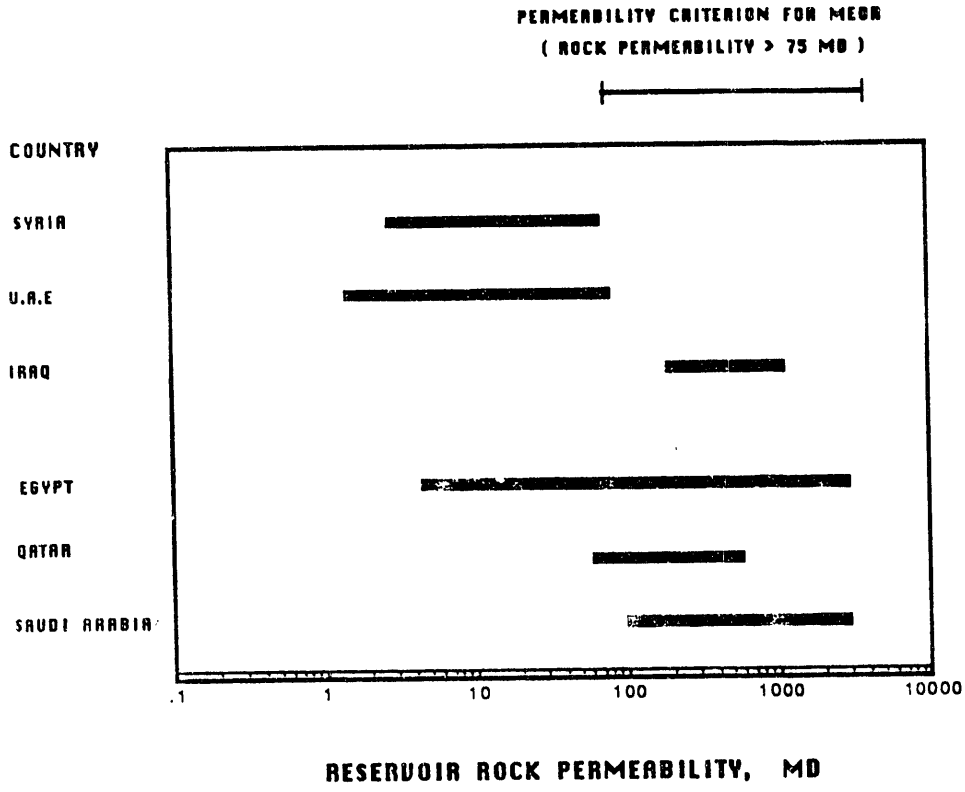


Figure 4. Rock permeability for different crude oil reservoirs.

