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Microbial Enhanced Oil Recovery and
Wettability Research Program

Annual Report for FY 1991

By

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ABSTRACT

This report covers fiscal year 1991 research results for the Microbial Enhanced Oil Recovery (MEOR) and Wettability Research Program conducted by EG&G Idaho, Inc. at the Idaho National Engineering Laboratory (INEL) for the U.S. Department of Energy Idaho Field Office (DOE-ID). The program is funded by the Assistant Secretary of Fossil Energy, and managed by the DOE-ID and the Bartlesville Project Office (BPO). The objectives of this multi-year program are to develop MEOR systems for application to reservoirs containing medium to heavy crude oils and to design and implement an industry cost-shared field demonstration project of the developed technology. The program is inclusive of collaborative research efforts (established as subcontract agreements) in wettability with the New Mexico Petroleum Recovery Research Center at the New Mexico Institute of Mining and Technology, Socorro, NM, and reservoir souring at the Montana State University Center for Interfacial Microbial Process Engineering in Bozeman, MT.

Surfactants produced by Bacillus licheniformis JF-2 both in vitro and in situ are effective to some extent at recovering waterflood residual oil (ranging in gravity from 17.5 to 38.1°API [0.9459 to 0.8343 g/cm³ at 15.6°C]) in laboratory scale Berea sandstone cores. Bacillus licheniformis JF-2 produces a polymer (Levan) as well as a surfactant. Levan is an insoluble (aqueous) homopolymer of fructose that is of little value (no contribution in laboratory systems) for sweep improvement but of concern for facial plugging. The surfactant produced by Bacillus licheniformis JF-2 does lower the interfacial tension (IFT) of all oils tested equally well with a trend toward greater efficacy with heavier oils. All isolates collected during FY 1990 were typed by gas/liquid chromatography of fatty acid methyl esters (FAME). Two of the isolates (tentatively identified as Bacillus subtilis) appear to be as effective as Bacillus licheniformis JF-2 at emulsifying oil in shake flask experimentation. Data indicate emulsification by IFT reduction is the most likely mechanism for oil recovery mediated by Bacillus licheniformis JF-2 and the Bacillus subtilis strains.

A dual microsensor electrode for detection of sulfide based on silver/silver sulfide (for sulfide detection) and iridium/iridium oxide (for pH) has been developed. An electrode has been developed that has a sensitivity of less than 10⁻¹¹ M total sulfide. Other souring research initiated in 1991 includes efforts to establish multivariant analysis techniques for biocide efficacy. These efforts will augment research efforts on the mitigation and abatement of product and reservoir souring. Select biocides are being tested for efficacy under a variety of conditions including presence of sandstone, oil, bacterial concentrations, and biocide concentration. A consortia of sulfate reducing bacteria isolated from the heater treater at the Moorcroft West Unit and Desulfovibrio desulfuricans (ATCC 13541) were selected for experimentation.

Wettability research has resulted in techniques by which crude oil may be used to prepare cores with a wide range of initial wetting conditions for use in systematic reservoir wettability studies. Wettability can be varied systematically by control of key variables such as oil and brine chemistries, temperature and duration of exposure, and water saturation. The wetting states produced are fairly reproducible and persist for some time, although not indefinitely. Preparation of extremely oil-wet cores required more strenuous treatment and a method using asphalt dissolved in diesel was developed.

The assessment of the Powder River Basin in Wyoming as a suitable site for field application has progressed. It is estimated that there are 2.2 to 3.6 billion barrels of oil remaining in the basin that are potentially feasible for MEOR applications. Two small Minnelusa fields, the Moorcroft West Unit and the Schuricht Field, have been evaluated for potential application. Enhanced oil recovery (EOR) currently in place in the Moorcroft West field has been reviewed and field histories (Moorcroft West and Schuricht) documented.

