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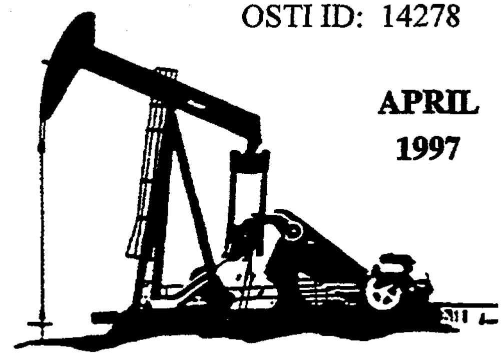
SUPPORTING TECHNOLOGY FOR ENHANCED OIL RECOVERY



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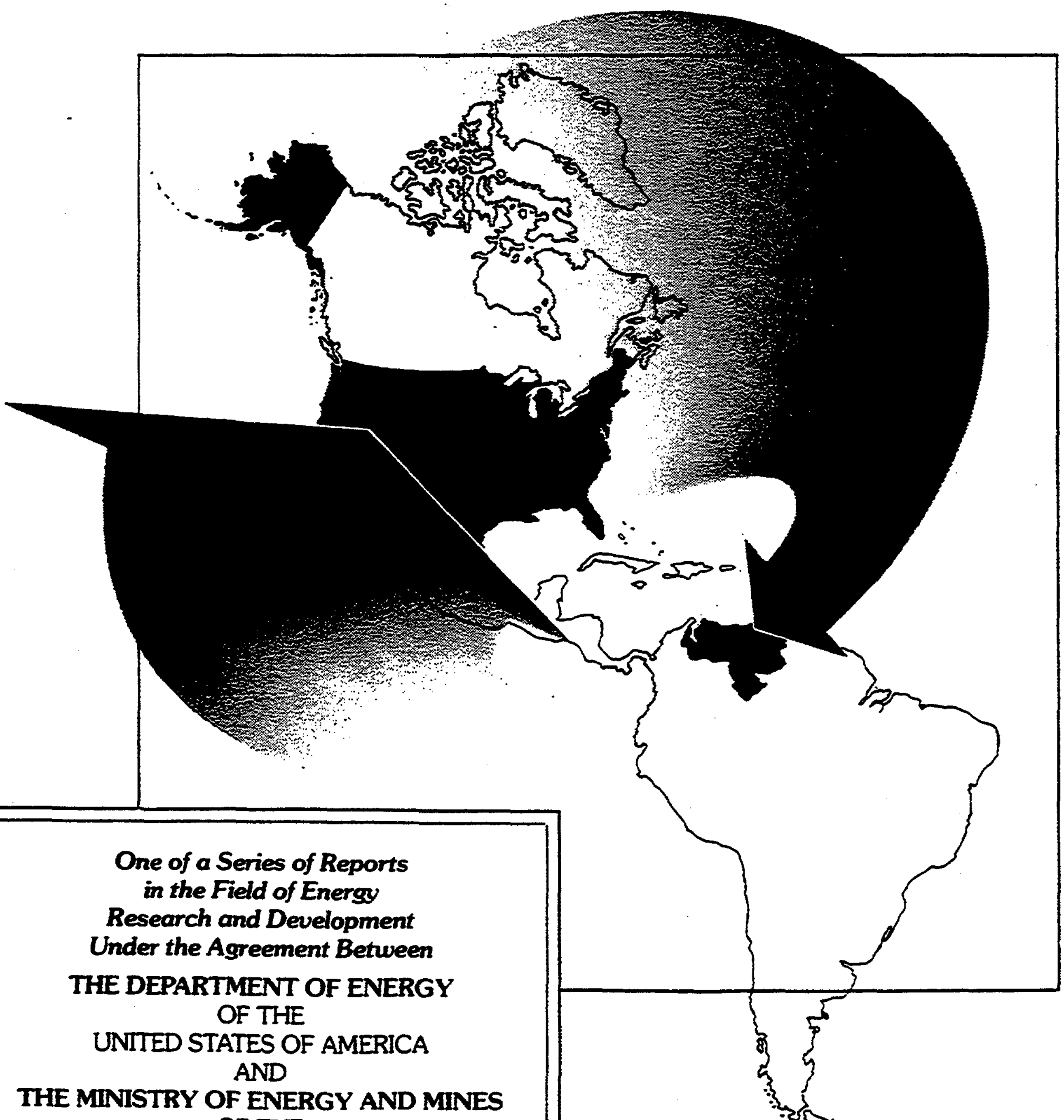
MICROBIAL EOR



One of a Series of Reports in the Field of Energy Research and Development Under the Agreement Between

THE DEPARTMENT OF ENERGY
OF THE
UNITED STATES OF AMERICA
AND
THE MINISTRY OF ENERGY AND MINES
OF THE
REPUBLIC OF VENEZUELA

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FINAL REPORT

ANNEX XIII–MICROBIAL ENHANCED OIL RECOVERY

THE DEPARTMENT OF ENERGY OF THE UNITED STATES OF AMERICA
and
THE MINISTRY OF ENERGY AND MINES OF THE REPUBLIC OF VENEZUELA

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DISCLAIMER

PREFACE

An agreement between the Department of Energy of the United States of America and the Ministry of Energy and Mines of the Republic of Venezuela to cooperate in Energy Research and Development was signed March 6, 1980. The object of cooperation under this DOE/MEMV Agreement was to promote a balanced exchange of energy technologies and to conduct joint projects in the areas of Petroleum, Solar Energy, Geothermal Energy, Hydroelectric Energy and Coal.

This agreement supported the Agreement for Scientific and Technological Cooperation between the two countries which was signed by the Secretary of State of the U.S.A. and the Minister of Foreign Relations of Venezuela on January 11, 1980.

The original DOE/MEMV Agreement was supplemented by six annexes to describe specifically the work to be done. Over the past sixteen years additional annexes have been signed, resulting in a total of seventeen annexes as of March 31, 1996. The Agreement has been extended to September 8, 1998. The annexes are:

I. Characterization of Heavy Crude Oils.
II. Supporting Research in the Area of Enhanced Oil Recovery.
III. Evaluation of Past and Ongoing Enhanced Oil Recovery Projects in the U.S. and Venezuela.
IV. Enhanced Oil Recovery Thermal Processes.
V. Drilling, Coring and Telemetry.
VI. Residual Oil Saturation.
VII. Petroleum Products, Use and Evaluation.
VIII. Coal Preparation, Combustion and Related Technology.
IX. Subsidence Due to Fluid Withdrawal.
X. On-Site Training of Petroleum Engineers.
XI. Energy Conservation.
XII. Geochemistry (Oil Generation, Migration and Accumulation).
XIII. Microbial EOR.
XIV. Exchange of Energy Related Personnel.
XV. Oil Recovery Information and Technology Transfer.
XVI. Oil and Petrochemical Ecology and Environmental Research.
XVII. Drilling Technology.

Each of these annexes has a document describing the work to be done as part of the cooperation. Amendments and Extensions to the Annexes are provided for in the Agreement.

Currently, eight annexes are active (Annexes I, IV, X, XI, XIV, XV, XVI, and XVII); one annex is inactive (VIII); and eight annexes have been completed (Annexes II, III, V, VI, VII, IX, XII, and XIII).

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EXECUTIVE SUMMARY

The results from Annex XIII of the Cooperative Agreement between the United States Department of Energy (DOE) and the Ministry of Energy and Mines of the Republic of Venezuela (MEMV) have been documented and published with many researchers involved. Integrated, comprehensive research programs in the area of Microbial Enhanced Oil Recovery (MEOR) ranged from feasibility laboratory studies to full-scale multi-well field pilots. The objective, to cooperate in a technical exchange of ideas and information was fully met throughout the life of the Annex.

Information has been exchanged between the two countries through published reports and technical meetings between experts in both country's research communities. The meetings occurred every two years in locations coincident with the International MEOR conferences & workshops sponsored by DOE (June 1990, University of Oklahoma; September 1992, Brookhaven; September 1995, National Institute of Petroleum and Energy Research). Reports and publications produced during these years are listed in Appendix B.

Several Annex managers have guided the exchange through the years. They included Luis Vierma, Jose Luis Zirritt, representing MEMV and E. B. Nuckols, Edith Allison, and Rhonda Lindsey, representing the U.S. DOE. Funding for this area of research remained steady for a few years but decreased in recent years. Because both countries have reduced research programs in this area, future exchanges on this topic will occur through ANNEX XV. Informal networks established between researchers through the years should continue to function between individuals in the two countries.

VENEZUELA

Activities conducted by Venezuelan researchers include screening of reservoirs suitable for MEOR, isolation of high temperature tolerant microbes, laboratory studies on promising microorganisms, and field tests of single well microbial treatments for paraffin removal.

THE UNITED STATES

The United States Department of Energy sponsored several National and private laboratories and universities to research many areas related to MEOR. It supported projects designed to improve the understanding of growth, mobility, and transport for a variety of microorganisms. This information was used to develop applications for use in field wide water flooding, single well stimulation, profile modification, and wellbore cleanup. Several field tests for the various applications were supported and the results documented.