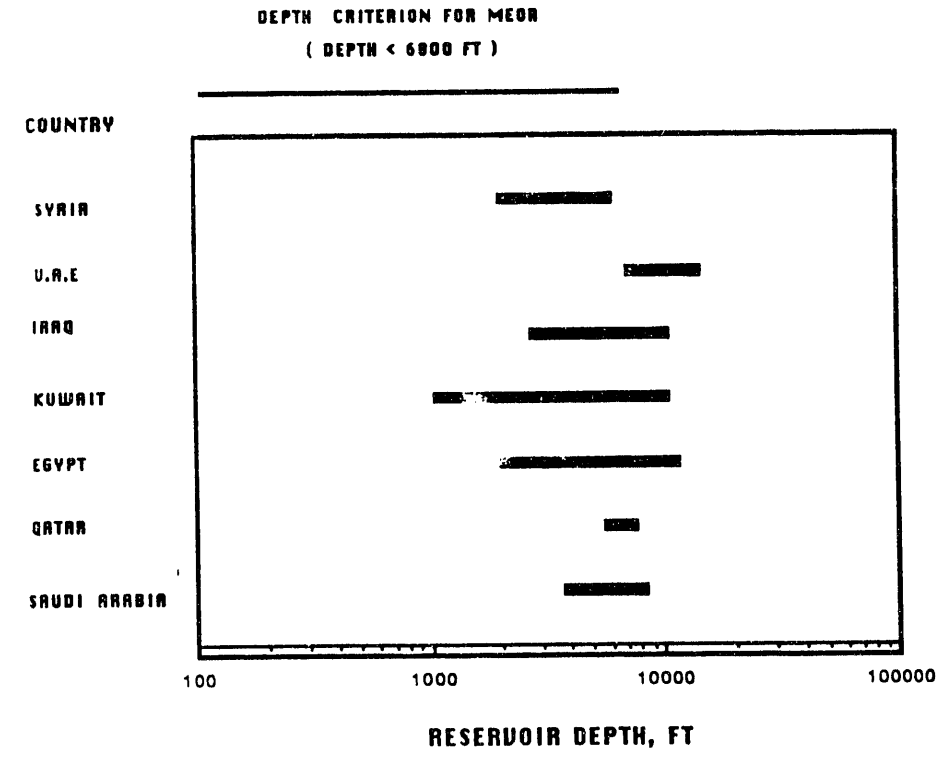


Figure 5. Depth for different Arab oil reservoirs.

as gases (methane, hydrogen, nitrogen, and carbon dioxide), polymers (polysaccharides and proteins), solvents and surfactants, and their environmental limits. There may be many undiscovered microbial systems that either already exist in deep reservoirs or are capable of existence there.

The data in Table 4 gives the characteristics of the Arab oil reservoirs used for possible MEOR field projects. The application of MEOR methods to improve oil recovery in depleted Egyptian and Syrian oil fields is very well suited to today's economic climate. The recent increase in interest in MEOR is attributed to its low cost compared to other EOR methods. Thus, with the present low oil prices and the high cost of other EOR methods, considering MEOR is justified.

5. CONCLUSIONS

From the analysis discussed in this study, we reached the following conclusions:

1. Extensive laboratory and field research should be carried out to develop new technologies for microbial enhanced oil recovery under reservoir conditions of temperature, pressure, permeability, and formation-water salinity.
2. Saudi, Iraqi, and Egyptian oil fields are good candidates for MEOR, while Qatar, Kuwait, and Syria have some potential. However, the reservoirs in the UAE have no potential for this process.
3. Depleted oil fields in Egypt, Syria, and Iraq could be activated by injection of microorganisms and produce more oil.
4. Although the application of MEOR may be limited due to the high formation salinity in the Arabian Gulf area, new bio-technology may solve this problem.

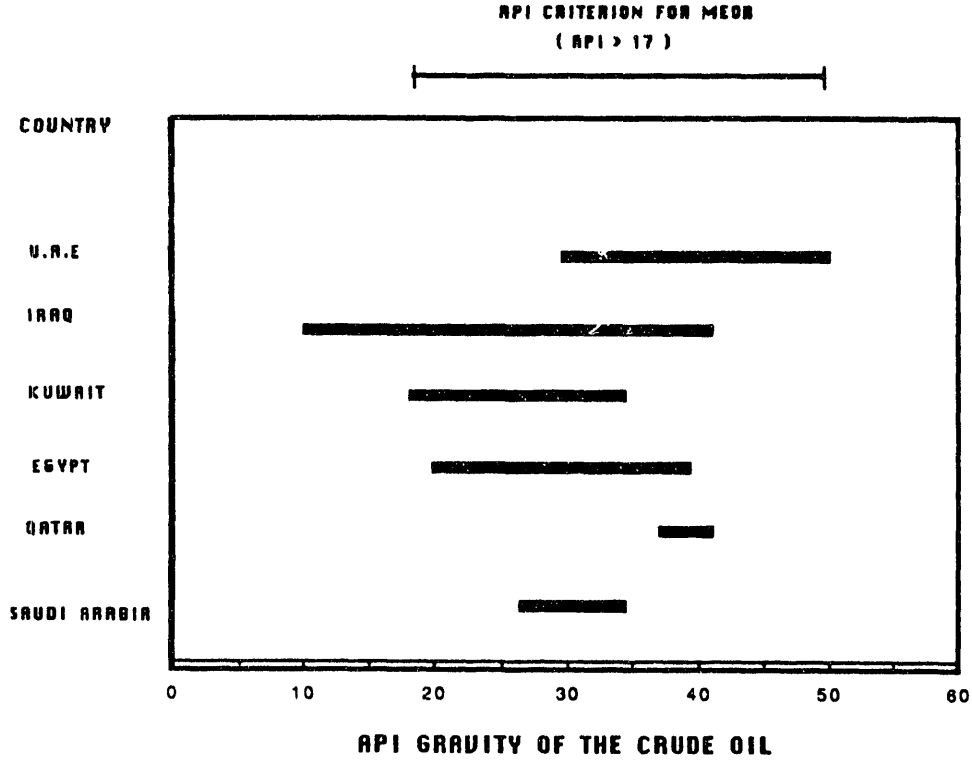


Figure 6. API gravity for different Arab crude oils.