SUMMARY

This report covers research results for fiscal year 1991 for the Microbial Enhanced Oil Recovery (MEOR) and Wettability Research Program conducted by EG&G Idaho, Inc. at the Idaho National Engineering Laboratory (INEL) for the U.S. Department of Energy Idaho Field Office (DOE-ID). The program is funded by the Assistant Secretary of Fossil Energy, and managed by DOE-ID and the Bartlesville Project Office (BPO). The objectives of this multi-year program are to develop MEOR systems for application to reservoirs containing medium to heavy crude oils and to design and implement an industry cost-shared field demonstration project of the developed technology. An understanding of the controlling mechanisms will first be developed through the use of laboratory scale testing to determine the ability of microbially mediated processes to recover oil under reservoir conditions and to develop the design criteria for scale-up to the field. Concurrently with this work, the isolation and characterization of microbial species collected from various locations including target oil field environments is underway to develop more effective oil recovery systems for specific applications. Research focus includes the study of biogenic product and formation souring processes including mitigation and prevention. Souring research performed in FY 1991 also included the development of microsensor probe technology for the detection of total sulfide in collaboration with the Montana State University Center for Interfacial Microbial Process Engineering (CIMPE). Wettability research is a multi-year collaborative effort with the New Mexico Petroleum Recovery Research Center (NMPRRC) at the New Mexico Institute of Mining and Technology, Socorro, NM to evaluate reservoir wettability and its effects on oil recovery. Results from the wettability research will be applied to determine if alteration of wettability is a significant contributing mechanism for MEOR systems.

The report is organized into six sections; *Introduction*, *Microbial (Bacillus licheniformis JF-2) Mediated Oil Recovery*, *Microbial Isolation and Characterization*, *Microsensor Probe Technology for the Direct Measurement of Sulfide*, *Reservoir Wettability and its Effect on Oil Recovery*, and *Target Reservoir Selection and Field Application*.

Microbial (Bacillus licheniformis JF-2) Mediated Oil Recovery. Moderate weight crude oils were recovered in laboratory systems to the same extent as light oils by washed cell preparations and cell free supernatants of Bacillus licheniformis JF-2 grown in Medium E. However, floods utilizing cell injections required a smaller total injection volume (about 1/3) to mobilize the same amount of oil as that resulting from cell-free injections. Organisms indigenous to Berea sandstone do not contribute to oil recovery. Bacillus licheniformis JF-2 surfactant(s) lowered the interfacial tension (IFT) of all oils tested regardless of composition. Although IFTs below 1 mN/m were not observed (even when the cells were grown in complete media), oil was recovered. Even the heaviest oils were affected to at least the same extent as the lightest. The biosurfactants were not inactivated by any of the crude oils tested. Polymer produced by Bacillus licheniformis JF-2 (levan) has no impact on oil recovery. Although specific mechanism(s) of surfactant mediated oil recovery are still not fully understood, it is clear that oil recovery in laboratory core floods is increased by microbial surfactants.

Microbial Isolation and Characterization. Application of MEOR processes in the field will rely intimately on adaptation of microbial physiology to specific reservoir environments. Microbial isolates

selected for analysis in the MEOR program were screened for phenotypic and genotypic traits that allow them to function in oil reservoir environments. New isolates were collected from several oil field and oil laden environments during FY 1991.

Organisms collected in FY 1990 have been tentatively identified by gas/liquid chromatography of fatty acid methyl esters (FAME). Two isolates appear to be as effective as Bacillus licheniformis JF-2 in oil emulsification. Both isolates are Bacillus subtilis species that could potentially produce the surfactant *surfactin*. IFTs mediated by these cultures grown on a complete medium [trypticase soy broth (TSB)] facilitated IFT values between 3 and 14 mN/m. All isolates were tested for their ability to lower the interfacial tension between Schuricht crude oil and cell free supernatants (aerobic overnight TSB cultures). Data indicate emulsification by IFT reduction is the most likely mechanism for Bacillus licheniformis JF-2, and two FY 1990 isolates.