Researchers in both the Venezuela and United States have greatly contributed to research efforts in MEOR. Integrated multi-disciplinary programs addressed aspects of MEOR ranging from theoretical consideration of the impact of such processes, to actual field demonstrations. MEOR involves a variety of processes that improve oil production. Work conducted by DOE and Venezuela have addressed these different processes.

I. INTRODUCTION

Mutual interest in the development of microbial methods to enhance oil production provided the motivation to create Annex XIII. Interest in applications of MEOR field technology increased significantly since 1982, when the lower price of crude oil as well as a more general acceptance of use of biotechnological processes probably contributed to this increase. Initially, projects involved universities and government laboratories without industry participation. Throughout the nine year history, research emphasis shifted due to industry influence, from water flood enhancement in a full-field and single-well stimulation to wellbore and near wellbore cleanup.

Conventional EOR processes rely on alteration of capillary and viscous forces to improve oil recovery. Chemicals used for EOR include CO_2, surfactants, polymers, and alcohols. Microorganisms can produce these chemicals by fermenting inexpensive raw materials, such as molasses or fertilizer. Chemicals used for EOR must be compatible with the physical and chemical environments of oil reservoirs. In the use of microorganisms *in-situ* for EOR, it is necessary to use microbial cultures that can survive and grow at the temperatures, pressures, and salinities present. For an *in-situ* MEOR process, the microorganisms must not only survive in the reservoir environment, but also produce the chemicals that are necessary for oil mobilization. The research produced a number of spinoff technologies in waste remediation and refining stream improvements.

The results from Annex XIII of the Cooperative Agreement between the United States Department of Energy and the Venezuela Ministry of Energy and Mines (Appendix A) have been documented and published by many researchers (Appendix B). Integrated, comprehensive research programs in the area of MEOR ranged from feasibility laboratory studies to full-scale multi-well field pilots. The objective to cooperate in a technical exchange of ideas and information was fully met. Funding for the research was outlined in Appendix C.

II. MINISTRY OF ENERGY AND MINES OF THE REPUBLIC OF VENEZUELA

A. INTEVEP, S.A.

1) Microbe Isolation

For the isolation of bacteria, co-produced water samples of the oils were taken at the wellhead of the producer wells. The samples were collected in sterile bottles.

The samples were enriched with a basal mineral and metals medium which consisted of: 2% Tanners minerals and 1% Tanners metals solutions. As carbon sources, different compounds were examined: molasses (2%), root beer (2%), glucose (0,5%), sucrose (0.5%) and starch (1%). All media were prepared anaerobically utilizing an atmosphere of CO_2:N_2 (20:80) and cisteine-HCI as a reductive agent.

The samples were incubated at the temperatures of each reservoir. Enrichments of the reservoirs GG-2 and C5-VLA-8 were also incubated at the reservoir pressures. For this purpose the enrichments were incubated in piston-like stainless steel cylinders at 1,200 psi under anaerobic conditions. Samples of the enrichments were taken periodically to determine growth by absorbency.

2) Growth and Metabolic Products

Despite the harsh conditions of the Venezuelan reservoirs (high temperature and pressure), bacteria were found in the three reservoirs studies. In general, all the carbohydrates used stimulated growth of the indigenous bacteria. The best growth was obtained with sucrose and starch for the reservoir GG-2, with molasses for C5-VLA-8 and glucose for reservoir LL-3. These observations suggested that every reservoir has its own type of microbiota. Microscopic observations showed the same result. In the C5-VLA-8 reservoir only one type of morphology was observed: bacilli-like, singles or in pairs, covered by a transparent film. During the stationary phase the bacilli turned into cocoid-like. In the GC-2 reservoir the microbial diversity increased including cocoid, cocobacilli, large and thin bacilli and also, but in less number, the bacilli-like with transparent film previously observed. The LL-3 reservoir has a larger diversity due to the lower temperature.

The rate of bacterial growth is slightly different for the three reservoirs, but the maximum growth achieved was similar for all of them, suggesting that each type of microorganism is adapted to LOCAL reservoir conditions.

The studies revealed that pressure affects the growth of the microorganisms. Bacteria of each reservoir grew more rapidly at high pressure, suggesting that the enzymatic complexes adapted well to high pressures. The stationary phase is achieved more rapidly and the biomass after 7 days decreases 30-35% with respect to the enrichments incubated at atmospheric pressure. Studies performed with barotolerant microorganisms suggest that these changes in biomass are due to changes in pH. In enrichments incubated under pressure the solubility of the CO_2 increases, this produces carbonic acid which lowers the pH in the broth, creating limiting conditions for growth.

Metabolites produced were identified by GC and HPLC. Organic acids like acetic, butyric and valeric were detected; alcohol like ethanol and butanol were also detected. Gases like CO_2, H_2 and CH_4 were also produced, increasing the pressure inside the bottles by 98%.

There were no changes in the viscosity on the surface tension in any other enrichments, suggesting no production of biopolymers or surfactants.

3) Coreflood Displacements; *In-Situ* Processes

Coreflood experiments injecting the indigenous bacteria from each reservoir were performed in Berea sandstone cores with residual crude oil. These experiments were conducted in the presence of residual oil and under the reservoir conditions of each case. The tests were performed on linear displacement equipment which consisted of a stainless steel coreholder, a pumping system, and a production fluid recollection system.

In all cases live bacteria were collected from the effluents, suggesting that the injected bacteria stayed alive inside the core and were transported through the porous media. Oil recovered for the LL-3 and C5-VLA-8 runs was negligible, and only 10% of the residual oil was recovered in the GG-2 reservoir test.

The Berea core treated with the microbes from reservoir GG-2, showed changes in its wettability; initially the core was highly water wet, changing to neutral after the treatment. Possibly, the fatty acids produced by these bacteria, and the lipopolysacharides from the bacterial membranes contributed to this change in wettability. Nevertheless, it is known that intermediate or neutral wettability favors oil recovery by water injection. Changes in wettability have been observed by other researchers. Therefore, the 10% residual oil recovered was attributed to these changes in wettability and also to a high volume of gas produced.

The studies performed in the Venezuelan reservoir samples revealed the presence of autochthonous microorganisms at extreme conditions of pressure and temperature, with a low concentration of nutrients and under an anaerobic environment. These conditions are limiting for the diversity of microorganisms, thus it is less probable to find organisms capable of producing surfactants or polymers, which are metabolites very important for tertiary oil recovery.

4) Coreflood Displacements: *ex situ* Processes

Due to the low residual oil recoveries in the coreflood experiments using the autochthonous microorganisms, metabolites produced ex-situ were also evaluated in the same type of experiments. In this case the microorganisms were grown in flasks outside the porous media. After growth was achieved, the broth was centrifuged and the cell-free broth was injected into the Berea cores.

A surfactant producer microorganism isolated from soil contaminated with crude oil was used to produce the broth. The microbial sample was enriched with a mineral medium and crude oil as the sole carbon source. From the enrichment, only one strain of bacteria was isolated, which belonged to the genera *Pseudomonas*, species *Ps. Fluorescent.*

Different carbon sources were evaluated to determine which gave the best surfactant production. The carbon sources evaluated were: glucose (0.5%), vegetable oil (0.5%), and kerosene (0.5%). After 7 days of incubation, cells were eliminated by centrifugation, and the supernatant (cell-free broth) was evaluated. It was found that the bacteria produced surfactant with both carbohydrates and hydrocarbons. After purifying the active compound and following the procedures established in the literature, three to four times more surfactant was produced in glucose that in kerosene and in vegetable oil, respectively. Similar results have been reported in the literature.

The behavior of the interfacial tension of the cell-free broth was evaluated as a function of variations in pH, ionic forces and co-surfactant (alcohol). It was found that butanol at 0.5% lowers the interfacial tension by one order of magnitude (0.05 mN/m). In the same way, after adjusting the pH of the cell-free broth to 5, interfacial tension is lowered one order of magnitude down to 0.04 mN/m. The variation of the ionic force did not appear to change the behavior in interfacial tension. Coreflood experiments were performed with the cell-free broth on Berea cores and non-consolidated cores, under reservoir conditions of the LL-3 reservoir. After leaving the cores under residual oil conditions, 2 pore volumes of the cell-free broth at pH 5 was injected. The cores were closed for 24 hours, and then water was injected to recover the released residual oil. All the experiments were carried out at $60^{O}C$, pressure of 500 psi and an injection velocity of 1.2 foot/day (0.086 cc/min). The results showed recoveries between 19 and 21% of the residual oil. These recovery values are superior to those reported using *Bacillus licheniformis* which were 7-16% of residual crude oil in Berea sandstones. Field applications of biosurfactant injection have not been performed to date, because of the high cost associated with purification. In this study and in other similar ones, the cell-free broth helps to recover residual oil without any need of purifying the active agent.

5) Single Well Microbial Stimulation: LL-3 Reservoir

Microbial well stimulation was performed in 16 wells which belong to LAGOVEN, S.A. The wells injected are offshore and located in the LL-3 reservoir, north-east of Lake Maracaibo.

The LL-3 reservoir has an average depth of 3,500'. Temperature around 140^{O}F, a pressure of 500 psi and it is a Miocene formation. The oil produced by the treated wells has an API gravity that ranges from 15 to 27. This reservoir was selected because it is highly depleted and has been water flooded for a long time.

