SUMMARY ANNUAL REPORT

TITLE: Annex V - Microbial Enhanced Oil Recovery Research
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PRINCIPAL INVESTIGATORS: Mukul M. Sharma and George Georgiou

OBJECTIVES

The objective of this work is to develop an engineering framework for the exploitation of microorganisms to enhance oil recovery. Specific goals include:

- i) investigation of the mechanisms of microbially induced oil mobilization,
- ii) the production, isolation, chemical characterization and study of the physical properties of microbially produced surfactants,
- iii) model studies in sandstone cores for the characterization of the interactions between growing microbially cultures and oil reservoirs,
- iv) development of simulators for MEOR, and
- v) design of operational strategies for the sequential injection of microorganisms and nutrient in reservoirs.

INTRODUCTION

The exact mechanism by which the growth of microorganisms *in situ* facilitates the flow of oil is uncertain. A critical evaluation of previous results was undertaken and the most relevant mechanisms of oil recovery have been identified. (Sarkar, 1989)

The formation of gases has been stressed in nearly all field trials as well as in laboratory experiments. However the amount of gas needed for pressurizing the reservoir is very large and cannot be easily generated from microbial growth. In CO₂ flooding it is not uncommon to use 20% CO₂ per unit weight of oil produced. A simple stoichiometric calculation reveals that the biological production of such large amounts of gas is not realistic. A very large amount of cell mass (approximately 6 % biomass wet weight per unit weight of oil) would have to be generated in order to produce the amount of CO₂ needed for satisfactory oil recovery.

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Using order-of-magnitude calculations it was concluded that the production of organic acids is not a primary mechanism for enhancing the flow of oil. Similarly, reduction in the viscosity of the oil because of *in-situ* degradation of higher molecular weight hydrocarbons is unlikely. The conclusion that can be drawn from these general arguments is that three microbial activities may play a critical role in microbial enhanced oil recovery.

- i) production of surfactants,
- ii) production of biopolymers, and
- iii) selective plugging of high permeability zones.

The significance of biosurfactants in MEOR depends on several factors including the effectiveness of the biosurfactant in lowering the interfacial tension, the amount necessary to obtain significant surface tension reduction, and the adsorption of the surfactant on the rock surface. Generally, the interfacial tension must be lowered to 10^{-2} to 10^{-3} mN/m, corresponding to a capillary number $N_{ca} = 10^{-3}$ to 10^{-4} , before significant oil recovery is achieved. Some biosurfactants are capable of reducing the interfacial tension to such values.

SUMMARY OF PROGRESS

Task 1 - Characterization of *Bacillus Licheniformis* and the Produced Biosurfactant.

Aerobic Fermentations

Batch fermentations were carried out in a 2-liter Multigen fermentor. The interfacial tension of the growth medium against decane was measured by the spinning-drop technique. A typical fermentation profile is shown in Fig. 1. A lag phase of approximately 4 to 5 hours is observed before the beginning of exponential growth. A final bacterial concentration of 1 g/L is obtained before growth stops and the optical density begins to decrease. At $T = 45^{\circ}\text{C}$, 1% glucose as the carbon source and 4.5% NaCl, the doubling time was 90 min. It can be seen in Fig. 1 that the reduction in interfacial tension (IFT) occurs during exponential growth. It remains stable during early stationary phase and then increases. This increase in IFT may be caused either by the release of cellular components in the medium which cause higher IFT's or by inactivation of the surfactant (e.g. degradation). Although the partially purified surfactant was found to be fairly stable, in the fermentation broth it may be subjected to degradation by microbial enzymes that are

produced at the late stages of the fermentation. Glucose is completely utilized during the exponential phase as seen in Fig. 2. The growth yield of biomass on glucose (grams of cells/gram of glucose consumed) for the fermentation in Fig. 1 is shown in Fig. 2.

The effects of the growth medium pH on the minimum interfacial tension of the growth medium against decane were studied. The effect of growth medium pH is shown in Table 1. The large increase in interfacial tension as the pH is raised from 7.0 to 8.0 is probably due to a decrease in surfactant production, since basic pH has little effect on interfacial tension of the partially purified compound. The effect of growth temperature on the minimum IFT is shown in Fig. 3. The minimum interfacial tension is obtained within the temperature range of 45° C and 50° C. Overall, the interfacial tension of the fermentation broth exhibits a relatively small variation with temperature in the 30 to 50° C range. The cells were grown in media supplemented with different concentrations of NaCl, and the biomass concentration was measured at the point when the minimum IFT was obtained (Fig. 4). Relatively low IFT values were obtained in the concentration range between 2% and 5% NaCl. However, high NaCl concentrations cause an increase in IFT.

