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INVESTIGATIONS OF MECHANISMS OF MICROBIAL ENHANCED OIL RECOVERY BY MICROBES AND THEIR METABOLIC PRODUCTS

Topical Report

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INVESTIGATION OF MECHANISMS OF MICROBIAL ENHANCED OIL RECOVERY BY MICROBES AND THEIR METABOLIC PRODUCTS

by K. L. Chase, R. S. Bryant, K. M. Bertus, and A. K. Stepp

ABSTRACT

Prior work at NIPER has identified the mechanisms of oil mobilization by certain microbial formulations. Mechanisms that have been shown to be important include wettability alteration, emulsification, solubilization, and alteration in interfacial forces. Experiments at NIPER have demonstrated that oil mobilization by microbial formulations is not merely the result of the effects of the metabolic products from the in situ fermentation of nutrient. Further investigation into the interfacial properties of microorganisms and the concentrations of metabolic products at the oil-water interface is needed to determine the specific mechanisms of oil mobilization.

A combination of two microorganisms, *Bacillus licheniformis*, NIPER 1 (ATCC No. 39307), and a *Clostridium* species (NIPER 6), was determined to be an effective microbial formulation for the recovery of residual crude oil in porous media. Flask tests with various nutrients and environmental conditions were used to evaluate the growth and metabolite production of NIPER 1 and 6. Several interfacial tension (IFT) measurements were conducted using certain metabolic products from the combined microbial cultures NIPER 1 and NIPER 6. Nonane was used as the oil for these experiments, since crude oil from Delaware-Childers field emulsifies very easily. Only propionic acid in the nonane system gave an IFT in the range of 1 mN/m by the spinning drop method.

The interfacial tensions of a selected microbial formulation were measured with two different crude oils using brines of varying salinities. Comparisons were made with saline brines containing only the nutrient and with microbial metabolite solutions from which the active cells have been removed by filtration to isolate the specific effects of the microbial cells. A significant change from the controls was not apparent in interfacial tensions using varying salinities or filtered products. However, a correlation between salinity and surface tension was observed; an increase in surface tension was observed with increased salinity.

Etched-glass micromodel studies showed that the microbial formulation effectively mobilized crude oil trapped after waterflooding. The observations from the micromodel indicate that significant interfacial effects occurred. Some oil mobilization by gas displacement was observed in the pores.

Because the interfacial tension values were not in the range (10^{-2} to 10^{-3} mN/m) necessary to cause a sufficient increase in capillary number, other mechanisms including wettability alteration were also investigated. Certain microbial formulations appear to shift the wettability of Berea sandstone core samples toward a more water-wet condition. The microbial cells appear to be involved in this wettability alteration since no change was observed in samples tested with a microbial formulation that had been filtered to remove cells.

Unsteady-state relative permeability tests were performed with microbial formulations in Berea sandstone cores. A decrease in relative permeability to water and an increase in relative permeability to oil was usually observed in microbially flooded cores causing an apparent curve shift toward a more water-wet condition. Samples tested with the microbial formulation exhibited higher oil recovery before water breakthrough and lower residual oil saturations.

INTRODUCTION

Laboratory and field experiments have demonstrated the potential for mobilization of oil with microbes and microbial products, but the actual mechanisms involved in the process have not been fully identified. Mechanisms shown to be important in most enhanced oil recovery methods involve lowering the mobility ratio and/or increasing capillary number. A significant increase in capillary number is achieved by lowering the interfacial tension between the displacing and displaced fluids. It has been reported that a 100 to 1,000-fold decrease in IFT is required to cause a decrease in oil saturation.[1]

Prior work has indicated that certain microbial formulations exhibit optimal behavior at specific salinities similar to surfactant systems.[2] Interfacial tensions of selected microbial formulations with crude oils were measured to determine the effects of salinity and composition of the microbial formulation.

Another significant mechanism of microbial oil mobilization is wettability alteration. The displacement of a wetting fluid by a nonwetting one is less efficient than the displacement of a nonwetting by a wetting fluid. For a microbial flooding process, wettability alteration could be a result of adsorption of a metabolic product or microbial cells. Tests were performed to determine wettability using the Amott[3] imbibition and USBM[4] centrifuge methods. Unsteady-state relative permeability tests were also performed to observe wettability alteration effects on relative permeability and oil recovery.

MATERIALS

Microorganisms

Results from previous studies have shown that a combination of *Bacillus licheniformis*, NIPER 1 (ATCC No. 39307), and a *Clostridium* species (NIPER 6) is one of the most effective formulations for the recovery of residual oil. This microbial formulation was chosen for further interfacial tension and wettability studies. *Bacillus licheniformis* is a facultatively anaerobic, sporeforming rod that produces alcohols, biosurfactant, and some organic acids when fermenting molasses. The anaerobic sporeforming *Clostridium* is a member of the butyric acid group that produce acetone, butanol, ethanol, isopropanol, butyric acid, acetic acid, propionic acid, carbon dioxide, and hydrogen when fermenting sucrose. This particular *Clostridium* species also produces a biosurfactant.

Several combinations of microorganisms were used in the USBM wettability tests. NIPER Bac 1 is the combination of four microorganisms used in a microbially enhanced waterflood field project in the Mink Unit of Delaware-Childers field near Nowata, Oklahoma.[5] NIPER Bac 1 is a combination of NIPER 1, NIPER

2, NIPER 3, and NIPER 4. NIPER 2 is a species of *Bacillus* that produces surfactant, acids, and some carbon dioxide. NIPER 3 is a species of *Clostridium* similar to NIPER 6 that produces carbon dioxide, ethanol, butyric acid, and surfactant. NIPER 4 is a Gram (-) negative, facultatively anaerobic rod that produces carbon dioxide and acids when fermenting sucrose.

NIPER 1 alone was used for relative permeability and Amott wettability tests because USBM centrifuge wettability tests have shown NIPER 1 to be the most effective in alteration of the wettability index. NIPER 6 was eliminated from these tests because the abundant production of gas would interfere with the tests.

Nutrient

The molasses used in these experiments was obtained from Pacific Molasses Company in Oklahoma City, and its mineral content is as follows: total ash, 6.1%; calcium, 0.8%; phosphorous, 0.08%; magnesium, 0.35%; potassium, 2.4%; sulfur, 0.8%; and sodium, 0.2%. The amount of total suspended solids is 74%, of which 3% is total protein, 48% is total sugar (sucrose), and the remaining 23% is fiber. The concentration of molasses used in the experiments was 4% vol/vol in tap water with 0.1% wt/vol ammonium phosphate added to facilitate microbial metabolism.

For relative permeability and Amott wettability tests, 3% wt/vol potassium chloride was added to the molasses solution. Microbes were cultivated in filtered molasses (0.45 μ Millipore filter) solution with an antibiotic added to prohibit growth of the normal molasses flora. Microbes were cultivated in tryptic soy broth (TSB) for the imbibition phase of the USBM wettability centrifuge tests.

A modified Medium E (Mod E) solution was used in some of the determinations of interfacial tension and for flask tests. The solution contained: sodium chloride, 5%; glucose, 1%; ammonium sulfate, 0.1%; sodium nitrate, 0.1% ; magnesium sulfate, 0.05%; and yeast extract, 0.05%.[6]

Crude Oil and Brine

Delaware-Childers (DC) crude oil was obtained from the Bartlesville Sand formation in Delaware-Childers field in northeastern Oklahoma. Delaware-Childers oil has a gravity of 31° API and a density of 0.87 g/cm^3. Chelsea-Alluwe (CA) crude oil was obtained from the Bartlesville Sand formation of the Chelsea-Alluwe field. Chelsea-Alluwe oil has a density of 0.852 and a gravity of 34.6° API. Brine with a concentration of 3% sodium chloride by weight was used for USBM wettability tests. Because of the possible presence of clays in the Berea sandstone cores, a concentration of 3% wt/vol potassium chloride was used for Amott wettability and relative permeability tests.

Core Samples

Blocks of Berea sandstone were obtained from Cleveland Quarries (Amherst, Ohio) and cut into cylindrical cores of 1.5 inches (3.8 cm) in diameter and 3 inches (7.6 cm) in length for Amott wettability and relative permeability tests. Core plugs for the USBM centrifuge tests were drilled 0.75 in. (1.9 cm) in diameter and 1.2 in. (3 cm) in length.

METHODOLOGY

Flask Tests

Flask tests were conducted in 500-mL nephelometric flasks. Optical densities were measured at 530 nanometers. The standard microbial plate count method[7] was used to enumerate microorganisms. Selective media developed and tested at NIPER were used for enumeration of NIPER 1 and NIPER 6.