The microorganisms used in this reservoir were supplied by Micro-Bac International, Inc. The microbiological product called Para-Bac is designed to produce mostly fatty acids, gases, alcohol and surfactants. All these metabolites are well known to enhance oil recovery.

This product was evaluated at the laboratories of INTEVEP, S.A., and it was found that the "cocktail" consists mostly of bacteria of the type *Acinetobacter* and *Pseudomonas*. It was also found that the product was able to degrade paraffins of the LL-3 oil under aerobic and anaerobic conditions, and also reduced the interfacial tension from 17 mN/m (crude oil + distilled water), to 0.7 mN/m.

The well treatment process consisted of injecting 600 barrels of water from the Lake (previously treated with biocide), blended with 110 gallons of Para-Bac and 55 gallons of Corroso-Bac. The solution was injected directly to the wellhead. The wells were then shut-in for 7 days.

The first group of treatments was performed in November 1993, in 5 wells, a second group of treatments was performed on three additional wells and in two wells that were previously treated in November. A third treatment was performed in 7 new wells in February 1995. Finally, a fourth but different type of treatment was performed in one well, where the product was not injected after being diluted in water. In this case the microorganisms were blended with sand and oil, which was used to do a sand squeeze in the well.

Out of the 16 wells injected, 12 wells had good production results, representing a 75% success. Table 1 and 2 show the production results of 11 wells treated, presented as the additional oil gained in each well with respect to the production prediction calculated from the decline curves, if no treatment was done. The rest of the wells are not presented, because due to operational changes, the productivity variations cannot be attributed only to the microbial treatments. Each treatment has a cost per well of $10,000 including the cost of the offshore injection barge which represents half of the expenses.

TABLE 1. NINE MONTHS PRODUCTION (BPD)

WELL	STIMUL. PROD.	PROD. DECLINE	ADIC. OIL
PB-465	44,640	49,946	-5,306
TJ-788	12,090	8,169	3,921
TJ-173	45,600	21,149	24,451
LR-246	41,310	19,509	21,801
LR-278	33,060	31,538	1,522
PB-410	10,860	510	10,350
TOTAL	**187,560**	**130,821**	**56,739**
Monthly Average (per well)	**3,505**	**2,423**	**1,051**

TABLE 2. TWENTY MONTHS PRODUCTION (BPD)

WELL	STIMUL. PROD.	PROD. DECLINE	ADIC. OIL
PB-295	47,790	18,479	29,311
PB-288	71,250	61,555	9,695
PB-253	71,580	25,873	45,707
PB-234	101,820	63,114	38,706
PB-472	58,020	19,960	38,060
TOTAL	**350,460**	**188,981**	**161,479**
Monthly Average (per well)	**3,505**	**1,890**	**1,615**

Five wells on production for 20 months have produced in average 1,615 barrels/month/well of additional oil for a total of 161,479 Bbl. representing an 85% increment in oil production. Six wells on production for 9 months have produced an average of 1,050 barrels/month/well of additional oil for a total of 56,739 Bbl. This represents an increment of 43%. To have an idea of the attractiveness of implementing the single well stimulation treatments in this field, it is important to establish that the produced oil has a market value of $14.50/Barrel (February, 1996).

6) Paraffin Control in La Concepcion Field Using Microorganisms

Throughout the production history of La Concepcion Field, paraffin deposition has been one of the main problems responsible for frequent plugging of the production lines at the surface and the production tubing in the wells. Pump rod failure is another problem also caused by the increased friction due to the presence and accumulation of paraffin in these wells.

In the past, efforts to control this problem only consisted of cleaning procedures rather than deposition prevention methods with more lasting results. Circulating steam through the annulus has been used to heat the tubing in order to dissolve the paraffin depositions. Also the injection of solvents has been used for the same purpose and with the same localized and temporary effects as steam. The most common treatment used is the mechanical cleaning of the tubing. Nevertheless, this cleaning procedure is also temporary and its application needs the pulling out of the production string.

The use of microorganisms has been proposed to clean and prevent the deposition of paraffins. In order to test this process, seven producer wells of La Conception field with severe paraffin deposition problems were inoculated with microbes every two weeks for 10 months. The microbes used were supplied by Micro-Bac International, Inc. which were specified to produce bio-surfactants that could keep the heavy molecular weight paraffins in suspension, and which could also selectively metabolize certain n-alkane heavy fractions of the oil to reduce the average molecular weight of the oil. Results are not available at the time of this report.

III. U.S. DEPARTMENT OF ENERGY

A. National Institute for Petroleum and Energy Research (NIPER)

1) Databases

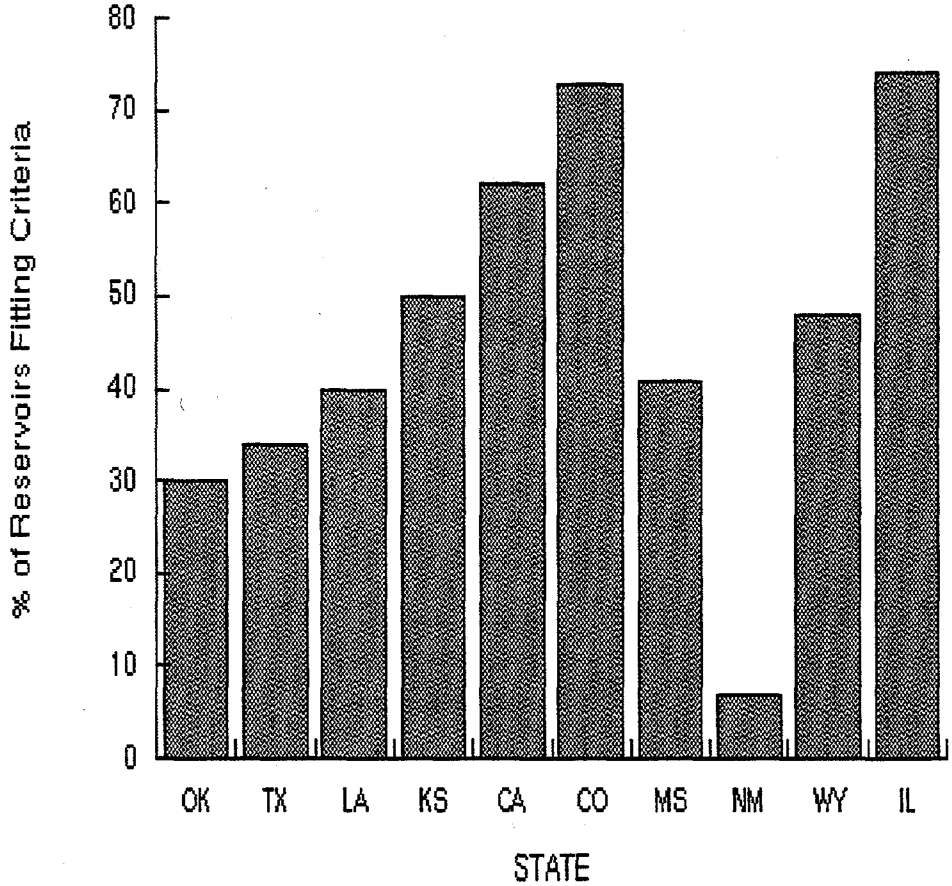
Bryant and Burchfield (1989) published an overview of MEOR technology. Since that time, data from some MEOR field trials have now been made public by researchers from countries such as the USSR, Germany, Romania, and China. Reports from some of these field trials were published in the Proceedings: The 5th International MEOR Conference.

NIPER has continued to maintain a data base on all available information regarding MEOR field tests in the United States and in other countries. The U.S. DOE Reservoir Data Base (public copy) was used to screen several oil-producing U.S. states for reservoirs with original oil in place greater that 20 million bbl that satisfy the following criteria: injected and connate water salinities less that 150,000 ppm, rock permeability greater than 50 millidarcies, depth less than 7,700 ft. and a bottomhole temperature limitation of about 80^{O} C. Table 3 shows the number of reservoirs that satisfied these parameters, and a graph of the percent of reservoirs in each state that satisfied these limiting criteria, and the total, is shown in Figure 1.

TABLE 3. NUMBER OF RESERVOIRS BY STATE WITH POTENTIAL FOR MEOR TECHNOLOGY

State	Total no. of reservoirs	No. of reservoirs that fit the criteria	%
			-
OK	107	32	30
TX	685	233	34
LA	195	77	40
KS	44	22	50
CA	197	122	62
CO	40	29	73
MS	49	20	41
NM	71	5	7
WY	71	34	48
IL	46	34	74
(AVG)			46

FIGURE 1. PERCENT OF RESERVOIRS IN EACH STATE



2) Biotechnology for Conformance Control

Applying permeability modification treatments can significantly extend the productive lives of active oil recovery projects. Improved effective modification of reservoir sweep can improve the economics of an oil recovery process. These permeability modification treatments can extend the productive lives of active recovery projects, curtailing the prospect of premature abandonment. Current state-of-the-art technology in this area uses cross linked polymer technology to alleviate problems associated with reservoir heterogeneity. Treatments in both injection and production wells have been applied, with about a 50 percent chance of success. Limitations of technology are being addressed by continued research efforts in the area, but many of the current applications still rely on the use of cross linking agents that may pose an environmental hazard. Development of alternative methods using biotechnology for permeability modification was initiated. A small improvement in volumetric sweep efficiency such as offered through the microbial-based processes can have a significant impact on the overall efficiency and economics of an oil recovery process.