Anaerobic Fermentations

To characterize the growth and surfactant production under conditions which resemble the reservoir environment, we studied the growth of *Bacillus licheniformis* JF-2 under anaerobic conditions, 5% NaCl and T=45° C. The results for these fermentations are shown in Fig. 5. A long lag, typically 2 to 3 days, occurs before the onset of growth. The long lag is consistent with and explains some earlier results of MEOR experiments in cores. However, further experiments showed that the lag phase could be reduced to 10 to 20 hours by starting the fermentation with acclimated cells grown under anaerobic conditions. Typical bacterial concentrations of 0.8 mg/ml were obtained before growth ceased and the optical density began to decrease. A typical doubling time of 5 hours was observed.

It can be seen in Fig. 5 that the reduction in interfacial tension occurs during exponential growth. As shown in Fig. 6, glucose is not completely utilized during anaerobic fermentation of *B. licheniformis* JF-2. Typically, between 40 and 95 % of the glucose is used depending on the composition of the growth medium. The yield of biomass on glucose consumed does not vary much with time. Values around 0.1 mg cells/mg glucose consumed were calculated.

The effects of temperature and NaCl concentration on the minimum interfacial tension and final bacterial concentration have been determined. As the temperature is decreased to 30° C or increased to 50° C, a drastic increase in minimum interfacial tension is observed (Table 2). The final bacterial concentration values show that at 30° C the cells

grew well but little surfactant was produced whereas at 50° C the high interfacial tension value can be attributed to very poor growth. The effect of NaCl concentration under anaerobic conditions is shown in Fig. 7. The dependence of the IFT between the fermentation broth and decane to the salt concentration is similar for both aerobic and anaerobic cultures.

In summary, we have shown that the biosurfactant is produced in a wide range of conditions including salinities up to 6% to 7% and temperatures in the range between 30° and 50°C. The microorganisms also produce several growth byproducts such as organic acids (lactic, acetic and formic) and organic solvents such as acetone, butanediol and ethanol (the latter is produced in cultures grown without oxygen). These compounds can facilitate oil recovery *in situ*, possibly by acting as co-surfactants. The data from the fermentation studies have been used to derive parameter values for microbial growth that are employed in the simulator discussed below.

Structural and Chemical Characterization of the Biosurfactant.

The aerobically and anaerobically produced surfactant was extracted from the growth medium by acid precipitation at a pH of 2.0. About 0.1 g of acid precipitate was obtained per liter of culture broth. A few milligrams of the surface active compound were dissolved in 10^{-2} M NaOH, subjected to thin layer chromatography on Bakerflex cellulose F sheets and developed in chloroform, methanol, acetic acid, water (25:15 4:2, vol/vol). Samples obtained from either aerobic and anaerobic cultures showed the presence of a major component with an R_f value of 0.55 in each case, suggesting that the same biosurfactant was produced under aerobic and anaerobic conditions. The UV spectrum of the acid precipitate showed one major absorbance peak at 226.5 nm. The biosurfactant is soluble in alkaline water and in many kinds of organic solvents including methanol, ethanol, acetone, pentanol, ethylacetate, chloroform, methylene chloride but insoluble in acidic water and decane.

Knapp and co-workers (Javaheri, 1985; Jenneman, 1983) have suggested that the surfactant is a lipopeptide compound presumably consisting of a fatty acid esterified to a short peptide. However, in contrast to previous studies, we found that the surface active compound produced by our microorganism, *Bacillus licheniformis* is not a lipopeptide. The surfactant was partially purified from the fermentation broth by extraction with ethylacetate. After evaporation of the solvent an oily precipitate was collected and resuspended in buffer, pH 7.0 with 5% NaCl. This solution gave an interfacial tension against decane of 0.016 mN/m. This is the lowest IFT value ever reported for a biologically produced compound and compares favorably with the values obtained with

synthetic surfactants. The NMR spectrum of this compound indicated that the surfactant contains fatty acid(s), a polyhydroxyl group and an amine. No signal corresponding to amide protons was observed, indicating that this compound does not contain an amino acid chain. This result was confirmed from the IR spectrum.