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On Towards the Real World

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Abstract

Converting an interesting research idea into a profitable technology requires three principal components: a sound scientific base, proper integration with the relevant engineering, and a good marketing strategy. Some people would add luck. Underlying all this is high quality management at every stage of the process. But however favorable the initial signs, not all good ideas will be successful in the marketplace; for many techniques, their time will come (and go) as the economics of their competitors deteriorate (and improve). Applying these concepts to petroleum microbiology in a broad sense, how have some of the major projects in the field measured up? Look at three cases:

Single-cell protein was certainly based on elegant science combined, in the course of time, with impressive engineering. Yet it has largely failed in the marketplace - economic conditions changed over a long incubation period as they so often do and the world view of food priorities was by no means the same in 1985 as it had been in the 1960s. Were managements at fault? Could they have done better?

Or, consider the control of sulfate-reducing bacteria. Their biochemistry and physiology have been studied for decades and no doubt their genetics will soon be equally well mapped. The problems they pose are believed to be widespread in oil production, even critical to some operations. Biocides galore have been marketed. Yet still the problems are said to persist, more readily acknowledged by some operators than by others, preventative measures preferred in one case, remedial procedures, when necessary, in another. The market appears obvious and the science seems to be ready - but has it neglected to acquire a convincing engineering dimension? Have economic cost-benefits not been properly assessed? Have managements failed to take the steps needed actually to control souring? Or is the whole problem not really acute enough to be worth bothering about in the light, perhaps, of more serious difficulties confronted by producers?

And what of MEOR? For close on half a century a band of stalwarts, now centered on one side of the Atlantic, now on the other, with offshoots at the ends of the earth, have battled away with very limited success to get their ideas adopted by the industry. The science is neither revolutionary nor contentious. But the engineering links are weak: few scientists working on MEOR seem to have been able to integrate well with qualified and experienced engineers. And of commercialization, there is hardly a breath: just a trace here and there. Is this, too, a management problem? Is dependence on government funding inevitable? Why does MEOR progress so slowly when some forms of innovation rapidly succeed by their own efforts?

Questions like this have certainly been asked before [1] - perhaps some of the answers will emerge in this volume.

1. INTRODUCTION

The title of this paper is intended neither to mislead nor to denigrate: the world of academic science is real enough to those who practice it but, in the context of the present analysis, the "real world" is taken to mean competitive existence outside the groves of academe where one has to fight for commercial success. The reality of that world is of immediate and vital importance to almost every aspect of petroleum microbiology; there are, to be sure, many fundamental questions worthy of academic study but the need for applied, commercial and industrial involvement is overwhelming, not least because it is the reason for most of the funding.

Industry, and government when acting on its behalf, rarely funds science for its own sake. Support of R & D may begin by paying for basic research but the intention is very much to ensure that projects reach the development stage which, invariably, is several times more costly than the initial science. Notwithstanding the loose way in which, when it suits them, many people use the word "technology" as being almost synonymous with "science" (the view, for example, that biotechnology is virtually identical with genetic engineering), most applications of petroleum microbiology are only now going through that phase of development implied by the term "R & D," the "science" becoming "technology" and emerging into a procedure capable of being commercialized in the marketplace.

This conference has concerned itself with many aspects of petroleum microbiology, all with strong applied implications. It is fair to ask, with respect to each of them, whether we are already dealing with a viable technology, whether we may be doing so in the future or, indeed, whether that technology, or something like it, has already been put to the commercial test. And if not, why not - what is holding things up?

Because the future is always difficult to foretell, and the author is not courageous enough to try, this discussion will be confined to one technology from the past and a couple of possibles from the present.