3) <u>Single Well Microbial Stimulation</u>

NIPER is conducting supporting research on the development of improved microbial methods for the control, treatment and prevention of formation damages attributed to paraffin and asphaltene deposition. Research is being conducted to determine if laboratory observations of the microbial treatment methods can be adapted to field situations. It is anticipated that different treatment protocols will be developed for wells having different problems as defined by laboratory observations and analyses.

A single well stimulation field test was conducted in 1994. Monitoring and evaluation of the three microbial single-well stimulation tests conducted to make a systematic comparison of treatments and nutrients was completed. The three production wells are located in Creek County, OK. Well #45-9 was injected with molasses and an adapted microbial formulation from BDM-Oklahoma's laboratory. Well #12-6 was injected with molasses as a nutrient control. Well #15-4 was injected with inorganic nutrients. The wells were shut in for one week, then put back on production. Barrel testing for oil/water ratios and sampling of the produced fluids was started 24 hours after the wells were put back on production. Sampling and barrel testing were done on a weekly basis for eight weeks. Samples were analyzed for microbial counts, SRB, molasses concentration, pH, and TDS. Results indicate #45-9, treated with NIPER's microbial formulation, was the only well that was favorably affected by the stimulation treatments. The microbial formulation effectively cleaned out the near-wellbore region, and increased the relative permeability to water. Since these wells were placed back on water flood, the microbial formulation was then essentially flushed out, which limited the effect of the stimulation. The other two wells showed no oil production response to being treated with nutrients.

4) <u>Wellbore Cleanup</u>

A project not completed at the time of this report provides a laboratory and field test program that will demonstrate a cost effective biotreatment technology to prevent and remove well damaging agents such as paraffin, H2S/FeS, and scale. The biotreatment technology is based on the beneficial stimulation of the indigenous microbial populations by addition of alternated electron acceptor chemicals which favorably alters the reservoir ecology. Field data and well response to treatment will be collected and reported. All information and results will be presented for technology transfer to the oil industry. Field tests will emphasize effective low cost treatments to prevent the premature abandonment of problem wells. A part of the project work is being performed under subcontract by Geo-Microbial Technologies, Inc. (GMT) of Ochelata, Oklahoma. Wells included in the field test will be in the Permian basin of West Texas, and independent leases in Kansas, Oklahoma, and the on-shore Gulf Coast area of Texas.

5) Simulation

Methodology development for applying microbial technology for improving oil recovery requires an integrated laboratory and field research effort to identify and understand the mechanisms of oil recovery, to determine the relative importance of these mechanisms in oil mobilization by laboratory experimentation, to develop mathematical correlations and models to describe the physical phenomena, and to develop and apply a mathematical computer reservoir simulator to match laboratory corefiooding results and ultimately match and predict oil recovery performance in field applications. Although several attempts have been made to modify existing reservoir simulators to describe microbial processes, no model has yet fully incorporated all of the complex phenomena that are believed to be important. NIPER developed a three-dimensional, three-phase, multiple-component numerical model to describe the microbial transport phenomena in porous media (Bryant et al., 1990). Unfortunately, a number of the parameters required to validate the simulator are not readily available. An experimental program to provide these parameters, to test the simulator against coreflood and field tests, and to improve the various mathematical representations of physical and biological phenomena included in the simulator continues at NIPER.

6) Field Research

To determine the feasibility of improving oil recovery and the economics of microbial enhanced water flooding in mature oil wells in the United States, two field pilots were conducted. A specific microbial formulation was selected that was compatible with the chosen reservoir environment and had been shown to recover oil after water flooding Berea sandstone and field core. The microbial formulation was designed to improve microscopic oil displacement efficiency by surfactant, gas and acid production from fermentation of molasses. A 20-acre pilot test was initiated in October 1986 and completed in December 1989. Results from this point demonstrated that microorganisms could be injected into an ongoing water flood and that such injection could increase the oil production rate by at least 13%. A larger test (380 producing acres) was completed in the same formation to evaluate the feasibility of commercial application of the technology. This field pilot was injected with microorganisms and molasses from a centralized injection station in June 1990. Although microorganisms were injected only once, nutrient injection continued throughout the project life. All 19 injection wells were treated, and oil production was monitored from the 47 production wells. Treatments were similar to procedures for the field pilot demonstration. No operational problems were encountered. At the end of May 1993, the oil production rate was improved by almost 20%. The patent resulting from this work will likely be commercialized.

B. <u>University of Oklahoma</u>

1) <u>Microbial Stimulation *in-situ* in Hyper Saline Reservoirs</u>

A multi-well, microbially enhanced oil recovery field pilot was conducted in the Southeast Vassar Vertz Sand Unit in Payne County, Oklahoma. The primary purpose of the pilot was to determine whether *in-situ* microbial processes could preferentially plug high permeability zones, and thereby improve sweep efficiency.

Studies were performed to determine the reservoir chemistry, the types of indigenous microbial populations, and the geological and petrophysical properties of the reservoir. Laboratory experiments showed that a molasses-nitrate-based nutrient mixture resulted in large increases in microbial growth and gas production without stimulating the detrimental activities of sulfate-reducing bacteria. A specific pilot area behind an active line of injectors was selected. A tracer test identified a large water channel that cause communication between the injector and the rest of the reservoir. Pressure interference tests showed significant permeability variations between the injector and production wells within the pilot area.

No oil was recovered in any of the three production wells after six months of operation prior to the initiation of the microbial pilot. Surface facilities were designed and installed. Injection protocols of bulk nutrients were developed to facilitate uniform distribution of nutrients within the pilot area. By the end of December 1991, 82.5 tons (75 tonnes) of nutrients had been injected into the reservoir.

Nutrient injection stimulated microbial activity in the reservoir as evidenced by an increase in the alkalinity of the produced fluids. *In-situ* growth and activity of microorganisms resulted in tertiary oil recovery. Two of the wells produced about one half to one barrel of oil per day after nutrient injection. A total of 82.5 barrels of tertiary oil was recovered. After nutrient injection, the interwell pressure interference tests showed that the significant permeability reductions occurred, and that the interwell permeabilities were made more uniform after *in-situ* microbial growth. A second tracer test showed that the major water channel was blocked. This study showed that a microbial plugging process is technically feasible under actual reservoir conditions and that the stimulation of denitrifying bacteria by nitrate addition will control souring.

2) MEOR in Carbonate Reservoirs

About one-half of the world's oil supply is produced from carbonate reservoirs. A review of the literature indicated that two common microbial activities, acid and gas production, may provide useful mechanisms for microbially enhanced oil recovery from carbonate reservoirs. Analysis of reservoir data from 10 states showed that over 7,790 of the 19,297 carbonate reservoirs in the database had environmental conditions favorable for *in-situ* microbial growth and activity.

Studies on the ecology of four carbonate reservoirs showed that each contained a small but metabolically diverse population of mesophilic microorganisms. The low population levels were due to the lack of available nitrogen, phosphate, and probably energy sources. However, the numbers of microorganisms were sufficient to support *in-situ* microbial processes without the need to inject cells into the reservoir . Microorganisms had predictable effects on the permeability and porosity of sandstone and limestone cores. Microbial growth and biomass production resulted in a decrease in the permeability and porosity of sandstone cores. Polymer-producing microorganisms also lowered the permeability and porosity of limestone cores. However, vigorous acid and gas production increased the permeability and porosity of limestone cores, probably by the dissolution of the carbonate matrix. In one case, an acid producing microorganism penetrated a core in two weeks into which fluids could not be injected. Recovery of residual oil by microorganisms was demonstrated in four systems packed with chips of carbonate rocks. Residual oil was recovered only after microbial growth and activity occurred. The largest amount of residual oil recovery occurred when these systems were inoculated with biosurfactant-producing or a vigorous acid-producing bacterium. A first generation mathematical model was developed that incorporated the effect of acid and gas production on the carbonate matrix into a mathematical model developed for microbial oil recovery in sandstone cores.

3) Simulation

During the first year of the project, the metabolism and nutrition of fermentative, halophilic, anaerobic bacteria were studied. Fermentative, halophilic anaerobes were the most numerous organisms present in the Vassar oil reservoir. All of the isolates used carbohydrates, and fermented glucose to hydrogen, carbon dioxide, ethanol, and acetate.

A simple porous chamber was designed to obtain statistically reliable data on the *in-situ* rates of microbial growth, substrate consumption, and product formation. This system consisted of a small, plastic tube packed with sand or glass beads and sealed at each end with rubber stoppers. Studies showed that the rate of growth was slower inside of the chambers compared to growth in liquid cultures. The stoichiometry of galactose metabolism by the bacterium was the same in the chamber as in liquid culture. Without fluid flow, bacterial strains were able to penetrate the chamber at rates up to 0.1 m/d. Cells moved through the chambers in a bandlike fashion. The propagation of non-motile strains required a critical cell density in a section of a core before cells were detected in the next section.