The effect of the composition of the aqueous phase on the interfacial tension against decane was investigated. In water at pH 7.0 the IFT was five times higher compared to brine. It was found that 1% butanol can act as a co-surfactant, whereas the addition of ethanol or butanediol had no effect on the IFT. Lowest IFT values were obtained in the pH range between 6.0 and 11.0. At pH 4.0 the IFT was four-fold higher than at pH 6.0.

Task 2 - Core Flood Experiments

An experimental system has been designed to investigate the effect of microbial injection and growth on parameters such as permeability and fractional oil recovery from Berea sandstone cores. Cylindrical cores were placed in a core holder linked to a flow assembly that allows simultaneous pressure measurements along the core length as well as collection and analysis of effluent samples. Initial experiments were conducted to evaluate the breakthrough concentration profiles of tritiated water and nutrients (glucose). It was shown that the nutrients are not significantly adsorbed on the rock. In subsequent experiments different initial concentrations and volumes of bacteria suspended in nutrient solution (up to one pore volume) were injected into the core. After 5 to 12 days the suspended bacteria were flushed with brine. The effluent bacterial concentrations were determined by plating and by measuring the number of microbial particles using a particle counter (Coulter counter). Relatively small numbers of bacteria were detected in the effluent in all experiments. This indicates that most growth occurred by microorganisms that have adhered to the rock. The growth of the microorganisms was demonstrated indirectly from the consumption of glucose in the core. After 12 days approximately 60% of the glucose had been consumed. In contrast, in control experiments in which a nutrient solution was injected without any cells, no consumption of glucose could be detected. These results demonstrate that microbial growth takes place within the pores of the rock. Experiments are currently being conducted to simulate the coupled transport of bacteria, nutrients and metabolites.

Task 3 - Simulation of MEOR Processes

A detailed simulator for MEOR has been developed. To our knowledge this the first mathematical model for MEOR and describes all the relevant processes. A paper providing some of the details of the model was presented at the International MEOR Conference (Sarkar et. al., 1990). The model includes mass balance equations for the species of interest and rates for the appropriate source/sink terms:

$$(\text{Rate of accumulation}) + (\text{Flux}) = (\text{Rate of adsorption and consumption by microorganisms}) - (\text{Rate of generation of/ by microorganisms})$$

Specifically, mass balance equations have been written for free flowing and adsorbed bacteria, oil, limiting nutrient, biosurfactant and for the major growth byproducts (metabolites).

For the microorganisms, the flux terms include both convection and propagation due to self-propelled motion (chemotaxis). Separate equations have been written for the trapped and flowing bacteria to account for the difference in the rates of growth and metabolite production of the microorganisms in suspension or adsorbed in rock. The absorption of bacteria in the reservoir has been considered in some detail. The adsorption of microbes through porous media has been modelled as a filtration process where sand grains or clay particles serve as adsorption sites and straining pores. The rate of microbial growth depends on the concentration of the limiting substrate (in this case glucose) and the inhibitory metabolite. These relations have been described by Langmuir-type kinetics. The source terms for the production of the surfactant and inhibitory metabolite have been written as functions of the rate of growth and concentration of carbon source. The mathematical form of these functions was determined from the experimental data reported above.

The model has been based on a radial geometry to model single well treatments. The main mechanism of oil mobilization was postulated to be the production of the surface active species and the concomitant decrease in capillary number. An empirical correlation between capillary number and surfactant concentration has been used. Overall, the model contains 13 partial differential equations that are solved simultaneously by finite differences.

Task 4 - Bioengineering Specific MEOR Processes

The model developed above is now being used for studying the effect of various parameters on the MEOR process. Factors such as the concentration and volume of the injected slug of bacteria, the concentration and the volume of the nutrient preflush and

postflush can be evaluated. The results should accurately simulate the process of bacteria injection and growth since most of the input parameters have been experimentally measured. In addition an attempt is being made to simulate the process of selective plugging in heterogeneous media.

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SIGNIFICANT ACCOMPLISHMENTS

1. Systematic discussion of the mechanisms important in MEOR processes.
2. Measurement of the growth characteristics of *Bacillus Licheniformis* under various conditions of pH, temperature and salt concentration for both aerobic and anaerobic growth.
3. Measurement of interfacial tension reducing ability of the biosurfactant under different conditions of pH and salt concentration.
4. Development of some preliminary methods to concentrate and characterize the biosurfactant.
5. Development of a compositional numerical simulator for MEOR processes.
6. Measurement of the lowest IFT value reported for biosurfactants to date. Demonstration of the fact that the low IFT values required for oil recovery can be attained with biosurfactants.