Surface and Interfacial Tension

Surface tensions and some of the interfacial tensions were measured with a Fisher Instruments semi-automated ring tensiometer by the ASTM method[8] using the DuNuoy principle. Interfacial tensions were measured with the spinning drop interfacial tensiometer by the method of Wade, et al.[9]

Gas Chromatography

Compositional analyses were performed using a Hewlett Packard 5980A gas chromatograph equipped with a flame ionization detector. A 6-ft (1.83-m) glass column packed with Poropak QS (800-100 mesh) was used for all analyses. A temperature program of 95° to 195° F (35° to 90.6° C) gave the best separation of compounds. Standards used were 0.1 or 1% by weight alcohols or fatty acids.

Coreflood Apparatus

Three-inch-long core samples for Amott wettability and relative permeability tests were encased in rubber sleeves and confined in a hydrostatic coreholder at 350 psi, net confining pressure. The samples were waterflooded at constant pressure using nitrogen to drive a piston-type fluid reservoir.

Core Preparation

All core samples were evacuated and then saturated with brine. Initial water saturation (S_{wi}) was established in USBM centrifuge samples by placing the samples in Delaware-Childers crude oil and centrifuging at approximately 3,000 RPM which is equivalent to a pressure gradient of approximately 10 psi. One set of the centrifuge plugs was stored for 7 days submersed in the crude to establish initial wettability conditions. Other sets of core plugs were left in the oil overnight.

Samples were prepared for Amott wettability and relative permeability tests by flushing with a refined mineral oil (45 cP, viscosity). The mineral oil was then displaced with crude oil by flushing with brine. Permeability to oil at initial water saturation was determined using Darcy's law. All core samples to be used for wettability and relative permeability tests were aged with the crude oil in place to establish initial wettability conditions. The samples were stored submersed in the crude for 7 days.

Micromodel Experiments

Glass micromodels used in this study were previously described by Chatzis[10] and Bryant and Douglas.[11] The flow rate was adjusted to 0.01 mL/min, which corresponded to approximately 8 ft/day. The micromodels were brine-saturated (0.5% sodium chloride), oil-saturated with crude oil from Chelsea-Alluwe field, and waterflooded to residual oil saturation before microbial injection.

Centrifuge USBM Wettability Tests

For these tests, core samples were stored at initial water saturation and submersed in the crude oil for 7 days. Other samples were stored for 24 hours. Imbibition and drainage capillary pressure curves with brine and crude oil were generated by increasing rates of rotation to facilitate oil displacement with brine followed by brine displacement with oil. For other samples, imbibition and drainage capillary pressure curves were generated using a microbial formulation in TSB. The displacement curves of average saturation versus pressure were used to calculate the wettability index. A schematic of the procedure for USBM wettability is presented in figure 1, and a schematic showing wettability determination is presented in figure 2.

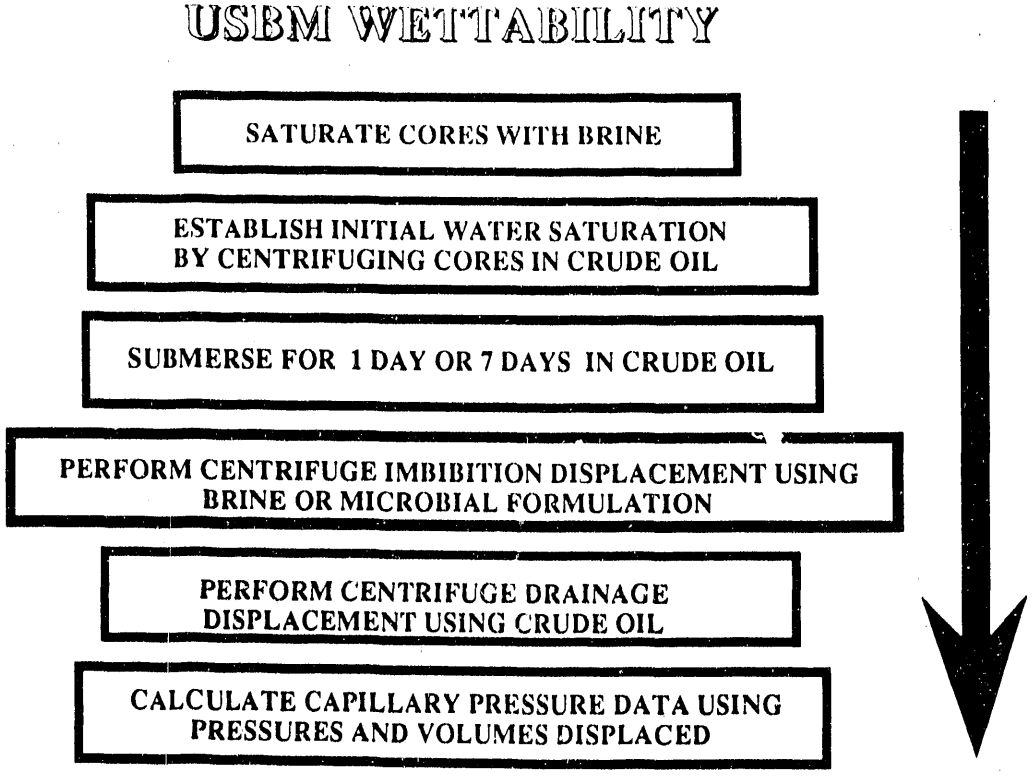

FIGURE 1.– USBM wettability procedure.

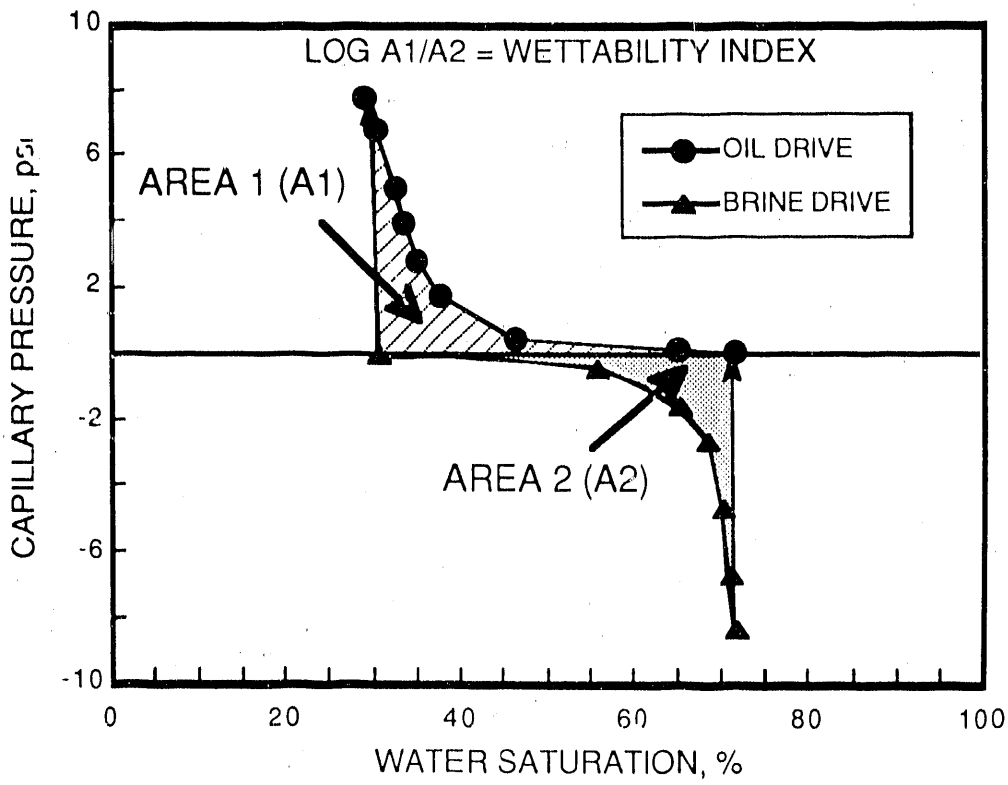

Figure 2.– USBM wettability determination.

Amott Wettability Tests

Following a 7-day period submersed in crude oil, the cores were placed in imbibition tubes. One core was placed in a tube full of brine solution (A1), and the other core was placed in a tube full of the microbial formulation (A4). The cores were left in the tubes and monitored daily until no more oil was produced. Both samples were stored in the tubes for about 3 weeks.

Each sample was then removed from the wettability tube and loaded into a coreholder. The samples were flooded with either brine or microbial formulation to determine the additional volume of mobile oil that could be displaced dynamically. Sample A4 was flooded with the microbial formulation. The samples were then placed in imbibition tubes full of crude oil. Again, the samples were left in the tubes until equilibrium had been reached, then both samples were flushed with crude oil until no more brine (or microbial formulation) was produced. Residual water saturation was verified by a toluene distillation method. Results are presented in terms of a wettability index to both oil and water. A brief outline of the Amott wettability procedure is presented in figure 3.