Microsensor Probe Technology. Microbially-induced formation souring occurs by sulfate reducing bacteria (SRB) converting sulfate to hydrogen sulfide gas (H_2S) in the formation. Other forms of souring include both electrochemical and geochemical reactions. If souring is sufficiently severe, it is necessary to remove H_2S from the produced gas and oil to meet product specifications, environmental constraints, and to prevent corrosion of the product handling system. Injection of nutrients into the formation to enhance oil recovery could aggravate souring by increasing the carbon source for SRB either directly or through metabolic products from other organisms that feed on the primary substrates.

The fundamental process in souring is the production of H_2S . To adequately study the souring phenomenon and monitor possible means of control, it is essential to be able to measure the activity of SRB spatially within the porous medium. To accurately determine the spatial distribution of microbial activity it is necessary to detect differential changes in conversion, which is more easily performed through product (sulfide) detection. Sampling of product streams for off-line analysis involves several problems that are difficult to overcome. Consequently, in situ, online measurements are preferred.

To provide a key tool needed for microbially-induced product souring research, the development of a small pH-insensitive, online detector was initiated. The goal of the program was to develop microsensor technology amenable to laboratory and, ultimately, field scale applications for the online, in situ detection of total sulfide. The objectives were to develop a detector that could measure total sulfide concentrations independent of the pH of the aqueous medium, and use the detector technology to develop a probe and thoroughly test it for monitoring the total sulfide concentration in core experiments containing oil, brine, and microorganisms.

A combination probe was constructed from an IrO_2 pH electrode, a Ag/AgS sulfide electrode, and a Ag/AgCl reference electrode. The detection limit is $\approx 10^{-18}$ molar $S^{=}$ in the pH range normally found in reservoir environments. At a pH of 7, this sulfide ion concentration would correspond to a total sulfide concentration of 10^{-11} molar using Equation 1 (Section 4.2.3) and a $pK_2 = 13.7$.

Future research will include testing the probe under a variety of conditions normally encountered in laboratory coreflood experimentation.

Target Reservoir Selection and Field Application. It is estimated that there are 2.2 to 3.6 billion barrels of oil (BBO) remaining in the Powder River Basin in reservoirs that are potentially feasible for MEOR applications. Field application of MEOR requires selection of appropriate sites as well as

specifically designing, engineering, and applying a system for the selected field. Analysis of select, small Minnelusa Sand formations in the Powder River Basin in Wyoming was conducted in FY 1990. Further information has been gathered and experiments performed indicating the applicability of MEOR in these fields. Enhanced oil recovery technology currently in place has been evaluated and field histories have been documented for two such fields (Schuricht and Moorcroft West).

Reservoir Wettability. Fluid distribution in oil reservoirs and the displacement of oil by water or other recovery fluids is governed by interfacial phenomena between the fluid and solid substrates of the system. This interaction is commonly known as wettability. While the importance of saturation history is recognized, the once common expectation that all reservoirs should be water wet (because water was the first fluid to occupy the pore space) has given way to the realization that a wide spectrum of wetting conditions occur. This includes strongly and weakly wetted systems (by both oil and water) as well as mixed wet systems. The condition most likely to occur in most reservoirs is one of mixed wettability. Research is being conducted to achieve a better understanding of the process by which one fluid immiscibly displaces another from a porous media. To this end, investigations have focused on surface phenomena, wetting changes induced by crude oils on solid surfaces, factors causing wettability changes, and the influence of these changes in wettability on displacement efficiency. Key to recent advances was the use of crude oils to alter wettability. Selected crude oils have been used to prepare cores with a wide range of initial wetting conditions for use in systematic reservoir wettability studies. The wettability produced in a core sample by exposure to crude oil can be systematically varied by control of key variables such as oil and brine chemistries, temperature and duration of exposure, and water saturation.

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1. INTRODUCTION

This report covers research results for FY 1991 for the Microbial Enhanced Oil Recovery (MEOR) and Wettability Research Program conducted by EG&G Idaho, Inc. at the Idaho National Engineering Laboratory (INEL) for the U.S. Department of Energy Idaho Field Office (DOE-ID). The program is funded by the Assistant Secretary of Fossil Energy, and managed by the DOE-ID and the Bartlesville Project Office (BPO).