SIGNIFICANCE TO EOR RESEARCH PLANS

Use of EOR technologies for additional oil recovery is driven by their economic feasibility. MEOR provides an economical alternative with low capital and chemical costs. There are however major technological hurdles to be overcome before the process becomes accepted by industry. The two major issues that need to be addressed are: can biosurfactants reduce interfacial tensions sufficiently to affect oil recovery; and how can these biosurfactants be effectively utilized in the oilfield i.e. by an ex-situ or an in-situ process. We have shown that biosurfactants hold the greatest promise for enhancing oil recovery through microbial treatments. Furthermore we have demonstrated that biosurfactants can reduce interfacial tensions sufficiently to affect oil recovery. We are currently working on better understanding the nature of these biosurfactants so that they can be optimally extracted and used. We are also actively pursuing the question of how these biosurfactants can be used in an in-situ process. This problem is intimately tied to the problem of bacteria transport and growth in rocks.

FUTURE RESEARCH PLANS

No major changes from the original proposal are envisioned in the upcoming year.

PUBLICATIONS

1. Goursaud, M.S.: "Production of a Biosurfactant by *Bacillus Licheniformis* JF-2," M.S. Thesis, The University of Texas at Austin (1989).
2. Sarkar, A., J.C. Goursaud, M.M. Sharma and G. Georgiou: "A Critical Evaluation of Microbial Enhanced Oil Recovery," *In-Situ* (1989) 13, 207-238.
3. Sarkar, A.K., M.M. Sharma and G. Georgiou: "Compositional Numerical Simulation of MEOR Processes", International MEOR Conference Proceedings, 1990.
4. Lin, S., G. Georgiou and M.M. Sharma: "Production and Characterization of a Lipopeptide Surfactant from *Bacillus Licheniformis* JF-2", American Chemical Society Meeting, Chicago, 1989.
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Figure 1: Typical fermentation profile of *Bacillus lienchiformis* JF-2 grown in a 1-liter batch fermentor under aerobic conditions at constant pH. *Bacillus lienchiformis* JF-2 was grown in medium E supplemented with 1% glucose and 5% NaCl. The temperature was kept constant at 45° C.

Figure 2: Glucose concentration and yield of biomass on glucose consumed during a batch fermentation of *Bacillus lienchiformis* JF-2 under aerobic conditions. The growth medium was medium E supplemented with 1% glucose and 5% NaCl. The temperature was kept constant at 45° C.

Figure 3: Minimal interfacial tension obtained during an aerobic batch fermentation of *Bacillus lienchiformis* JF-2 as a function of temperature. The organism was grown in medium E supplemented with 1% glucose and 5% NaCl.

Figure 4: Effect of NaCl concentration on the doubling time of *Bacillus lienchiformis* Jf-2, grown aerobically in shake flasks. The organism was grown at 45° C in medium E supplemented with 1% glucose.

Figure 5: Typical fermentation profile of *Bacillus lienchiformis* Jf-2 grown in 1-liter batch fermentor under anaerobic conditions at constant pH in medium E supplemented with 1% glucose, 5% NaCl and 1% NaNO₃. The temperature was kept constant at 45° C.

Figure 6: Glucose concentration and yield of biomass on glucose consumed during a batch fermentation of *Bacillus lienchiformis* JF-2 under anaerobic conditions at 45° C. The organism was grown in medium E supplemented with 1% glucose, 5% NaCl and 1% NaNO₃.

Figure 7: Minimal interfacial tension and bacterial concentration obtained during aerobic batch fermentation of *Bacillus lienchiformis* JF-2 as a function of NaCl concentration. Bacterial concentration was measured when the minimum value of the interfacial tension was reached. The organism was grown at 45° C in medium E supplemented with 1% glucose.

pH	interfacial tension (mN/m)
6	0.50
7	0.30
8	3.65

Table 1: Minimal interfacial tension during an aerobic batch fermentation of Bacillus lienchiformis JF-2 as a function of the pH of the growth medium.

T (°C)	Minimum interfacial tension (mN/m)	Final bacterial concentration (mg/ml)
30	5.81	0.71
45	0.51	0.83
50	8.06	0.20

Table 2: Minimal interfacial tension and final bacterial concentration obtained during an anaerobic batch fermentation of Bacillus lienchiformis JF-2 as a function of the temperature.

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