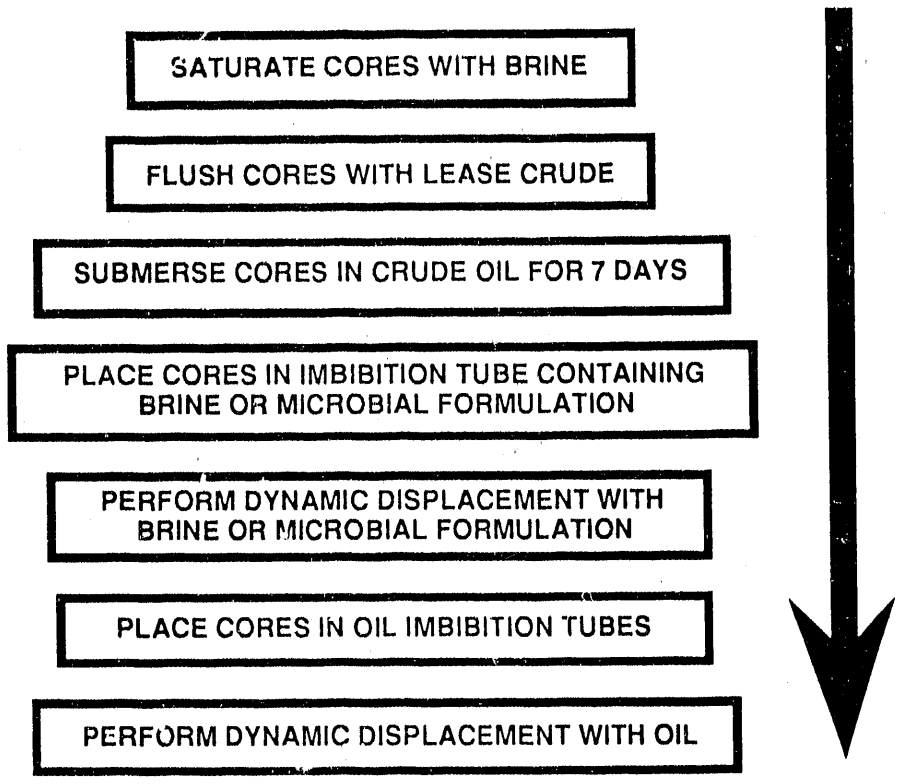

FIGURE 3.– Amott wettability procedure.

Unsteady-State Relative Permeability Tests

After the cores were aged in crude oil for 1 week, the crude oil was displaced dynamically with mineral oil. Permeability to mineral oil at initial water saturation was determined. The core was immediately waterflooded with either the 3% by weight potassium chloride brine or the microbial formulation. The cores were waterflooded at a constant pressure calculated from a theoretical flow rate of 0.1 cm^3/sec to overcome end effects. The brine (or microbial formulation) was injected at constant pressure, and water and oil volumes produced were measured as a function of time. These data were used to calculate relative permeability values for water and oil using the method of Johnson, Bossler and Naumann.[12] A schematic of the procedure for sample preparation and relative permeability is presented in figure 4.

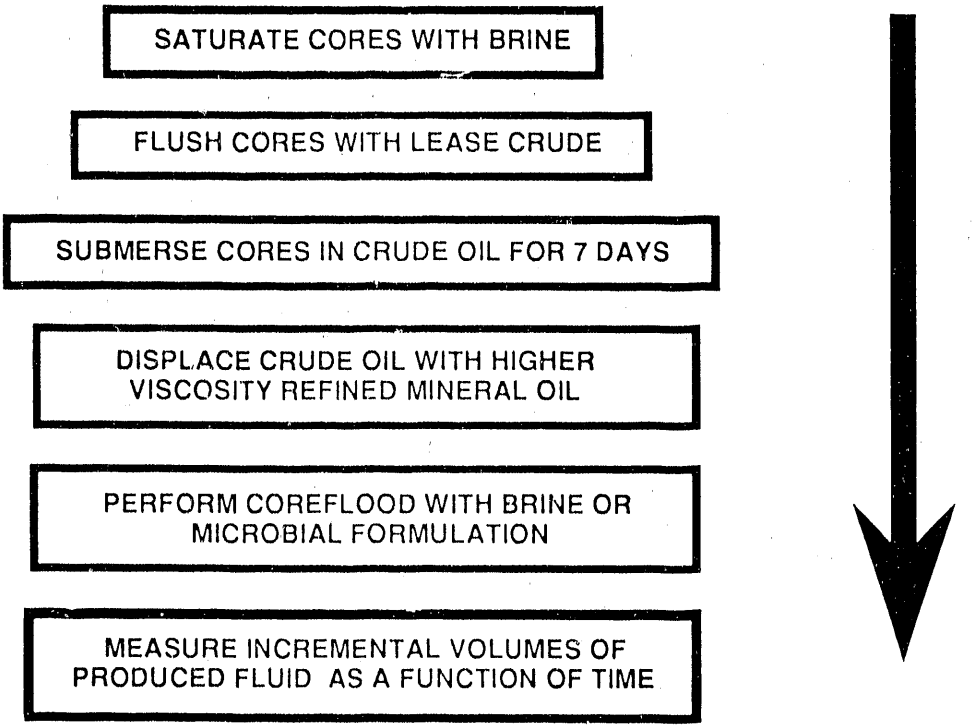

FIGURE 4.– Unsteady-state relative permeability procedure.

RESULTS AND DISCUSSION

Flask Tests

A series of flask tests was conducted with NIPER 1 and 6 using different nutrients and salt concentrations to determine the differences in microbial growth and metabolite production. One flask test compared TSB with molasses, another compared differences obtained with crushed Berea sandstone and 1% sodium bicarbonate in the flasks, and the last flask test compared NIPER 1 and 6 microbial growth and metabolite production in modified Medium E with the results in molasses.

The results from a comparison of NIPER 1 and 6 in TSB vs. molasses are presented in tables 1 and 2 and figures 5 and 6. Although the microbial counts for both NIPER 1 and NIPER 6 were consistently 10 times higher in TSB than molasses, the metabolites detected by gas chromatography were almost twice as high in the molasses flask. The metabolite responsible for most of this increase was butyric acid. However, NIPER 1 also appears to produce butyric acid, so it is unclear which microorganism was stimulated by the molasses.

TABLE 1.– Microbial counts and metabolites detected by gas chromatography from NIPER 1 and 6 in molasses

Time, hr	NIPER 1 Aerobic, cfu/mL	NIPER 1 Anaerobic, cfu/mL	NIPER 6, cfu/mL	TGC,[1] wt%
[2]0	1.2×10^6	9.8×10^5	1.6×10^6	0.25
4	2.9×10^5	8.5×10^5	2.2×10^6	0.24
8	2.8×10^7	6.2×10^6	2.8×10^7	0.24
12	1.9×10^6	4.6×10^7	2.8×10^7	0.24
24	8.0×10^7	5.6×10^7	4.0×10^5	0.24
48	8.0×10^8	1.4×10^7	5.5×10^6	0.31
72	5.0×10^7	1.3×10^8	1.2×10^7	0.43
96	1.3×10^8	1.0×10^8	1.5×10^7	0.53
120	1.1×10^7	3.1×10^8	1.7×10^6	0.57
144	3.3×10^6	1.1×10^7	6.5×10^5	0.63
168	4.5×10^8	1.5×10^8	5.5×10^5	0.68
216	4.3×10^7	1.6×10^7	3.0×10^6	0.90
312	2.1×10^6	2.1×10^6	5.5×10^3	1.07

[1]Total amount of metabolites detected by gas chromatography.
[2]0 hr designates the sample taken after microbial inoculation of the flask.

TABLE 2.– Microbial counts and gas chromatographically detectable metabolites from NIPER 1 and 6 in tryptic soy broth (TSB)

Time, hr	NIPER 1 Aerobic, cfu/mL	NIPER 1 Anaerobic, cfu/mL	NIPER 6, cfu/mL	TGC[1] wt%
[2]0	1.6×10^6	1.3×10^6	8.5×10^5	0.18
4	6.4×10^5	1.5×10^6	2.3×10^6	0.23
8	1.0×10^8	([3])	1.1×10^8	0.24
12	6.9×10^6	([3])	3.3×10^8	0.26
24	3.6×10^{10}	3.9×10^{10}	([3])	0.23
48	1.8×10^{10}	5.3×10^9	3.1×10^7	0.24
72	1.7×10^9	1.6×10^9	4.6×10^8	0.24
96	3.2×10^9	2.4×10^9	4.9×10^8	0.25
120	3.9×10^9	2.9×10^9	2.8×10^6	0.24
144	1.7×10^5	6.2×10^8	2.1×10^6	0.26
168	2.8×10^8	3.4×10^8	2.3×10^8	0.25
216	([3])	3.2×10^8	3.4×10^8	0.25
312	5.7×10^7	4.2×10^7	1.9×10^5	0.24

[1]Total amount of metabolites detected by gas chromatography.
[2]0 hr designates the sample taken after microbial inoculation of the flask.
[3]Counts were unavailable.