1.1 Program Purpose

The objective of this project is to develop microbial enhanced oil recovery (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 must be completed. These subtasks are

- Determination of MEOR mechanisms of recovery and laboratory testing of MEOR systems
- Compatibility of MEOR with other EOR methods that may have been used previously in the target reservoir(s)
- Evaluation of the impacts of MEOR application with respect to biogenic product and formation souring
- Target reservoir(s) evaluation and characterization for potential oil recovery
- Economic evaluation of a potential MEOR system
- Single well injectivity testing and back-flow production of a potential MEOR system
- Industry cost-shared field demonstration of a potential MEOR system.

An understanding of the controlling mechanisms must first be developed through the use of laboratory scale testing to determine the ability of microbial processes to recover oil under reservoir conditions. Further studies will define nutritional requirements, temperature extremes, salinity ranges, and synergistic activities for potential organisms. A multi-disciplinary geological, biological, chemical, and engineering characterization of the target reservoir will be conducted to ensure a successful design and application of the process. The technical and economic potential of enhanced oil recovery in the target reservoir type will be determined to provide an evaluation of the ultimate technology potential. The feasibility of combining microbial systems with other EOR methods such as gas and chemical flooding will be addressed. The effects of following other EOR processes such as polymer flooding with an MEOR application also needs to be determined because many reservoirs of interest may have undergone tertiary oil recovery processes such as chemical, gas, and polymer flooding. This information will contribute to using MEOR in conjunction with other tertiary recovery methods. The applicability

and oil recovery potential of MEOR processes in actual reservoir environments will be evaluated by single well injectivity testing and back-flow production before initiation of an MEOR flood.

Current research at the Idaho National Engineering Laboratory (INEL) is intended to define and report specific microbial mechanisms responsible for oil displacement. Comprehensive understanding of the microbial mechanisms and the chemical and physical attributes of the reservoir and reservoir fluids controlling the microbial processes and mechanisms are being studied. Understanding the impacts of MEOR application in the field is of paramount importance and therefore the effect of MEOR application on produced oils (including potential souring) is being studied. The intention of this research is to gain a better understanding of how and why MEOR works. The results reported are for non-optimized microbial systems. Efforts have been made to perform realistic and definitive laboratory testing, to collect and preserve oil samples that will best represent reservoir composition, and to carefully conduct all experimental protocols to answer the appropriate questions. The amount of oil that may be recovered under optimum laboratory conditions is of little value unless a fundamental understanding of MEOR is developed through realistic laboratory simulation. Oil recoveries reported herein are not necessarily indicative of the oil recovery obtainable with optimized, specifically designed microbial systems.

It was not the objective of the research reported here to devise an optimized recovery system for a specific reservoir but to lay the foundation necessary to achieve that goal.

Several contributions to the permanent literature have been made as a result of this program. To the extent possible, reference is made to these publications and the results are not repeated in the text of this report. This report is organized into six sections; Microbial (*Bacillus licheniformis* JF-2) Mediated Oil Recovery (Section 2), Microbial Isolation and Characterization (Section 3), Microsensor Probe Technology for the Direct Measurement of Sulfide (Section 4), Reservoir Wettability and its Effect on Oil Recovery (Section 5), and Target Reservoir Selection and Field Application (Section 6).

Extensive efforts have been made to interact with industrial scientists and engineers to facilitate technology transfer and obtain critical program evaluation. Collaborative research programs have been established with the New Mexico Petroleum Recovery Research Center and the Montana State University Center for Interfacial Microbial Process Engineering. Both programs are jointly funded by the petroleum industry and academic institutions and are discussed in sections 4 and 5.