2. THE MATTER OF SINGLE-CELL PROTEIN (SCP)

Single-cell protein was perceived in the 1960s as offering a way out of the eternal problem of the chronic food shortages, particularly of protein, which repeatedly affect large numbers of people in some parts of the world and which result from a combination of natural disasters, poor agricultural practice, and unhelpful political and economic policies. At that time, the price of crude oil was low and few people anticipated the dramatic rises which were to occur in the following decade.

It did not seem unreasonable to look for ways of converting abundant, and therefore cheap, hydrocarbons into food supplements for malnourished populations. Several large chemical and petrochemical corporations explored technologies for cultivating various microorganisms on hydrocarbon feedstocks and processing them into acceptable food supplements: thus, "Pruteen" was developed by ICI, "Toprina" by BP, and "Provesteen" by Philips. The microbiological and biochemical science was good, and spectacular advances in fermenter technology enabled large-scale production to take place [2].

But in spite of being based, as they were, on good science and equally good, if not even better, engineering, why did these products largely fail as commercial ventures, the test of a real technology? There were many contributory reasons [3]:

- the price of crude oil went up while that of such agricultural sources of protein as soy bean meal did not rise as fast;
- new varieties of basic food plants gave greatly enhanced yields;
- technical problems intervened in the manufacturing processes;
- the presence, particularly in Japan, of consumer antipathy to oil-based SCP products;
- in some cases, licenses and approvals were not granted. Some said politics were involved - perhaps they were right.

Only in countries unable to make use of alternative agricultural sources did single-cell protein production continue on a large scale and most, if not all, facilities in western industrial countries have probably now been decommissioned. The commercial failures were not so much questions of being "somebody's fault" (although, with hindsight, people might be seen to have made wrong decisions); rather, the economic and other business factors turned out to be unfavorable. In spite of the good science and engineering, the profitable product in too many cases was not forthcoming.

3. SULFATE-REDUCING BACTERIA (SRBs) AND THE SOURING OF CRUDE OIL

Nobody attending this meeting needs to be reminded of the risk that souring presents in oilfield operations nor of the costs incurred either in trying to stop it or in repairing the damage it may already have caused.

"Souring", the production of hydrogen sulfide (H_2S) from oil and gas wells [4], is a well-known phenomenon which may occur at the start of hydrocarbon recovery although, for a complex of reasons, it often begins later. Sometimes the stimulus is unclear while on other occasions it appears to result directly from introducing extraneous materials into the reservoir - the injection of surface waters for pressure maintenance or waterflooding as well as a variety of chemicals for scavenging oxygen, inhibiting scale and corrosion, enhancing the viscosity or reducing the interfacial tension of a waterflood, and controlling the microflora (even biocides themselves are believed by some to be a contributory factor). As well as being highly toxic, H_2S may cause severe matrix plugging by the precipitation of insoluble metallic sulfides, resulting in the corrosion of production and processing equipment, and giving rise to difficulties during refining. Because of the additional production, refining and other costs, the value of "sour" oil is generally less than that of "sweet" oil of corresponding quality.

There seems to be no clear-cut industry-wide consensus of just what to do about it. Most authorities seem to agree that souring arises from the activity of SRBs within the reservoir matrix. But not everyone subscribes entirely to that view and there are good arguments for believing that non-biological factors contribute significantly. There is evidence that H_2S may originate in several chemical reactions, not all of them involving biological catalysis: they include thermochemical sulfate reduction, decomposition of sulfide rock materials, breakdown of sulfurous oil components, and the excessive use of such oxygen scavengers as sodium sulfite and ammonium bisulfite [5]. Nevertheless, the reduction of sulfate to sulfide by SRBs is widely held to be a, perhaps the, major factor in the generation of H_2S.

Many studies of SRBs have been reported, most of them dealing with bacterial nutrition, biochemistry, and ecology [6]. The widespread souring of reservoirs has led to further attempts to acquire a better understanding of its causes and has prompted the development of control strategies. Earlier work focussed mainly

on mesophilic SRBs but recent work has been directed more towards the thermophiles isolated from some production fluids [7-9]. Thermophilic SRBs are known to survive in the hostile temperatures and pressures of reservoir environments. Other studies have explored their relation to biofilms with respect to the origins of souring and from the viewpoint of SRB-associated corrosion.