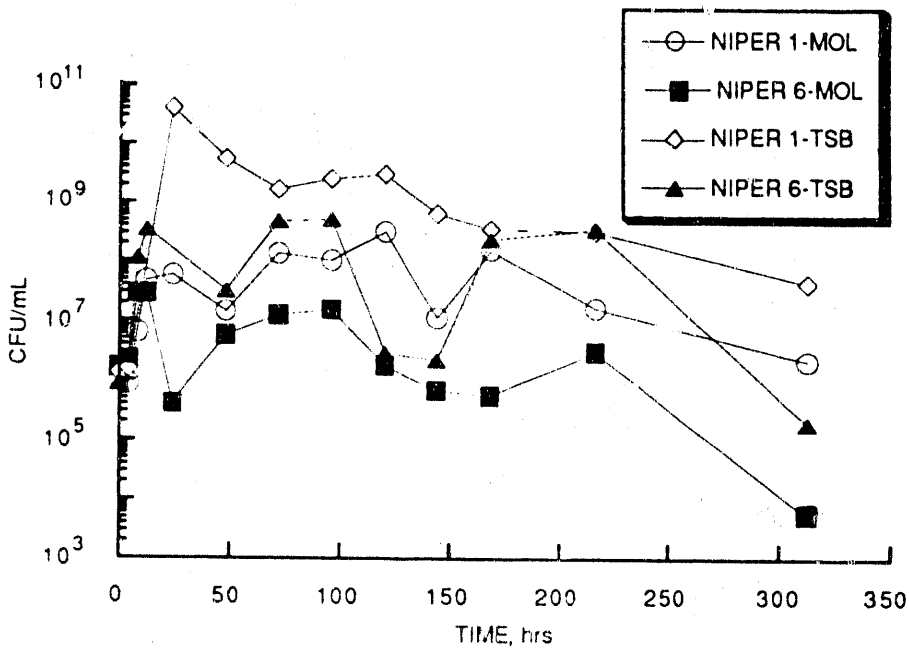

FIGURE 5.– Anaerobic microbial counts of NIPER 1 and 6 in TSB and molasses.

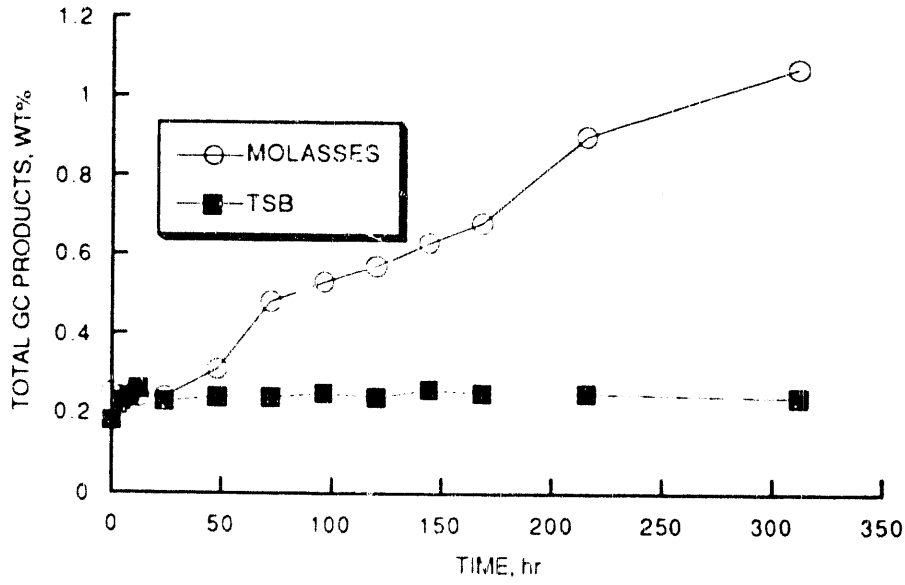

FIGURE 6.– Metabolites detectable by gas chromatography from NIPER 1 and 6 growing in molasses and TSB.

Previous work conducted by NIPER in FY89 showed that use of 1% sodium bicarbonate was an effective preflush for improved microbial oil recovery efficiency.[2] It was postulated that perhaps sodium bicarbonate was altering the amounts or types of metabolites produced by NIPER 1 and 6, or perhaps increasing their growth rates. Flask tests and corefloods with 1% sodium bicarbonate and NIPER 1 and 6 showed that there may be a complexity of effects of sodium bicarbonate, both on microbial metabolism and perhaps oil adsorption. To determine the effects of Berea sandstone on microbial metabolism, a second flask test was designed to measure the microbial counts, viscosity, and metabolites with sodium bicarbonate, and with Berea sandstone. Flask tests were conducted in the usual manner, with 1% sodium bicarbonate and approximately 10 grams of Berea sandstone added to the designated flasks. The results are presented in tables 3 and 4 and figures 7 and 8.

TABLE 3.– Microbial counts and viscosity measurements from flask tests with Berea sandstone and sodium bicarbonate and NIPER 1 and 6

		CFU/mL	
	Viscosity, cP	Aerobic	Anaerobic
		TIME = 0 days[1]	
Flask 1	1.0	1.6×10^5	1.7×10^5
Flask 2	1.1	2.6×10^5	4.0×10^5
Flask 3	1.0	4.8×10^5	3.0×10^5
Flask 4	1.1	4.3×10^5	5.0×10^5
		TIME = 14 days	
Flask 1	1.0	$< 1.0 \times 10^5$	3.2×10^6
Flask 2	1.1	1.9×10^7	2.4×10^7
Flask 3	0.9	$< 1.0 \times 10^5$	1.1×10^7
Flask 4	1.1	2.4×10^7	4.3×10^7

[1]0 hr designates the sample taken after microbial inoculation of the flask.

Flask 1 = Control NIPER 1 & 6.

Flask 2 = 1% sodium bicarbonate added.

Flask 3 = 10 g crushed Berea sandstone added.

Flask 4 = 1% sodium bicarbonate + 10 g crushed Berea sandstone added.

TABLE 4.– Gas chromatographic products from flask tests with Berea sandstone and sodium bicarbonate and NIPER 1 and 6

	Flask 1	Flask 2	Flask 3	Flask 4
Product		Conc., wt %		
		TIME = 0 days[1]		
Methanol	0	0	0	0
Ethanol	0.018	0.018	0.016	0.018
Acetone	T	T	0	0
Isopropyl alcohol	T	T	0	0
Acetic acid	T	T	T	T
Propionic acid/				
n-butyl alcohol	0	T	T	T
Butyric acid	0	0	0.023	0
2,3-Butanediol	0.058	0.056	0.065	0.056
		TIME = 14 days		
Methanol	0	0	0	0
Ethanol	0.037	0.048	0.019	T
Acetone	0	0	0	0
Isopropyl alcohol	0	0	0	0
Acetic acid	T	0.121	T	0.362
Propionic acid/				
n-butyl alcohol	0.026	0.01	0.025	0.011
Butyric acid	0.173	0.365	0.336	0.343
2,3-Butanediol	T	T	T	0

[1]0 days designates the sample taken after microbial inoculation of the flask.

T = Trace amount, for all compounds: < 0.01%; except acetic acid and 2,3-butanediol, which are < 0.05%.

Flask 1 = Control NIPER 1 & 6.

Flask 2 = 1% sodium bicarbonate added.

Flask 3 = 10 g crushed Berea sandstone added.

Flask 4 = 1% sodium bicarbonate + 10 g crushed Berea sandstone added.

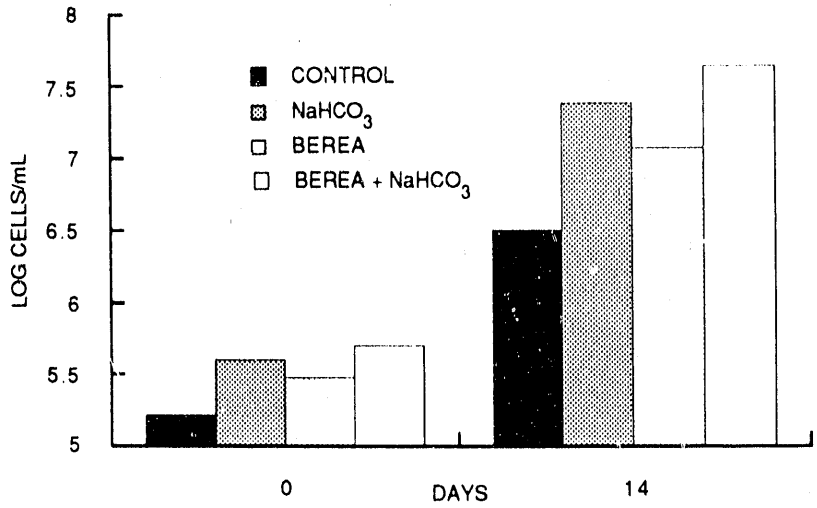

FIGURE 7.– Effect of Berea and $NaHCO_3$ on microbial counts of NIPER 1 and 6.