These data suggested that the transport of nonmotile cells through porous materials may occur by a physical displacement mechanism where progeny of cells are forced into less populated regions of the chamber.

In the second year of the project, a new non-invasive method to monitor microbial activity in porous materials was developed. The rate of *in-situ* microbial activity was inferred from the rate of change in the pore pressure of the chamber. The pore pressure that was monitored continuously using an electronic transducer system. This method helped show that the grain size of the porous material affected the rate and extent of glucose degradation and the extent of substrate utilization by microorganisms. The rate of hydrogen production, the final amount of hydrogen produced, and the cell concentration decreased with a decrease in grain size. This shows that grain size (pore size) of the porous material is an important factor controlling the reproduction and metabolism of microorganisms in porous materials.

A one-dimensional, three-phase, multiple-component, numerical simulator was developed to investigate transport and growth of microorganisms in porous media and the impacts of microbial activities on oil recovery. The simulator was verified using analytical solutions and other simulators. Special cases regarding growth inhibition, chemotaxis, microbial plugging, biopolymer production, biosurfactant production, and gas production were used to test the simulator and investigate the mechanisms involved in microbial oil recovery. Simulation studies showed that additional oil was recovered by the following mechanisms: selective plugging, mobility control due to polymer production, and interfacial tension reduction due to biosurfactant production. These studies also showed that microbial gas production did not recover residual oil. Apparently, the amount of gas produced during an *in-situ* microbial process was insufficient to recover residual oil.

C. Idaho National Engineering Laboratory (INEL)

Microbial research at the INEL has been historically diversified over a wide range of problems with a common focus on MEOR systems for application in reservoirs containing medium to heavy crude oils, and the design and implementation of an industry cost-shared field demonstration project of MEOR technology. In order to achieve this objective, several subtasks have been addressed including: 1) Determination of MEOR mechanisms of recovery and a laboratory testing of MEOR systems, 2) Compatibility of MEOR with other EOR methods that may have been used previously in the target reservoirs, 3) Evaluation of the impacts of MEOR application with respect to biogenic products and formation souring, 4) Target reservoir(s) evaluation and characterization for potential oil recovery, 5) Economic evaluation of a potential MEOR system, and 6) An industry cost-shared field demonstration of a potential MEOR system.

1) Surfactant Based Enhanced Oil Recovery

Surfactants are hypothesized to enhance the recovery of oil through a reduction of the interfacial tension (IFT) of the organic and aqueous interfaces. A reduction in the IFT between these interfaces is believed to cause oil displacement by overcoming capillary forces and displacing oil from the rock pores into the mobile liquid phase. Microbially mediated reductions of IFT's have been reviewed.

Laboratory research to determine and quantify mechanisms by which crude oil is recovered from oil reservoirs using a naturally occurring surfactant producing microorganisms was investigated. Microorganisms represent a replenishable *in-situ* source of surfactants and other beneficial metabolites such as acids, gas biopolymer, or solvent which can be supported and manipulated by the addition of inexpensive nutrients that are not tied to crude oil prices. A naturally occurring, readily available surfactant producing microorganism, Bacillus licheniformis JF-2 (ATCC 39307) and a sucrose based nutrient were studied in an experimental system using Berea sandstone cores (85-510 md). The effectiveness of this microbial system was reported for a variety of crude oils in terms of oil recovery efficiency (E_r) and correlated with IFT's , viscosity, crude oil type, and the effect of MEOR on oil composition.

The surfactant produced by Bacillus licheniformis JF-2 is thought to be physicochemically and structurally similar to that produced by Bacillus subtilis. The proposed structure of these biosurfactants is very different from that of common anionic petroleum sulfonates. While the sulfonate surfactants are anionic, the biosurfactant produced by Bacillus licheniformis JF-2 is zwitterionic and relatively large in comparison.

For four crude oils (19.1 to 38.1^o API (0.8343 g/cm^3 at 15.6^{o}C)) used in coreflood experiments, no direct correlation of oil recovery efficiency with crude oil type was observed. Oil was recovered equally well using viable washed cells or cell-free supernatants for core injections. The surfactant produced by Bacillus licheniformis JF-2 lowered the IFT's of five oils tested (17.5 to 38.1^o API (0.9459 to 0.8343 g/cm^3 at 15.6^{o}C)) and aqueous systems equally well, and there was no adverse effect on the comparison of the crude oil during microbial emulsification. Measurements of IFT's were made using an interfacial tensiometer based on digital imaging of an inverted pendant drop.

Although IFT reductions measured with the pendent drop apparatus were not low enough to be an effective oil recovery process according to current theory, both cell and cell-free injections mobilized residual oil. The amount of oil mobilized was about the same for all oils tested. Data from a bacterial adherence to hydrocarbon assay indicate that oil mobilization was due to produced metabolic products and not the physical presence of the bacterial cells. This is further substantiated by oil recovery obtained using the cell free supernatant injections. However, floods using cell injections required Ç 1/4 less injection volume to mobilize the same amount of oil attained using cell-free injections. This indicates that *in-situ* cell metabolism is effective in producing sufficient quantities of surfactants to mobilize oil. Scanning electron microscopy data indicate a complete distribution of cells in the core that follows flow direction.

Two strains of Bacillus subtilis (ATCC21331 and ATCC21332) 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.

Cell-free extracts of B.subtills grown in ATCC 1502 Medium E (supplemented with 2.5% NaCI and 1% sucrose) recovered Schuricht crude oil from cores in the laboratory. Interfacial tension 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 mNlm. Like Bacillus licheniformis, neither strain of Bacillus subtilis was able to degrade the oils tested, or adhere to hydrocarbons in a standard bacterial adherence to hydrocarbon (BATH) test. Both strains of Bacillus subtilis recovered more water flood residual oil than Bacillus licheniformis. 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 (potato) was the best carbon substrate for surfactant production.

2) Measurement of Interfacial Tensions

Measurement of IFT's has been accomplished by a variety of methods, including pendant drop, sessile drop, and spinning drop methods. These methods place a drop of oil into a surfactant solution, and then measure the shape of the drop formed. Current developments in personal computers, coupled with the ability to digitize video images enables the researcher to automate the process, and provides an improved process to determine the IFT. Video digital methods have been applied to the measurement of the IFTs of bacterial biosurfactants. A simple, fast, automated system for measuring interfacial tensions using the pendant drop method was developed at the INEL.

3) Evaluation of Biocide Efficiency For Controlling Sulfate-Reducing Bacteria In Petroleum Reservoirs

The objectives of this study are: 1) Measure differences in the efficacy of biocides used to control the activity of sulfate reducing bacteria (SRB), using an assay that employs characteristics of a petroleum reservoir to challenge biocide efficacy called the Challenge Assay. Crushed Berea sandstone (representing the host rock matrix) and crude oil were used to represent physical reservoir properties in this study. 2) Evaluate differences, if any, in the response to biocide treatments between a wild-type consortium (K3-3) of SRB and a pure culture of Desulfovibrio desulfuricans (ATCC # 13541). 3) Compare microbial activity for all biocide treatments using the standard minimum biocidal concentration (MBC) assay and the Challenge assay.

D. desulfuricans and K3-3 were used to evaluate the efficacy of five different biocides within two different systems (Challenge or MBC). Contact time and biocide concentration were the key factors affecting the efficacy of all biocides screened. D. desulfuricans and K3-3 did not respond in a significantly different way to any of the biocides except DMDM hydantoin. K3-3, though uncharacterized, was similar to D. desulfuricans in a number of ways; K3-3, as a consortium, did not contain spore forming species as indicated by the viability test. Morphologically, the members of the K3-3 consortium resembled D. desulfuricans, and their growth and activity was similar. Morphologically similar strains, however, may respond differently under different test conditions.

The systems made a significant difference in microbial response to sodium hypochlorite and sodium o-phenylphenate. With both biocides, microbial activity was inhibited to a greater degree in the MBC system than in the Challenge. The sandstone and/or the oil in the Challenge system may have decreased biocide efficacy by chemically reacting or sorbing to the biocide.

This research has demonstrated that a standard MBC assay will not necessarily perform competently for uncharacterized petroleum reservoir. A Challenge assay is more indicative of reservoir conditions and as such, is a better indicator for biocide efficacy than the MBC assay.

4) Microsensor Probe Technology For The Direct Measurement Of Sulfide

Several sulfide detectors are available that can be used for on-line measurements. Notably, all detect sulfide ion ($S^{=}$) rather than total sulfide ($H_2S + HS^- + S^{=}$), which is the measure of souring activity. In the normal pH range (5-9), the fraction of total sulfide present in the ion form (S^-) is in the 10^{11} to 10^{-5} molar range. Therefore, these detectors sense only a minor portion of the total sulfide. At the low levels of total sulfide present in produced waters, the sulfide ion level is generally below the detection limit unless the pH is adjusted to high values. To provide a key tool needed for microbially-induced product souring work, the development of a small pH-insensitive/online detector was proposed. This work utilized the detector development expertise, SRB experience, and process analysis capabilities of the Montana State University Center for Interfacial Microbial Process Engineering.