3.1. The operators' views

Most operators who seek to control SRBs in their reservoirs do so by the addition of chemicals although one of them operating offshore installed a bank of ultraviolet lamps in an attempt to sterilize the injection waters. A couple of years ago this author inquired of several major oil companies working in the North Sea what problems they encountered and what preventative or remedial measures they were taking. The answers were not uninteresting:

Company One said that operators accept souring as a fact which they can do nothing to reverse; when necessary, the wells are completed for "sour service," using corrosion-resistant materials. Some operators do that anyway; it is said to be not much more expensive.

Their main concern with H_2S is safety and there is always a risk of a sudden and dangerous release. Sour gas is, therefore, flared on the platform: exporting the gas for onshore use might cause trouble if the permitted sulfur limits were to be exceeded. The informant was convinced that H_2S is a microbiological problem; biocide is pulsed into the water injection system but there is no massive treatment and the company seemed not to be worried about H_2S except as a safety hazard - for unknown reasons H_2S levels had reached a stable plateau. The company was much more concerned about scale formation and spent large sums annually on controlling barium and calcium scales in the risers.

Company Two treats the injection waters by a membrane system to remove inorganic sulfate - the technique is expensive and the intent is primarily to control scaling, not SRBs. The H_2S is believed to be indigenous and not the result of recent/contemporary microbiological activity. The field in question was sour *ab initio* so the H_2S is not of recent biological origin and perhaps not biological at all. Knowing the field to be sour, the appropriate corrosion-resistant equipment was installed from the start and the operators learned to live with the situation.

It was suggested that operators who start with sweet fields often do not install corrosion-resistant equipment and try to keep the fields sweet ("it won't happen to me"). Once a field does go sour there is a feeling that little can be done about it: the microbiological implications may be that late-onset H_2S is indeed bacterial in origin and the reason it cannot be controlled by biocide is that established SRBs are too well protected in biofilms. Thus, the company felt, as long as metabolizable material is present downhole and the injection waters contain sulfate, H_2S production will continue.

Company Three takes the view that all operators are likely to get SRBs in their systems, particularly with seawater flooding. Topside (in the piping) they are under control but not eliminated. Occasional problems are encountered with "dead legs" but they are quickly identified and dealt with. There are few problems in annuli but some downhole souring is probable. There is a widespread sense that once souring does start downhole there are no really effective control measures: this feeling arises from the concept that the responsible organisms are somewhere out with the waterflood front and therefore cannot be reached by further injected biocide. Nevertheless, the company does attempt to control SRBs by injecting biocide once a month and agrees that any improvement in control methods would be welcome.

3.2. Is there a technology waiting to emerge?

With the wealth of existing knowledge about the biochemistry and physiology of SRBs, and the growing interest among microbiologists in their genetics and ecology, the stage seems set to tackle the problem systematically and come up with both a good understanding of the origins of H_2S in individual reservoirs and effective methods for its control. These have to be conceived in terms compatible with existing production activities and be cost-effective compared with those other approaches already in use. The science is largely in place; can a technology develop? Several organizations appear now to be trying to do just that. Among them a consortium led by Archæus Technology Group Ltd., together with the Universities of Aberdeen and Exeter, Corex Ltd., and NOWSCO Well Service Ltd., all collaboratively funded by the Offshore Supplies Office of the UK Department of Trade and Industry, began a major project in August 1992 to attempt to resolve the outstanding problems and develop cost-effective preventative regimes.

4. SO WHAT ABOUT MEOR?

The third example, the major topic of this conference, has been relegated to the end because it is in many ways the most complex. "MEOR" is always a problem because different people clearly use it to mean different things. Although the term is, of course, derived from the concept of "enhanced oil recovery" (EOR) well known in the industry, MEOR is often used to suggest a single activity (for example, some folks ask "What is MEOR and how does it work?") even though it refers in reality to a series of procedures, each designed to solve a different specific problem. It is often applied equally to production problems (single-well stimulation and coning control) and to such tertiary oil recovery procedures as polymer- and surfactant-flooding, although non-microbial EOR is not used in this way. Fashions in acronyms may be changing: aside from the suggestion by Hitzman [10] that *Microbial Oil Recovery Enhancement (MORE)* would be more appropriate, some people [11] prefer the more Germanic *Microbial Enhanced Hydrocarbon Recovery (MEHR)*, while the increasingly popular *Microbial Improved Oil Recovery (MIOR)* might be a better all-embracing phrase and get away from the specific implication of tertiary recovery procedures.