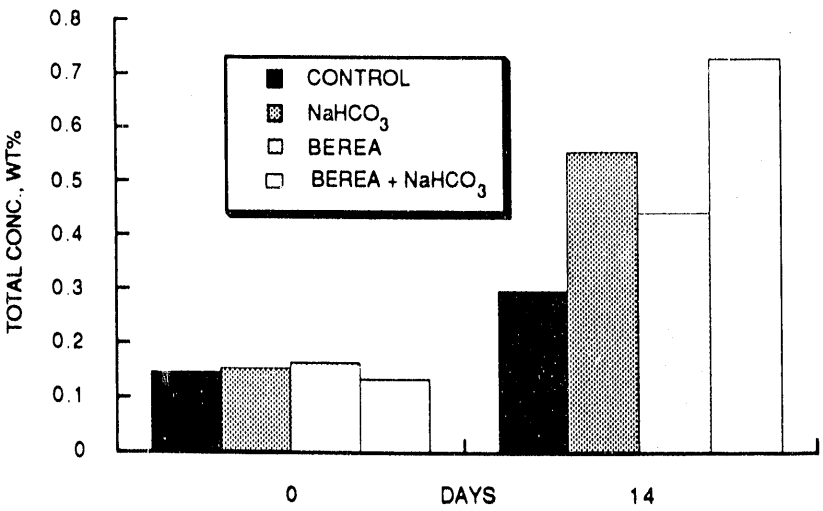

FIGURE 8.– Effect of Berea and $NaHCO_3$ on total metabolites detected by gas chromatography of NIPER 1 and 6.

The microbial counts, viscosities, and gas chromatographically detectable products from each flask were determined at the initiation of the experiment, and at the end of 2 weeks incubation. The presence of Berea sandstone and sodium bicarbonate does make a significant difference in the amount of certain metabolites produced by NIPER 1 and 6. In particular, the production of butyric acid and acetic acid is stimulated. Further investigations were conducted measuring the IFTs of these individual chemicals with crude oil; no significant lowering of IFT was achieved.

Discussions with researchers using the same strain of *Bacillus licheniformis* have emphasized the differences that nutrients can have on metabolic activity of the microorganisms. An experiment was conducted to ascertain whether NIPER 1 made different metabolites using a modified Medium E nutrient than when using molasses. Interfacial tensions of NIPER 1 in different media using two different hydrocarbons were also measured (table 5). The nutrient medium E is currently used by the University of Texas (Austin) laboratory for their experiments, and was first described for this microbe by the University of Oklahoma. The filtered molasses as a nutrient stimulated greater metabolite production by our strain of *Bacillus licheniformis* (figure 9). In particular, the production of a tentatively identified butyric acid by NIPER 1 was surprising. Later gas chromatographic analyses indicate that this may be lactic acid. There are two major differences in these media, one is that modified Medium E is buffered to a pH of 7.0, while filtered molasses has a pH of around 6.0. Modified Medium E also contained 5% sodium chloride, which may inhibit our strain of *B. licheniformis*.

TABLE 5.– Interfacial tension (IFT) measurements of NIPER 1 in modified Medium E and molasses containing 5% NaCl

	IFT, mN/m	
Incubation time, hr	Decane	Dodecane
	MEDIUM E	
38	([1])	([1])
64	([1])	([1])
136	4.22	5.78
160	6.03	2.48
184	7.39	4.11
	5% NaCl MOLASSES	
38	([1])	([1])
64	([1])	([1])
136	6.23	4.68
160	5.33	6.76
184	([1])	([1])

[1]Culture produced IFT's greater than could be measured on spinning drop apparatus.

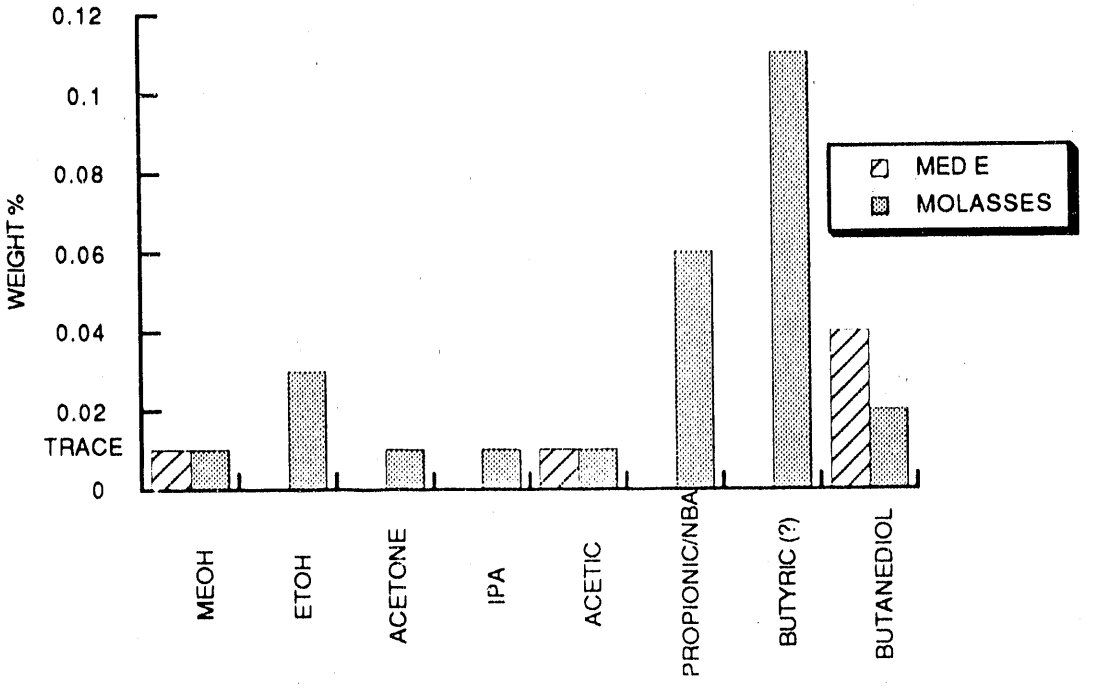

FIGURE 9.– Total metabolites detected by gas chromatography of NIPER 1 grown in Medium E and sterile molasses.

Table 6 and figure 10 show the results of a microbial growth flask test using NIPER 1 and 6, where the optical density and microbial counts were taken. The counts were surprisingly very low in Medium E, particularly for NIPER 6. After about 10 days, the counts appeared to begin increasing, but the lag time for this flask test is much too long for Medium E to be considered a good nutrient for NIPER 1 and 6. Since this experiment was conducted, Medium E flasks have been inoculated with NIPER 1 and 6 cultures that were adapted to higher salinities, and the results appear to be more favorable. The results from these flask tests emphasize the difference between microbial species and even strains of bacteria. Obviously the strain of *Bacillus licheniformis* (NIPER 1) that we have been using is different from the one being used at the University of Texas. NIPER 1 has been used in coreflooding experiments at NIPER for more than 5 years. Presumably the microbial strain has continued to adapt during this time and has the capability of transport in porous media, as well as improved oil mobilization properties.[2]

TABLE 6.– Microbial counts and optical densities from NIPER 1 and 6 in modified Medium E

Time, hr	NIPER 1 Aerobic, cfu/mL	NIPER 1 Anaerobic, cfu/mL	NIPER 6, cfu/mL	OD_{530}[1]
[2]0	6.9×10^5	4.1×10^5	4.3×10^3	0.0
4	4.3×10^5	8.0×10^3	8.0×10^2	0.001
8	9.2×10^5	6.0×10^4	1.2×10^4	0.010
12	1.2×10^5	5.0×10^7	7.5×10^4	0.015
24	1.0×10^5	1.5×10^6	1.5×10^6	0.000
48	4.0×10^5	1.0×10^5	1.0×10^4	0.020
72	2.5×10^5	2.5×10^4	3.0×10^4	0.025
96	9.5×10^4	1.7×10^4	4.7×10^3	([3])
120	8.0×10^4	5.7×10^4	3.0×10^1	([3])
144	1.6×10^5	3.2×10^4	1.4×10^3	0.010
168	3.2×10^5	7.2×10^4	1.5×10^3	0.005
216	5.5×10^4	3.9×10^5	1.4×10^3	0.020
336	6.5×10^7	2.6×10^7	1.2×10^4	0.090
360	1.7×10^7	8.4×10^7	1.0×10^2	0.155
384	7.6×10^6	2.4×10^7	([4])	0.190
408	3.9×10^7	3.7×10^7	1.2×10^6	0.210
480	2.0×10^4	4.3×10^7	7.7×10^4	0.185
504	2.4×10^4	6.9×10^6	4.7×10^4	0.205

[1]Optical densities measured at 530 nanometers.
[2]Time 0 hr designates the sample taken after microbial inoculation of the flask.
[3]Spectrophotometer readings were unreliable.
[4]Counts were unavailable.