The preferred approach to developing a pH- independent total sulfide detector was to find a method of adjusting, the pH to the highly basic range where sulfide ions are the dominate species. This approach would require a sulfide ion electrode, a pH electrode, and a reference electrode to monitor the effectiveness of the pH adjustment. As a secondary approach (in the event that the pH could not be made basic enough for the quantitative conversion of the total sulfide to the sulfide species) the pH and sulfide concentrations could both be measured and the total sulfide calculated. This approach also requires a compatible set of pH, sulfide, and reference electrodes. Thus, the initial step was to develop a set of compatible electrodes and then combine them into a single probe.

A detector was developed that can measure total sulfide concentration independent of the pH of the aqueous medium. The technology relies on measuring both pS and pH and then calculating total sulfide concentrations.

5) Evaluation of Reservoir Wettability And Its Effect On Oil Recovery

Research continued to achieve a better understanding of the process by which one fluid immiscibly displaces another from a porous media. Understanding the interactions of crude oil/brine/rock systems is important. Investigations focused on surface phenomena, wetting changes induced by crude oils on solid surfaces, factors causing wettability changes, and the influence of these changes in wettabillity on displacement efficiency. Identifying and understanding conditions for optimum oil recovery by waterflooding is a primary objective.

D. Brookhaven National Laboratory

1) Thermophilic and Thermoadapted bacteria

At the Brookhaven National Laboratory (BNL), systematic studies have been conducted which dealt with the effects of thermophilic and thermoadapted bacteria on the chemical and physical properties of selected types of crude oils at elevated temperatures and pressures. Particular attention was paid to heavy crude oils such as those from Venezuela, California, Alabama, Arkansas, Wyoming, Alaska, and other oil producing areas. Current studies indicate that during the biotreatment several chemical and physical properties of crude oils are affected. The oils are 1) emulsified; 2) acidified; 3) there is a qualitative and quantitative change in light and heavy fractions of the crudes; 4) there are chemical changes in fractions containing sulfur compounds; 5) there is an apparent reduction in the concentration of trace metals; 6) the qualitative and quantitative changes appear to be microbial species dependent; and 7) there is a distinction between "biodegraded" and "biotreated" oils. "Biodegraded oil" is more suitable for changes which occur under natural conditions over geological periods of time, and the "biotreated" is more applicable to changes brought about by deliberately introduced microorganisms acting over short periods of time. Further, preliminary results indicate that the introduced microorganisms may become the dominant species in the bioconversion of oils.

These studies have also generated information which supports the view that the biochemical interactions between crude oils and microorganisms follow distinct trends, characterized by a group of chemical markers. Such markers are useful in the prediction of bioprocessing efficiency prior to core-flooding experiments and field testing. Core-flooding experiments based on these predictions have shown that compared to commonly used microorganisms, e.g. Clostridium sp., significant additional crude oil recoveries are achievable due to the biochemical action of thermophilic (thermoadapted) microorganisms at elevated temperatures similar to those found in oil reservoirs. In addition, BNL studies have also shown that the biochemical treatment of crude oils has technological applications in downstream processing of crude oils such as in upgrading of low grade oils and the production of hydrocarbon based detergents.

E. University of Michigan

In-situ growth of cellular material is known to cause formation damage to porous media. Bacterial reproduction and polysaccharide production are the key factors that segregate bacterial formation damage from fines and particulate damage. The transport of a model bacteria, Leuconostoc mesenteroids, through porous cores, was studied for different nutrient conditions. When the bacteria were fed glucose/fructose, there was no or minimal plugging of the porous cores. However, when the bacteria were fed sucrose, the porous cores plugged rapidly and the permeability was reduced to 0.001 its initial value. The plugging was a result of polysaccharide production when sucrose was used as a nutrient. The cellular polysaccharide production increases cell retention in porous media and causes an overall decrease in media permeability. Further, we have been able to use Leuconostoc mesenteroids for profile modification. Here we have been able to successfully divert flow from a high permeability layer of a porous medium to a low permeability layer for profile modification. This flow diversion due to permeability alteration, can be controlled by the inoculation strategy, nutrient concentrations, and injection rates.

In order to control the bacterial growth to produce selective plugging for bacterial profile modification, a reaction mechanism and a kinetic model for the model bacteria have been developed. Based on experimental observations, a phenomenological model has been developed to describe the inoculation of the porous medium, the *in-situ* growth of bacteria, polysaccharide production, and the permeability decline of the porous medium.

1) Mechanistic Study of Biomass Plugging and Movement in Porous Media

Previous work by Lappan and Fogler have demonstrated the feasibility of using exopolysaccharide producing bacteria in MEOR processes. Specifically, bacterium Leuconostoc mesentroides can be used to selectively plug high permeability zones (oil-depleted), thus diverting production fluids through low permeability zones (oil-rich). As bacteria undergo growth and exopolysaccharide EPS production, a biomass plug is formed in the porous media. Both packed bed and micromodel experiments were conducted to better characterize the biomass growth and plug development. Results indicate that under constant flow conditions, there are three pressure regimes which are dependent upon biomass morphology and distribution in the porous media. These regimes include initial no pressure changes as individual cell growth occurs, rapid pressure increases when contiguous biomass is produced, and pressure oscillations which correspond to the cyclic creation of biomass plugs and breakthrough of flow channels.

2) Retention and Transport of Multiple Bacterial Species

Processes such as microbially enhanced oil recovery and bacterial profile modification rely on the successful placement of bacteria at the oil-water interface. Developing an understanding for the retention and transport of bacteria in porous media is important for designing injection strategies capable of proper bacterial placement. However, indigenous bacteria that reside on subsurface minerals can affect the subsequent colonization of injected bacteria. Furthermore, injection fluids available in the field are likely to contain additional strains of bacteria which could further affect the retention and transport of injected bacteria. Work designed to gain a mechanistic understanding for the transport and retention of multiple bacterial species in porous media, used experimental results as baseline information for comparative studies. Once a mechanistic understanding of multi-species bacterial transport is achieved, strategies for the proper placement of injected bacterial strains can then be developed which exploit multi-species retention mechanisms.

3) Development, Evolution, and Stability of a Biomass Plug in Porous Media

Packed bed and micromodel experiments were conducted to elucidate the development, evolution, and stability of a biomass plug in porous media. It was determined that plugging by Leuconostoc meseneroides will not occur from cell growth in the absence of exopolysaccharide (EPS) production. Although maximum cell growth occurs under the morphology of the EPS-free biomass, it does not allow plugging to occur. Specifically, conductive flow channels are formed through regions of high biomass growth due to shearing of the biofilm. This shearing occurs because the cell-to-cell interaction in the absence of EPS is weak. As nutrient conditions are altered to induce EPS production, however, plugging does occur in porous media.

A characteristic pressure response curve is observed. Initially there is a lag time before the onset of pressure increase. The length of the lag time is dependent on the initial concentration of cells deposited in the porous media during inoculation. The onset of pressure increase occurs when a critical number of EPS-producing cells are present and this time is decreased with an increase of initial cell adsorption during inoculation. After the lag phase, the pressure increases. At this point, all the pore space is filled within plugged regions located near the inlet. Pressure continues to increase compacting the biomass in the plugged regions as a result of local redistribution of biomass.

At a critical pressure, the biomass yields and a channel breaks through the initial biomass plug. If a bacterial mat is present at the injection face, this channel will refill causing a pressure increase and as a maximum pressure is reached the channel will empty with a corresponding pressure decrease. Cyclic filling and emptying of the channel causes pressure oscillations. If the biomass plug is developed away from the injection face so that a bacterial mat is formed, pressure oscillations still occur. However, the channel is not refilled, but rather partial blockage occurs due to erosion of the biomass along the channel. This blockage is periodically removed at the critical pressure causing a subsequent pressure decrease that completes the pressure oscillation. Biomass removed from the breakthrough channels during oscillations is recaptured downstream of the initial plug due to mechanical straining. Straining occurs, because biomass aggregates formed from sheared EPS-producing biomass are rigid. As a result of this recapture, the biomass plug is propagated toward the distal end. Hence, the pressure oscillations indicated biomass transport through porous media.

4) Development of a Bacterial Profile Modification Technique

Before any successful application of a MEOR process can be realized, an understanding of the cells' transport and retentive mechanisms in porous media is needed. Cell transport differs from particle transport in their ability to produce polysaccharides, which are used by cells to adhere to surfaces. Cell injection experiments have been conducted using Leuconostoc cells to illustrate the importance of cellular polysaccharide production as a transport mechanism that hinders cell movement and plugs porous media.

It is proposed that core plugging phenomena by bacteria polysaccharide production be used for profile modification. Parallel core plugging experiments have demonstrated the ability of cells to selectively plug highly permeable regions. Flow diversion from the high to low permeable core occurred for a limited time only. This result was due to diversion of nutrients into the low permeability core which in turn allowed for cell growth and plugging. Hence, to be able to use a bacterial system for profile modification requires a set injection strategy. This strategy would have to state the concentration of cells initially injected, the number of cells injected, and the total mass and concentration of nutrients injected to allow for cell growth within the water swept zone only. Thus kinetic data is needed for the establishment of these parameters.