4.1. The past

After an initial suggestion by Beckman [12], the ideas on which *in situ* microbial procedures are based received their initial and most important stimulus from the work of ZoBell [13] and his collaborators in the period immediately following World War II.

It must, from the outset, have been conceived potentially as a commercial activity. ZoBell himself was sponsored in part by the American Petroleum Institute, an oil industry-affiliated organization. Those who gave his ideas their earliest expression in the field did so as oil company employees or with company support. But progress was slow - in the western world the price of crude oil was low and its supply so prolific, particularly as the vast natural resources of the Middle East increasingly came to be exploited, that there was little incentive to develop commercially viable methods. Rather, it was the command economies of eastern Europe that felt the most acute need for new, low-cost technologies based on domestic skills and resources. Those economies were always chronically short of foreign exchange and on the world market oil had to be purchased in dollars; only the Soviet Union could look to real domestic self-sufficiency of supply.

Table 1
What MEOR enthusiasts talk about at conferences

Year	Conference location	No. of titled papers	No. of papers reporting new "interventionist" field work	
			experimental	commercial
1979	San Diego	About 7	1	0
1981	Vancouver	16	0	0
1982	Afton	26	2	0
1984	Fountainhead	30	2	0
1986	Abilene	13	2	0
1987	Bartlesville	19	6	0
1990	Norman	34	8	(1?)
1992	Brookhaven	40	9 (10?)	(1? 2?)

Following the initial spurt lasting in the United States until the middle 1950s, interest shifted to Europe, to Czechoslovakia, the USSR, Hungary, Poland, Romania and East Germany - indeed, the current Romanian activity represents a continuous effort of more than 20 years duration, maybe the longest ever in any country. Only after the dramatic crude oil price rises starting in 1973 did interest reawaken in the west. Presently, it is possible to identify technological developments related to MEOR in more than 15 countries, located in every continent except, perhaps, Africa. What is the nature of these developments? Are they still mainly in the laboratory or coming out into the field? How close are they to commercial application, the hallmark of a mature technology?

4.2. The present

More than 45 years after ZoBell first published his ideas, the pace of field activity now seems to be growing significantly (Table 1). Countries such as Trinidad and the United Kingdom are reporting field trials for the first time. But most field work remains very much at the experimental stage of pilot trials, with no more than a hint of commercialization in one or two cases. Nevertheless, commercialization of *in situ* microbial procedures is clearly the next stage and there are signs that it is indeed now beginning to take place.

Commercialization is dependent on satisfactory field trials and two considerations often combine to require those trails to be at least moderately successful from the start. Firstly, operators hosting trials need confidence that the procedures to be tested will at best provide some improvement in oil recovery while at worst producing no deterioration. If those same people have little understanding of microbiology and its implications, it may be difficult to convince them to accept the trial in the first place and impossible to secure agreement for another attempt if the first goes wrong. Secondly, good field trials are complex to organize and perform as well as expensive in cash and in kind. Although the trial itself need not be as tightly cost-controlled as the subsequent commercial technology expected to develop from it, there is doubtful value in testing procedures so costly to carry out that it is difficult to see how they might ever become marketable services. Furthermore, resource limitations are likely to demand that without some measure of success at the