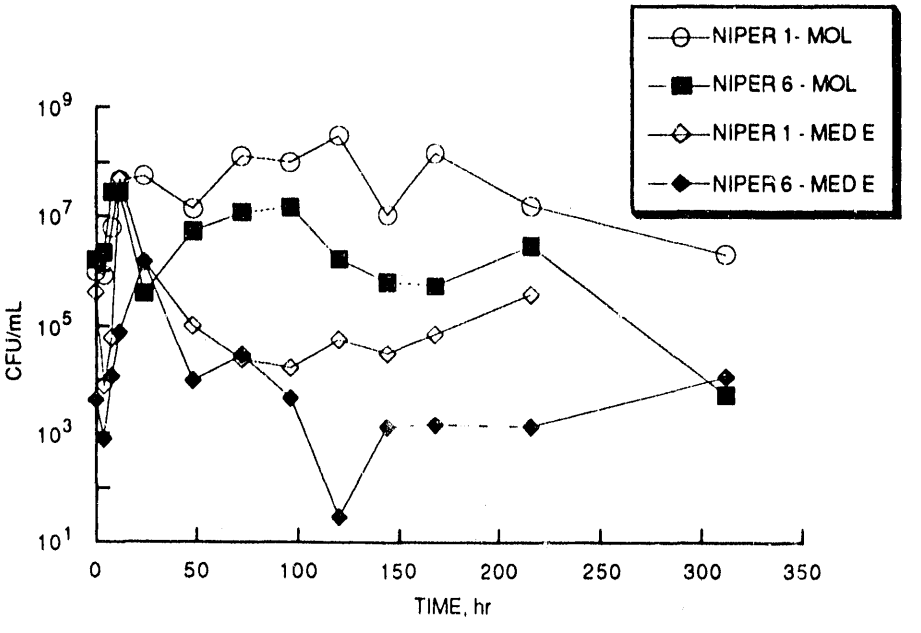

FIGURE 10.– Microbial counts of NIPER 1 and 6 in modified Medium E

TABLE 7.– IFT determinations between chemicals representing NIPER 1 & 6 metabolic products and nonane

Metabolite	Conc,wt %	IFT, mN/m
Spinning drop tensiometer		
Isopropyl alcohol	0.05	12
Propionic acid	0.05	1.09
Acetic acid	0.05	15.4
Ethanol	0.05	7.5
Butyric acid	0.05	15.2
Semi-automated ring tensiometer		
2,3 Butanediol	0.05	48.5

Interfacial Tension

Interfacial tension (IFT) measurements were conducted using chemicals known to be metabolic products from the combined microbial cultures NIPER 1 and NIPER 6 formulation including: methanol, ethanol, acetic acid, propionic acid, butyric acid, and 2,3-butanediol. Nonane was used as the oil for these experiments, since crude oil from Delaware-Childers field emulsifies very easily and could not be used as effectively with the spinning drop interfacial tensiometer. Nonane was previously determined to have the same equivalent alkane carbon number as that of Delaware-Childers crude oil. Only propionic acid in the nonane system gave an IFT around 1 mN/m by the spinning drop method (table 7). All other metabolites gave IFTs that were much greater than 1 mN/m. 2,3-butanediol has very little surface activity with nonane and had to be measured using the semi-automated tensiometer (ring detachment) method.

Interfacial tension was also determined using 5-day-old cultures of NIPER 1 and 6 grown anaerobically in unsterile 4% molasses nutrient containing varying concentrations of sodium chloride. Measurements were made using both the filtered (0.22 μ) microbial formulation and the unfiltered formulation to determine if there was a difference in IFTs with and without the cells present. Interfacial tensions were also evaluated using two different crude oils. Interfacial tension was slightly lower with crude from the Chelsea-Alluwe field than with oil from Delaware-Childers field. No significant difference was observed in IFT values for the microbial formulation containing varying salt concentrations or for unfiltered and filtered microbial formulation--without cells present (table 8). Interfacial tension values were not in the range that would significantly increase capillary number.

TABLE 8.– Interfacial tension determinations using varying salt concentrations

	IFT, mN/m at varying NaCl concentrations			
	0%	3%	5%	10%
Interfacial tension - NIPER 1 & 6 anaerobic molasses culture (5-days-old)				
Filtered[1]/DC oil	15.5	15.3	15.3	14.9
Unfiltered/DC oil	13.0	14.8	15.2	14.9
Filtered[1]/CA oil	10.7	10.9	11.1	8.7
Unfiltered/CA oil	9.3	11.0	10.6	8.6
Surface Tension - NIPER 1 - aerobic TSB culture (5-days-old)				
Unfiltered NIPER 1 in TSB	38.9	43.6	55.2	56.9

[1]Filtered with 0.22 micron syringe-filter to remove cells.

NIPER 1 was inoculated into tryptic soy broth (TSB) containing varying concentrations of sodium chloride. After aerobic incubation for 5 days, surface tension determinations were performed for each unfiltered culture solution. An almost linear increase in surface tension was observed with increased sodium chloride concentration (table 8).

NIPER 1 was also inoculated into filter-sterilized (0.22 μ pore size) molasses and incubated aerobically at 30° C. The culture was incubated until a valid reading could be taken using the spinning drop tensiometer. Two highly refined hydrocarbons were used, decane and dodecane, since these had been reported to give low IFT values of 10^{-2} mN/m with NIPER 1.[13] The results are shown in table 9. These IFT measurements, although lower than the values obtained with NIPER 1 and 6, were still not low enough to cause a significant increase in capillary number.

TABLE 9.– Interfacial tension values of two nutrients containing NIPER 1

	IFT, mN/m	
	Medium E	Molasses
136-hour-old culture		
Decane	4.2	6.2
Dodecane	5.8	4.7
160-hour-old culture		
Decane	6.0	5.3
Dodecane	2.5	6.8

Micromodel Experiments

A series of micromodel experiments was performed with a video recorder camera apparatus to document key observations. Previous work in this laboratory has shown that micromodel observations can be correlated with oil recovery data from Berea sandstone coreflooding experiments.[11] Microbial formulations that recovered a significant amount of crude oil in Berea sandstone cores also mobilized crude oil trapped after waterflooding in micromodels. Figure 11 shows residual oil trapped in the micromodel after waterflooding. After microbial injection and incubation, we observed gas droplets that moved into the pore throats containing trapped oil, thereby mobilizing the oil (figs. 12 and 13). Figure 14 shows changes in oil ganglia; elongation and pinching off of many of the oil droplets during waterflooding was observed. These changes indicate lowering of interfacial tension. Some solubilization of the trapped oil droplets was also observed (fig.15). Several oil droplets began to lighten in color at the edges, producing a type of halo effect.

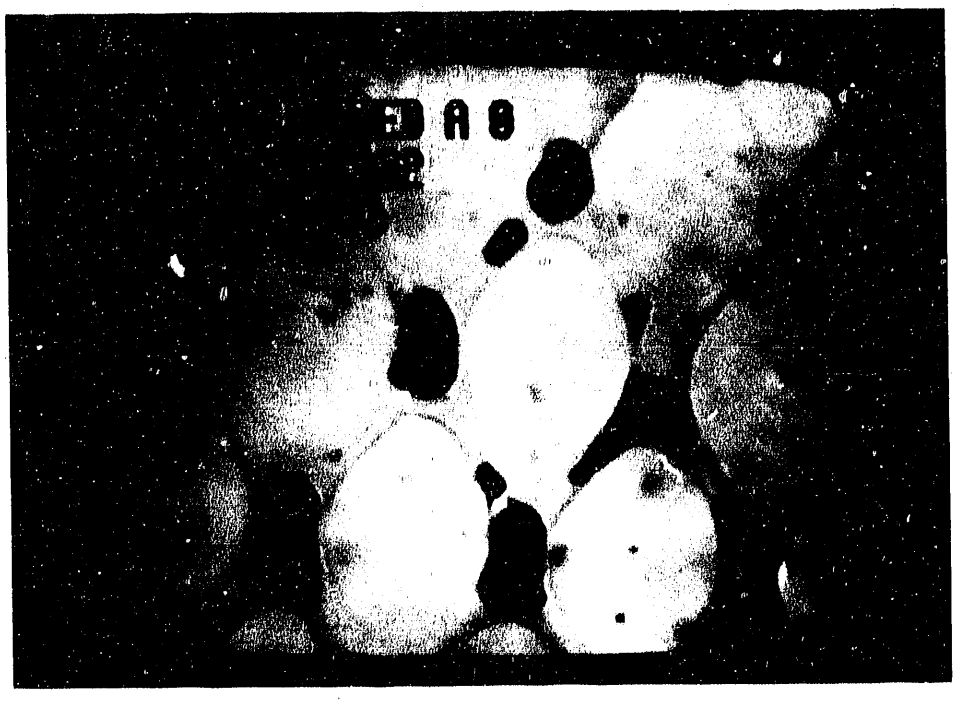

FIGURE11.– Residual crude oil after waterflooding micromodel.