Kinetic studies of the Leuconostoc cells have been carried out to further understand the plugging rates of porous media. Experimentally, it has been shown that the cells' growth rate is weekly dependent upon the sucrose concentration in the media, and strongly dependent upon the concentration of yeast extract. On the other hand, dextran yields were found to be dependent upon the sucrose concentration in the media. In addition, the synthesis of cellular dextran has been found to lag behind cell generation, thus indicating that the cells' need to reach maturity before they are capable of expressing the dextran sucrase enzyme and synthesizing insoluble dextran. These observations have been used to develop growth rate, polymer production, and substrate utilization models which support overall development of injection strategies for profile modification.

F. Mississippi State University and Hughes Eastern Corp.

1) Nutrient Studies

In laboratory studies it was shown that starved oil-utilizing microorganisms would biosorb both ammonium ions and orthophosphate ions. Berea sandstone cores (2 inches by 4 feet) were prepared to simulate depleted oil sands. Microorganisms were introduced into test cores with the simulated production water while control cores were sterilized with ethylene oxide. Experiments performed using these cores yielded the following results.

- The sequential addition of low levels of nitrate ions and orthophosphate ions to the cores resulted in significant increases in microbial numbers and activity without plugging the core.
- Excessive amounts of nutrients allowed the microflora in the core to proliferate to such an extent that the core became plugged.
- The sequential addition of nitrate ions and orthophosphate ions to the core caused the microorganisms in the core to increase oil recovery by 350%.
- Evidence of microbial activity in the cores was characterized by a depletion of the short-chain aliphatics and the production of a long-chain aliphatic (C24-25) and long-chain fatty acids.
- A U.S. Patent was obtained (U.S. Patent No. 4,475,390).

2) <u>Indigenous Microflora</u>

The overall purpose of the second project was to develop information on the microflora indigenous to subterranean oil reservoirs, with special emphasis on their potential role in MEOR.

With regard to the microbial residents of subterranean oil reservoirs, it was learned that:

- Significant numbers of microorganisms were present in all 13 reservoirs examined (ranging in depth from 805-14,492 ft.)
- Each reservoir had a somewhat distinctive miroflora.
- Of the 37 pure culture isolates examined, all grew anaerobically and produced one or more products of potential value to MEOR - gas, acid, emulsifiers, polymers, solvents.
- Microbial isolates would colonize stratal materials (limestone, sandstone, clay shale) and their growth in sandpacks caused alterations in the flow of water through the sandpack.
- None of the 13 reservoirs examined contained sulfate-reducing bacteria.
- Ultramicrobacteria were present in two reservoirs.

The results of this investigation support the concept that microorganisms indigenous to subterranean oil reservoirs are valuable for enhancing oil recovery. Toward this end, studies were conducted to test this concept under the most realistic conditions possible in the laboratory. Specifically, cores (1.5 in x 4 in.) from oil reservoirs were employed in core flood experiments. By so doing, the microorganisms, formation materials, oil, and water were in as close to their natural state as possible.

Simulated production water containing supplemental nutrients was then allowed to flow through these cores. No supplemental nutrients were added to the water flowing through the control cores. One test core and one control core were prepared from each of five cores obtained from different reservoirs and the following results were obtained when supplemental nutrients were added to the injection water.

- Oil was released from the test cores.
- There was an increase in the number of microorganisms present in the core effluent.
- The production of acid by the microflora resulted in the dissolution of large amounts of carbonate material and the development of new channels.
- Some plugging of the more porous zones occurred.
- Gas was produced in some cases.
- The addition of trace amounts of ethanol to the injection water greatly enhanced the release of oil and the dissolution of carbonated in the formation material.

3) Water flooding Field-Test-*In-situ* Microflora

The project began with the drilling of two wells to obtain live cores for laboratory work and to determine how well the reservoir was being swept by the existing water flood. The microbiological analyses of the core (top, middle, and bottom portions) suggest that the area of the reservoir in which the wells were drilled had not been impacted by the water flooding operations because no sulfate-reducing bacteria were found in the core. Sulfate-reducing bacteria were prevalent in production water from other wells in the reservoir.

Laboratory water flood experiments using live cores were performed. Simulated production water was allowed to flow through the cores from carboys situated 41 inches above the core. Control cores received simulated production water only while test cores received simulated production water supplemented with potassium nitrate and disodium hydrogen phosphate on a prescribed schedule. The flow rate of water through the control cores increased with time while the flow rate of water through the test cores decreased with time and ceased to flow on some occasions. The flow was restarted by slightly increasing the pressure on the influent. The presence of oil in the effluent was more frequent from the test cores. Data for these laboratory coreflood experiments were used to formulate the concentration of nutrients to be employed on the field tests and the schedule for their addition to the injection water.

The North Blowhorn Creek Unit is the site of the field demonstration. Four injector wells were selected for nutrient additions and the surrounding producer wells are being monitored. Results obtained in these tests will be compared to historical data for the wells and the data obtained from four additional injectors and their surrounding producers which are serving as controls. It was considered essential to the scientific integrity of the project that control wells be included in the experimental design and as a consequence will be included for the duration of the 37-month trial ending in 1997. Performance of the production wells is being monitored. Additionally, chemical, microbiological, and petrophysical characteristics of the injection water and produced fluids are being monitored.

After eight months, examination of the production in test pattern one has shown the following trends. The decline in oil production is less, in fact, it has stopped and appears to be increasing in one well. Perhaps more importantly, the water to oil ratio in one well has changed direction and is now decreasing, not increasing. In two cases, the water to oil ratio has slowed its upward trend. In the control wells, in three cases, the water to oil ratio is increasing and the other two cases it is on the same increasing slope.

The smooth operation of the nutrient injection systems being employed in the field demonstration is considered a significant accomplishment, as is the fact that no changes in injection pressure have been observed and there has not been any evidence of skin effect around the wellbore.

G. Geomicrobial Technologies (GMT)

1) Single Well Microbial Treatments for the Prevention and/or Removal of Paraffin Deposition and Mitigation of Scale Problems.

Initial research centered upon laboratory studies using the patented technology developed by GMT (U.S. patent # 5,405,531). This patent describes the use of salts yielding anions of nitrate, nitrite and molybdate to selectively and favorably alter the microbial ecology of subsurface environments such as would be encountered in an oil field. Laboratory studies using bottle tests, Berea sandstone cores, and sandpack tests have shown that established sulfate reducing bacterial (SRB) populations can be suppressed or eliminated by using this treatment technology. This treatment technology has been shown to not only prevent denovo formation of hydrogen sulfide, but also to mitigate existing hydrogen sulfide and iron sulfide (pyrite) problems which can cause severe problems in oil and gas field operations. Additionally, this treatment technology has been shown to replace the detrimental SRB population with a benign population of denitrifying bacteria which can help consume sulfide while producing known MEOR agents such as gasses, surfactants, etc. The results and treatment strategies from laboratory investigations are being applied toward preliminary field tests.

Field tests to date have been conducted in Oklahoma, Kansas and Wyoming. Initial, preliminary field tests were conducted in Montgomery Co., Anderson Co., Kansas, and Rogers Co., Oklahoma. In all tests, no damage occurred to existing operations. The effects of the treatment technology on isolated single wells on the Phoenix lease in Rogers Co. Oklahoma was monitored using a Port-A-Chek unit to gauge fluid production. Preliminary tests yielded several important results. Data recorded from the Port-A-Chek unit revealed a possible increase in total fluid and oil production resulting from a single well batch treatment. Field tests in Anderson Co., Kansas and at the National Petroleum Reserve #3, Casper Wyoming have revealed a significant, favorable change in bacterial populations following field treatments and a noticeable reduction in sulfide levels and a mobilization of iron sulfide (pyrite). The successful remediation noted in the Anderson Co., Kansas field test (ongoing) showed a possible drawback in that the treatment resulted in a good cleanout of iron sulfide which subsequently led to some surface plugging problems which were later corrected.

H. Alpha Environmental

Alpha Environmental completed an eighteen month research program for DOE to demonstrate a process using petroleum degrading microorganisms, a biocatalyst, formation water and inorganic nutrients to economically recover residual oil in reservoirs. Results were mixed and inconclusive.

IV. BENEFITS TO VENEZUELA AND THE UNITED STATES

Through the programs of both Venezuela and the United States, microorganisms have been shown to be beneficial to the oil and gas industry in a number of ways as shown in Table 4.

TABLE 4. A CLASSIFICATION OF DIFFERENT MICROBIAL RESERVOIR TREATMENTS

MEOR Process	Production Problem	Type of microorganism used
Well Stimulation	Formation damage	Generally surfactant, gas, acid and alcohol producers.
	Low oil relative permeability	
Waterflooding	Trapped oil due to capillary	Generally surfactant, gas, acid forces and alcohol producers
Permeability modification	Poor sweep efficiency	Microorganisms that produce polymer
		Channeling and/or copious amounts of biomass
Wellbore cleanup	Paraffin problems	Microorganisms that produce Scaling emulsifiers, surfactants, and acids Microorganisms that degrade hydrocarbons

Venezuela conducted research directed specifically toward the reservoir conditions in that country. Overall, the Venezuelan reservoirs are deeper and have greater bottomhole temperatures than most of the U.S. reservoirs targeted for microbial EOR. Thus, Venezuela has had to draw upon microorganisms that can tolerate high temperatures and pressures.