Table 2
The steps for the design and execution of field tests

- decide in principle the procedure to be tested;
- identify prospective fields;
- identify prospective operator(s);
- where appropriate, identify a prospective service company;
- design test procedures in outline;
- evaluate cost implications of the test - how can it be made most cost-effective?;
- consider all the effects of the procedure both downhole and on the surface environment;
- check on the need for official regulatory approvals;
- identify additional laboratory work needed for the trial;
- identify essential equipment modifications in the field;
- identify prospective suppliers of feedstocks and other materials;
- identify source(s) of funding;
- carry out laboratory modelling of test procedures under simulated reservoir conditions;
- carry out computer simulation of possible test protocols;
- design actual test protocols;
- set benchmark criteria for evaluation of success/failure;
- choose field(s)/well(s);
- secure adequate indemnity insurance cover;
- negotiate contractual details between primary participants (microbiological organization, operator, service company, funding source);
- carry out additional laboratory work as necessary;
- make provision for microbial inoculum, in house or via a specialist fermenter company - as appropriate, check viability of organisms after storage;
- identify personnel responsible for on-site injections and monitoring;
- negotiate feedstock prices and delivery schedules; secure import licenses and customs clearances as required;
- obtain permissions for treatment from regulatory agency;
- design monitoring procedures before and after the test - check methods where necessary;
- evaluate success/failure of test.

first attempt there will be no follow-up.

Recent experience of field testing at Archæus Technology Group [14], clearly demonstrated the many factors which had to be in place (Table 2 summarizes some of them) as well as the variety of participating specialists whose contributions needed to be coordinated and managed (Table 3).

Only with all these participants agreed upon a common course of action, each with a defined role to play and a contractual agreement to do so, was it possible actually to undertake a successful trial. Even so, as the paper reports, there were limitations and compromises: the field was not the ideal test site and the funding was not enough to allow for everything that really should have been done.

Our own experience suggests that field trials properly designed, executed and monitored are essential precursors to commercialization. Clearly a single test, however successful, will not be adequate either to prove the applicability and effectiveness of the technology under a variety of conditions, or to satisfy prospective clients. While provision must therefore be made for enough testing

Table 3
The people and organizations collaborating in field tests

- the microbiologists, with their various support facilities;
- the microbial products supplier to grow the inoculum;
- the operators who will host the trial in their field;
- the service company bringing in on-site facilities;
- the computer simulation expert to model the trial and provide the basis for the operational protocols;
- the suppliers of feedstocks and other essential materials who must deliver to the site at just the right time;
- the government department supplying part of the funding.

to generate a service which can be promoted in the commercial marketplace, each successive test, assuming reasonably progressive improvement and an absence of serious disasters, can be expected to strengthen the technology in an exponential manner. Services subsequently performed for commercial clients will continue to add to the track record and credibility of the technology.

For MEOR procedures, commercialization comprises selling a proven and successful field technology to willing purchasers. The field data from the pilot trials must provide the sound base needed to offer a technology for sale. All the proper commercial relationships and marketing strategies have to be evolved: protection of intellectual property, establishment of a price structure, conclusion of deals with raw material and other suppliers, arrangements for publicity and advertising, identification of the client base, development of possible partnership relationships with a service company, the negotiation of conditions of sale with individual clients and the provision of the after-sales and other back-up that most service operations require. Thus, from its beginnings as a laboratory-based scientific investigation, an MEOR project must progress through the stage of engineering development to become a fully operational commercial technology, or it is without meaning. The relative importance of the scientists originally responsible for starting the project necessarily diminishes with time but does not disappear: few technologies are set forever in a rigid mold and most will benefit from ongoing modification and improvement.

4.3. The future

I personally have been guilty several times of predicting that MEOR will become a commercial reality within five years. The last occasion that forecast was actually committed to paper was in November 1989 [15], but as the book in which the relevant chapter appeared was not published until July 1991, one could take the view that the prediction will not be invalidated before July 1996, still nearly four years away.

Commercial MEOR must mean ongoing profitable sales, not an occasional trial. There are already sufficient variants of MEOR far enough along the path of development and successful testing to give comfort to the view that, with the right business management in place, they will progress naturally into the market. Encouragingly, there also are indications that, in some organizations at any rate, the right management *is* in place. I now feel confident and brave enough to shorten the odds and predict commercial MEOR within four years of this presentation - by September 11, 1996!