FIGURE 12.– Microbial gas production in micromodel.

FIGURE 13.– Later view of same location showing gas bubble snapoff.

FIGURE 14.– Later view showing oil mobilization from gas production in micromodel.

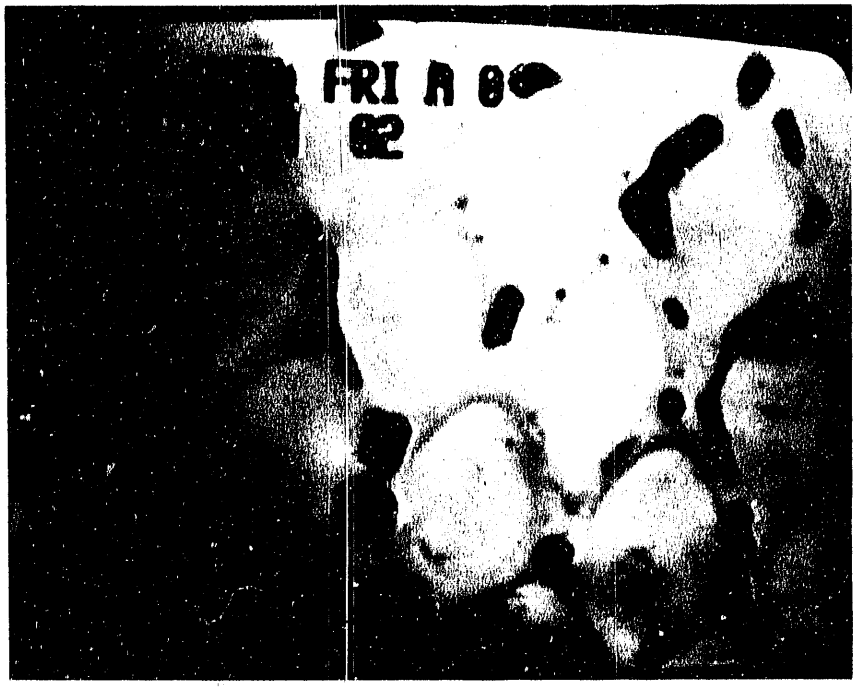

FIGURE 15.– Elongation and mobilization of oil ganglia in micromodel.

Centrifuge Wettability

Results of the USBM method centrifuge wettability tests are presented in table 10. NIPER 1 used either alone or in combination with other microbes appears to shift the wettability index significantly in the positive direction toward a more water-wet condition. The core treated with a filtered solution of products (cells removed) appeared to be in the same range of wettability index as the core used with the brine-oil system. The in situ microbial metabolism appears to be involved in wettability alteration since microbial products alone do not appear to alter wettability.

TABLE 10. – USBM centrifuge wettability tests

3% Sodium chloride brine

Sample No.[1]	Wettability Index
CP66	+0.153
CP67	+0.145
CP68	+0.267
CP69	+0.196
CP70	+0.223
CP71	+0.222

Average WI = +0.201 ±0.046

0.5% Sodium chloride brine

Sample No.[1]	Wettability Index
CP53	-0.064
CP54	-0.030
CP55	-0.082
CP56	+0.063

Average WI = -0.028 ±0.065

DC oil and different microbial solutions in TSB

Sample No.[2]	Wettability Index	Formulation
CP1	+0.315	Brine/ DC oil
CP2	+0.453	Brine/ DC oil
CP3	+0.739	NIPER Bac 1[3]
CP4	+0.992	NIPER Bac 1
CP23	+0.927	NIPER 1 and 3
CP24	+0.763	NIPER 1 and 3
CP9	+0.950	NIPER 1
CP10	+0.959	NIPER 1
CP15	+0.137	NIPER 2
CP16	+0.117	NIPER 2
CP11	+0.293	NIPER 3
CP12	+0.138	NIPER 3
CP13	+0.283	NIPER 4
CP 14	+0.262	NIPER 4
CP21	+0.355	NIPER 5
CP22	+0.500	NIPER 5
CP19	+0.310	NIPER 6
CP20	+0.314	NIPER 6

Delaware-Childers oil and NIPER 1 & 6 filtered products in TSB

Sample No.[2]	Wettability Index
CP60	+0.239
CP61	+0.117
CP62	+0.247
CP63	+0.176
CP64	+0.237
CP65	+0.100

Average WI = +0.186 ±0.065

[1]The core was aged for 7 days with Delaware-Childers oil at S_{wi}.
[2]Core was aged overnight with Delaware-Childers oil.
[3]NIPER Bac 1 is a consortium of 4 microbes used in field test; NIPER 1, NIPER 2, NIPER 3 and NIPER 4.
WI = wettability index

Amott Wettability

Results of the Amott wettability test are presented in table 11. The decrease in relative permeability to the microbial formulation at the end of the test shows a decrease in water mobility which would be seen in a trend to more water-wet conditions. The ratio of the permeability to water in the presence of residual oil to the permeability to oil in the presence of connate water (end-point k_{rw}) is sometimes used as an indicator of wettability--a value of less than 0.3 indicates water-wetness and a value near unity indicates oil-wetness [13]. The brine-oil core (A1) had a calculated end-point k_{rw} value of 0.355, whereas the sample tested with microbial formulation (A4) had a value of 0.220. The wettability index to water of 0.244 for control core A1 was very close to the USBM centrifuge wettability index values presented in table 4. The wettability index to water for the microbial sample was significantly higher; however, this core did imbibe some oil--the wettability index to oil was 0.162. The difference of 0.422 between the wettability index to the microbial formulation and the wettability index to oil is still twice the value of the wettability index to water for sample A1. The residual oil saturation for sample A4 was also 5.3% lower, which is consistent with the relative permeability results.

TABLE 11. Summary of Amott wettability test results

Sample Number	A1	A4
System	Brine-DC[1] Oil	Microbial formulation-DC Oil
Permeability to air, md	325	271
Porosity, %	19.2	19.2
Immobile water saturation, % PV	21.7	22.9
Permeability to oil at initial water saturation, md	163	131
Water[2] imbibed, % PV	12.2	27.7
Oil displaced dynamically, % PV	31.1	19.7
Total oil recovered, % PV	43.3	47.4
Immobile oil saturation, % PV	35.0	29.7
Permeability to water[2] at immobile oil saturation, md	58	29
Endpoint k_{rw}[3], fraction	0.355	0.220
Oil imbibed, % PV	0	6.2
Water[2] displaced dynamically, % PV	41.6	32.6
Total water recovered, % PV	41.6	38.8
Wettability index [4] to water[2]	0.244	0.584
Wettability index [4] to oil	0	0.162

[1]DC Oil = Delaware-Childers oil
[2]Denotes microbial formulation for sample A4
[3]k_{rw} =Effective permeability to water or microbial formulation (A4) at residual oil saturation/effective permeability to oil at initial water saturation
[4]Wettability index = Fluid imbibed/fluid imbibed + fluid displaced dynamically

Unsteady-State Relative Permeability Tests

Relative permeability measurements were conducted on adjacent samples from two sets of Berea sandstone that had different permeability ranges. Each set includes one sample with brine-oil relative permeability and another sample used for microbial formulation-oil relative permeability tests. The two samples from each group are presented together graphically for comparison studies. A summary of relative permeability test results is presented in table 12. Comparisons of the relative permeability fraction curves and ratio curves are presented in figures 16 and 17, respectively. A higher relative permeability to oil is apparent in both microbially flooded samples as compared to brine-flooded samples. The higher permeability core exhibited the effect much earlier in the flood. A slight shift to the right can be observed in the ratio curves of microbially flooded samples, which indicates that sample R8 had an increase in oil production as compared with the brine sample toward the latter half of the flood. Sample R2 exhibited a significant decrease in relative permeability to water throughout the flood. This higher permeability core, flooded with a microbial formulation that had been incubated 24 hours, exhibited an increase in oil production and relative permeability to oil immediately and continuing throughout the flood.

Oil recovery before water breakthrough was derived from the fractional flow equations using the viscosity of the Delaware-Childers oil. The samples waterflooded with the microbial formulation had a higher oil recovery before water breakthrough. A greater difference was observed in the relative permeability characteristics for the higher permeability sample that had been flooded with the 24-hour microbial culture.