In addition to the laboratory and simulation research efforts, the U.S. DOE Office of Fossil Energy co-funded several successful field pilot projects of MEOR involving injected organisms. At least four major oil companies and many independent operators eight have or are currently applying microbial technologies in the field to improve oil recovery.

The DOE co-funded two pilot projects, including a demonstration project using indigenous microorganisms to improve sweep of the reservoir by water flooding. In addition to increased oil production, these processes can significantly reduce operating costs to independent producers. Such treatments have been shown to reduce water production by 50 to 75% and associated lifting, separation and treating, transportation and disposal costs.

Literally thousands of wells have been treated in Venezuela and the United States with microbial systems that can clean up the wellbore and improve the productivity. Treatments such as these can decrease the costs associated with well workovers and cause savings to the independent operator of over 50% in operating costs. Although a significant number of new businesses have been established based on oil field applications, DOE is funding a small business through a subcontract with NIPER to provide documentation to the industry of the field application of microbial wellbore cleanup processes. This effort should make the technology more available to the more than 425,000 stripper wells (i.e., wells producing less than 10 bbl/d) which approximates 75% of the producing wells in the United States.

V. RECOMMENDATIONS - INCOMPLETE WORK

Several areas of research are recommended below that have been identified as needed or have incomplete results through this program.

- Perform well documented field tests, especially in reservoirs with substantial remaining oil saturation.
- Development of low-cost, consistent, and readily available nutrients.
- Continued development of profile-modification and well-stimulation methods.
- Development of salinity-and temperature-tolerant microbes, including those that can upgrade heavy oils.
- Improvement of simulator for MEOR processes.
- Improved understanding of recovery mechanisms for various microbial techniques and identify where combinations of processes will work.

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APPENDIX A
IMPLEMENTING AGREEMENT NO. XIII

IMPLEMENTING AGREEMENT NO. XIII

TO THE AGREEMENT

BETWEEN

DEPARTMENT OF ENERGY OF THE UNITED STATES OF AMERICA

AND

THE MINISTRY OF ENERGY AND MINES

OF THE REPUBLIC OF VENEZUELA

IN THE AREA OF MICROBIAL ENHANCED OIL RECOVERY

WHEREAS, the United States Department of Energy (hereinafter referred to as DOE) and the Ministry of Energy and Mines of Venezuela (hereinafter referred to as MEMV) desire to cooperate in the field of energy research and development;

WHEREAS, in the furtherance of their mutual interest DOE and MEMV entered into the Agreement in the field of Energy Research and Development signed March 6, 1980 (hereinafter referred to as the Energy R&D Agreement);

WHEREAS, DOE and MEMV have a mutual interest in technology exchange on the application of microorganisms for the recovery of petroleum;

WHEREAS, DOE and MEMV have a mutual interest in developing modeling capability to predict the recovery of petroleum via microorganisms from applicable petroleum reservoirs;

The Parties shall carry out a series of tasks over an initial period of 18 months. Further work may be required to complete all tasks at the end of the initial 18 month period. Any further work shall be the subject of a future Amendment and Extension to this Implementing Agreement.

A. Technical Information Exchange

The DOE and INTEVEP shall provide an annual summary of MEOR activities in their respective countries.

B. INTEVEP Experimental Tasks

Task 1 - Applied Venezuelan Microbial Laboratory Work

INTEVEP shall identify and select strains of surfactant producing bacteria such as Pseudomonas Aeruginosa. Results of experiments with these bacteria show that surfactant is easily liberated to the culture media instead of being associated with the bacterial surface. This capacity could prove to be of economical importance in an industrial extraction process. Experimental studies are being conducted to determine the influence of medium, temperature, pH, salt type, surface tension reduction and emulsifying capacity of the bacteria in order to optimize the production of surfactant.

Task 2 - <u>Evaluation of INTEVEP Reservoirs for MEOR</u>

INTEVEP shall analyze samples of various types of oils, effluent from bituminous sands, and other types of stratum for which MEOR could be used. INTEVEP shall analyze the reservoir data and the chemical composition and physical properties of the reservoir material therefrom and shall select candidate reservoirs for further screening studies. INTEVEP shall provide this information to DOE.

Task 3 - <u>Screening and Selection of Microorganisms</u>

INTEVEP shall screen and select several microbial strains that have potential for EOR using the samples from the candidate reservoirs selected in Task 2. Compatibility studies shall be performed to evaluate the microbial species and metabolic products shall be determined under the reservoir conditions selected by INTEVEP and DOE. INTEVEP shall provide this data for DOE.

Task 4 - <u>Bioprocessing of Waste Materials</u>

INTEVEP shall determine the efficiency of bioprocessing waste materials as nutrients in surface reactors to produce metabolic products suitable for use in EOR processes.

C. DOE Experimental Task

Task 1 - Microbial Transport and Porous Media Evaluation

DOE shall perform studies in porous media, cores and micromodels, to investigate the transport of selected microbial species, and to determine the oil recovery efficiency of the selected microbial strains, resulting in improved understanding of the fundamental mechanisms of mobilization and displacement of oil by microorganisms.

Task 2 - Effect of Nutrient on Oil Recovery Efficiency

DOE shall determine the metabolic products responsible for oil recovery improvement and nutrient screening and selection shall be done to optimize the production of these key microbial metabolities.

Task 3 - Development of Numerical Process Model

DOE shall obtain improvement in predictability and displacement sweep efficiencies by the development of a numerical simulator model of the MEOR process.

Task 4 - Profile Modification Field Test

DOE shall conduct a profile modification field test to demonstrate selective plugging of the swept zone of a reservoir to allow flooding of the by-passed mobile oil in the unswept zone.

All work under Paragraphs A and B, excluding Task 3 & 4, is to be completed by the end of the 18 months of the project. The Project Managers shall report to the Steering Committee at the end of the first year and propose the effort in man-years and funds required by the participants to complete the project. Effort beyond the 18-month duration of this Implementing Agreement shall be the subject of an amendment to the Implementing Agreement.

ARTICLE 3

A. The performance of Article 2, Paragraph B shall be by INTEVEP and all costs pertaining to Paragraph B shall be borne by INTEVEP.

B. The performance of Article 2, Paragraphs C, Tasks 1, 2, 3 and 4 shall be by DOE and all costs pertaining to Paragraphs C shall be borne by DOE, with the exception of shipping costs for any samples of oil, effluent from bitiminous sands, and other types of stratum required, which shall be borne by INTEVEP. Obtaining and shipping the samples from Venezuela to Bartlesville, OK, shall be the responsibility of INTEVEP.

ARTICLE 4

The Parties shall support the widest possible dissemination of information arising from this Implementing Agreement in accordance with Article 2 of the Annex to the Energy R&D Agreement. If a Party has access to proprietary information as defined in Article 2 of the Annex to the Energy R&D Agreement which would be useful to the activities under this Implementing Agreement, information shall be accepted for the task only on terms and conditions as agreed in writing by the Parties.

ARTICLE 5

Rights to any invention or discovery made or conceived in the course of or under this Implementing Agreement shall be distributed as provided in Paragraph 1 of Article VI of the Energy R&D Agreement. As to third countries, rights to such inventions shall be decided by the Joint Steering Committee.

Each Party shall take all necessary steps to provide the cooperation from its inventors required to carry out this Article. Each Party shall assume the responsibility to pay awards to compensation required to be paid to its own nationals according to its own laws.

ARTICLE 6

The existing terms and conditions of the Energy R&D Agreement shall continue and remain in full force and effect notwithstanding the terms of this Implementing Agreement.

ARTICLE 7

This Implementing Agreement shall enter into force upon the later date of signature and shall remain in force for a period of 18 months. It may be amended or extended by mutual written consent of the Parties in accordance with Article V of the Energy R&D Agreement.

ARTICLE 8

This Implementing Agreement may be terminated at any time at the discretion of either Party, upon six (6) months advance notification in writing to the other Party by the Party seeking to terminate. Such termination shall be without prejudice to the rights which may have accrued under this Implementing Agreement, to either Party up to the date of such termination.

Done in Caracas & Washington this day 29 Feb, 1988.

THE JOINT STEERING COMMITTEE

On behalf of DOE

Member Marvin Singer

Member George Stosur

Member Thomas Wesson

Feb 29, 1988

Date

On behalf of MEMV

Member Enrique Vásquez

Member Manuel Alayeto E.

Member Pedro Luis Díaz

Feb. 26, 1988

Date

APPENDIX B
LIST OF RESEARCHERS

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BROOKHAVEN NATIONAL LABORATORY

Participating Team: R&D Staff: Eugene T. Premuzic, Mow S. Lin, Bettie Sylvester, Lori Racaniello, Jeffrey Yablon, J.-Z. Jin, Yao Lin, Karlene Hamilton, Guo Kin Ji, Xu Qian Fan, Wei Min Zhou, Lei Shing (Rina) Wu, Ludmilia Shelenkova, and Hsienjen Lian.

Note: Courtesy of other programs, notably the Educational and Minority programs, contributions of many participants are also acknowledged. These include all the students/minorities/teachers/visiting faculty and joint programs with the Department of Chemical Engineering at Howard University.

IDAHO NATIONAL ENGINEERING LABORATORY (INEL)

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INTEVEP

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