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Comparison of the Properties of Commercial Xantham Gum with a Xanthan Gum Produced by *Xanthomonas campestri*[tm] Using Lactose as Sole Source of Carbon*

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Abstract

Considerable interest was shown recently in xanthan gum produced by bacterial fermentation of industrial waste containing different forms of sugars like lactose, sucrose, and fructose. Xanthan gum is a thickening agent for water used in secondary recovery operations carried out in the oil industry. Proper concentrations of xanthan gum added to water or brine produce viscous solutions, which are relatively stable under the conditions prevailing in subsurface oil reservoirs. Using a viscosity controlled solution instead of water or brine normally employed in waterflooding projects gives a favorable mobility ratio between the oil in the reservoir and the liquid phase used for displacement.

Generally, xanthan gum is produced by the fermentation of glucose or corn syrup by *Xanthomonas campestri*[tm]. This paper discusses the characteristics of xanthan gum produced by a mutant of *Xanthomonas campestri*[tm], which can degrade lactose in glucose and galactose with enzyme /3-galactosidose. The newly produced xanthan gum has been characterized by nuclear magnetic resonance (NMR), thermogravimetric analysis (TGA), and infrared radiation (IR); the results were compared with commercial products (Xanflood). Additionally, the rheological properties were determined and compared with commercial products. NMR and IR showed that molecular structure and distribution of functional groups are very similar to those observed in xanthan gum produced by pure glucose fermentation.

However, comparison of viscosity properties with other commercial products showed that the new xanthan gum and these products have similar characteristics in terms of response to salinity, temperature, and concentration. Finally, the new product was tested for biodegradability using strains (*Pseudomonas*, *Bacillus*, and *Enterobacterum*) isolated from reservoir LL-3 in Maracaibo Lake, Venezuela. All three strains were able to metabolize the xanthan gum, decreasing its viscosity. However, the bulk viscosity of the solution did not change appreciably, which may be due to the presence of biomass. We conclude that the quality and general properties of this product are comparable to commercial xanthan gum.

*Because of Patent considerations, only the Abstract of the paper is included in this publication.

A Mathematical Model to Optimize Fermentation in *Xanthomonas campestris**

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Abstract

This work presents a mathematical model to control and optimize the production of xanthan gum by *Xanthomonas campestris* by fermentation of the waste from lactic industrial processes as nutrients instead of saccharose; the clone of *X. campestris* used was developed at INTEVEP, S.A.

Our main objective was to incorporate the model parameters that can be set up in the bioreactor, such as the stirring velocity and temperatures, to get an optimum final concentration of xantham gum in the least time.

To achieve this goal, several mathematical approaches were developed to model the effect of each parameter. Several mathematical fermentation models were formulated depending on the representation chosen for each parameter; all the models are based on the description of bacterial growth by a logistic representation, and xanthan production and lactose consumption by Luedeking-Piret equations, where the coefficients depend on the fermentor's parameters.

The models were evaluated using data obtained from fermentation experiments, and then compared with the aims of statistics estimators. In another experiment, the growth in complexity of the final model showed a significant number of known parameters; to do that, a particular strategy of estimation that combined empirical and least square methods was applied.

The comparison with real data shows that the mathematical model selected described very well the fermentation process under the different conditions.

*Because of Patent considerations, only the Abstract of the paper is included in this publication.

Thermophilic Bacteria from Petroleum Reservoirs*

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Abstract

Thermophilic bacteria were isolated from petroleum reservoir waters which originated from an oil field located in the Surat Basin of Queensland, Australia. Enrichments incubated under simulated reservoir conditions led to the isolation of species from the genera *Thermoanaerobacter*, *Thermoanaerobium*, *Fervidobacterium*, an isolate resembling *Dictyoglomus* sp. and several other isolates not yet identified. In subsequent studies, similar isolates were recovered from petroleum reservoirs located in Bahrain, Venezuela, the United Kingdom, and the United States, plus two additional species, tentatively identified as belonging to the genera *Thermotoga* and *Thermosipho*. The petroleum reservoirs examined had the following characteristics: depth 1000 - 2000 m, temperature 60°C - 130°C, and salinity 1 - 10%. The isolation of these organisms has been described previously from geothermally heated environments, such as continental hot springs and submarine hydrothermal vents. Petroleum reservoirs are a newly recognized source of these bacteria. We speculate on their activity and survival within this habitat.

*Because of Patent considerations, only the Abstract of the paper is included in this publication.

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