TABLE 12.– Summary of unsteady-state relative permeability tests

			Initial conditions	
Sample[1]	Permeability to air, md	Porosity, %	Water saturation, % PV	Effective permeability to oil, md
[2]R1	981	22.6	30.5	650
[3]R2	1,240	22.8	30.4	837
[2]R7	322	18.9	25.3	209
[4]R8	323	19.0	25.8	202

	Terminal conditions		Oil recovered		
Sample	Oil saturation, % PV	Effective k to water, md	Percent PV	Percent OIP[5]	Before Breakthrough[6]
R1	30.3	127	39.3	56.5	23.5
R2	24.7	107	44.9	64.4	31.6
R7	32.2	17	42.5	56.9	26.7
R8	28.2	19	46.0	62.0	28.2

[1]All cores were aged for 1 week with Delaware-Childers oil.
[2]Brine/oil (mineral oil) relative permeability.
[3]24-hour microbial solution/oil (mineral oil) relative permeability.
[4]5-day old microbial solution (optimal biosurfactant production)/oil (mineral oil) relative permeability.
[5]OIP = Oil in place.
[6]Calculated using viscosity of Delaware-Childers oil.

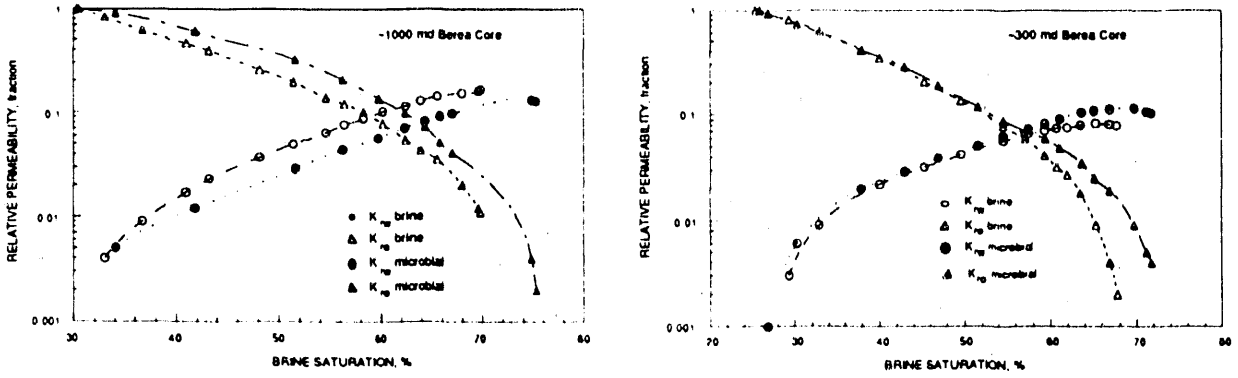

FIGURE 16.– Unsteady-state relative permeability fraction curves for cores with different permeability values.

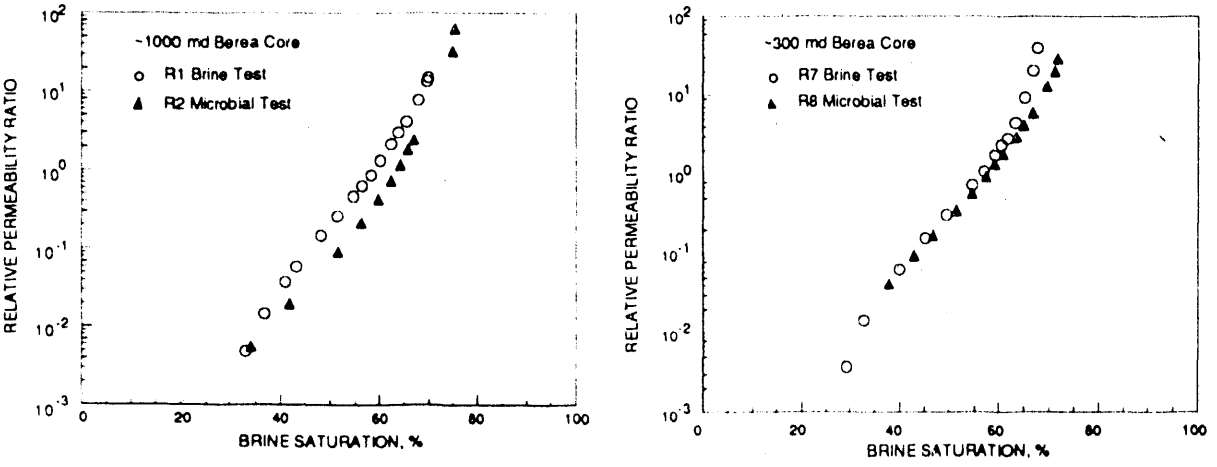

FIGURE 17.– Unsteady-state relative permeability ratio curves for cores with different permeability values

CONCLUSIONS

Data from several different flask tests have shown that by altering the nutrients and/or some of the environmental conditions, the metabolites produced by NIPER 1 and 6 can be changed. No one metabolite detectable by gas chromatography has been shown to significantly alter the IFT. The biosurfactants produced by NIPER 1 and 6 are probably the most important metabolites for oil mobilization, although even the metabolites themselves cannot significantly mobilize crude oil when the cells are not present. Flask experiments comparing microbial growth and metabolite production in molasses, molasses with sodium bicarbonate and/or Berea sandstone, and modified Medium E have demonstrated a great variety of microbial growth patterns and metabolite production. Although the interfacial tensions measured by the microbial cells with various crude oils and hydrocarbons are not in the range usually responsible for oil mobilization, there may be more of a variation in IFT behavior than can be measured at the present time. As an example, one of the keys to improved oil mobilization by NIPER 1

and 6 appears to be the presence of the metabolically active cells. Because the localized transient concentration of surfactant production cannot be measured at the oil/water interface, likewise, the IFTs at this interface may be transient and much lower than the overall values obtained.

Several biochemical assays were examined for their ability to detect and quantitate the biosurfactants produced by NIPER 1 and NIPER 6 using a spectrophotometric method. The biosurfactant from NIPER 1 is reported to have a peptide and a lipid moiety which can be detected using thin-layer chromatography; however, to quantitate the amount of surfactant, a colorimetric assay would be the easiest and most effective method to provide a standard concentration curve. At this time, there is no rapid or easy method to quantitate the NIPER 1 and 6 biosurfactants.

Interfacial values determined using the spinning drop apparatus were not in the range to cause an appreciable increase in capillary number. However, results from the micromodel studies show that interfacial tension must have been lowered by the microbial formulation in order to effectively mobilize the crude oil. Gas production by the microbial formulation was also responsible for some oil mobilization; however, in previous micromodel studies we have shown that a microbial formulation that produces only gas does not mobilize a significant amount of crude oil. The microbial surfactant production is important for crude oil mobilization.

Studies are continuing to isolate and quantitate the biosurfactant produced by NIPER 1. Sarkar, et al.[14] are reporting values in the range of 0.03 mN/M for *Bacillus licheniformis* JF-2. At this point, we have not been able to get values in this range. One of the major differences appears to be the nutritional requirements for NIPER 1 versus the *Bacillus licheniformis* JF-2 strain obtained by the University of Texas, and recent reports from Idaho National Engineering Laboratory.[15] These nutritional differences do not appear to be related to oil mobilization, however, since we are able to achieve much better oil recovery efficiencies with the NIPER strain.

Microbial formulations containing NIPER 1 appear to shift the wettability index of Berea core samples from just slightly water-wet to a more positive value. The microbial cells are involved in this wettability alteration since no change was observed in samples tested with filtered microbial products (cells removed). In situ microbial metabolism appears to be involved in wettability alteration. Results of the Amott wettability test indicate that the wettability index to water for the microbially treated core was twice that of the value for the control core, indicating a more water-wet condition.

A significant decrease was seen in k_{rw} curves (a decrease in water mobility) for two of three samples containing microbial formulation. Both relative permeability test samples showed an increase in relative permeability to oil; although, one sample containing microbial formulation had a 20% higher initial effective permeability to oil. However, it has been shown that cores with varying permeabilities but the same rock type (pore geometry) have almost identical relative permeability characteristics.[16] A slight shift to the right was observed at the brine saturation at which oil and water relative permeabilities are equal (crossover). Samples tested with the microbial formulation had higher oil recovery before water

breakthrough and lower residual oil saturations. Steady-state water-oil relative permeability tests may provide more meaningful data since Delaware-Childers crude oil, which has a low viscosity of 7.5 cP, could be used instead of refined mineral oil.

Although all wettability tests with microbial formulations exhibited a change in the direction of a more water-wet condition, changes in the range of neutral wettability are very hard to distinguish. Berea sandstone is very water-wet, and any alteration toward a more oil-wet condition is difficult to attain. Tests performed on a core that is definitel in the range of oil-wet would be helpful in further evaluating the effects of microbial systems on wettability.

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