1.2 INEL Publications, FY 1991

- 1.) "Surfactant Based Enhanced Oil Recovery Mediated by Naturally Occurring Microorganisms," Thomas, C. P., Bala, G. A., and Duvall, M. L., *1991 Society for Petroleum Engineers Annual Technical Conference, Dallas, TX.*, SPE 22844, pp. 287-298.
- 2.) "Surfactant Based Microbial Enhanced Oil Recovery (MEOR)," Bala, G. A., Duvall, M. L., Barrett, K. P., Robertson, E. P., Pfister, R. M., and Thomas, C. P., in *Minerals Bioprocessing*, R. W. Smith and M. Misra, eds., *The Minerals, Metals & Materials Society*, 1991, pp. 121-132.

- 3.) "Surfactant Mediated Microbial EOR of Medium to Heavy Crude Oils," Bala, G. A., Herd, M. D., Jackson, J. D., Robertson, E. P., and Thomas, C. P., *1991 Eastern Oil Shale Symposium, Lexington, KY.*, in press.
- 4.) "Microbial Enhanced Oil Recovery and Wettability Research Program, FY 1990 Annual Report," Thomas, C. P., Bala, G. A., and Duvall, M. L. *DOE/ID 10326, (DE910022-48)*.

1.3 INEL Technical Presentations, FY 1991

- 11.) "Microbial Enhanced Oil Recovery and Wettability Research Program," Thomas, C. P., Bala, G. A., Duvall, M. L., and Morrow, N. R., January 1991. U.S. DOE Bartlesville Project Office, Bartlesville, OK.
- 2.) "Oil Recovery Mediated by Microbial Mechanisms," Thomas, C. P., Bala, G. A., and Duvall, M. L., January 1991. Phillips Petroleum Company, Bartlesville, OK.
- 3.) "Microbial Enhanced Oil Recovery" Thomas, C. P., Bala, G. A., and Duvall, M. L., January 1991. National Institute of Petroleum and Energy Research, Bartlesville, OK.
- 4.) "Microbial Enhanced Oil Recovery" Thomas, C. P., Bala, G. A., and Duvall, M. L., January 1991. Oklahoma University Department of Petroleum Engineering, Norman, OK.
- 5.) "Current Status and Future Direction of Microbial Enhanced Oil Recovery," Thomas, C. P., Bala, G. A., and Duvall, M. L., TIORCO, Inc. Englewood, CO, June 1991.
- 6.) "Mechanisms of Microbial Enhanced Oil Recovery by *Bacillus licheniformis* JF-2." Thomas, C. P., Bala, G. A., and Duvall, M. L., Unocal 76 Science and Technology Division, Brea, CA, June 1991.
- 7.) "Mechanisms of Microbial Enhanced Oil Recovery by *Bacillus licheniformis* JF-2" Thomas, C. P., Bala, G. A., and Duvall, M. L., Chevron Oil Field Research Co., La Habra, CA, June 1991.
- 8.) "Microbial Enhanced Oil Recovery Research Program," Thomas, C. P., Bala, G. A., and Herd, M. D., U.S. Department of Energy, Office of Fossil Energy, Washington D.C., August 1991.
- 9.) "Levan Synthesis in *Bacillus licheniformis* JF-2: Production Parameters and Implications for Enhanced Oil Recovery," Wells, A. D., and Bala, G. A., *1991 Associated Western Universities Summer Research Fellows Conference*.
- 10.) "Adaptation and Application of Enumeration Techniques for Monitoring Indigenous and Injected Microbial Populations Retained in and Produced from Berea Sandstone Cores," Brehm, M. A., and Bala, G. A., *1991 Associated Western Universities Summer Research Fellows Conference*.

1.4 INEL Abstracts, FY 1991

- 1.) "Isolation and Characterization of Surfactant Producing Bacterial Isolates for Microbial Enhanced Oil Recovery," Wells, A. D., Barrett, K. B., Jackson, J. D., Herd, M. D., and Bala, G. A., *1991 American Society for Microbiology Annual Meeting*, Dallas, TX.

Publications, presentations, and abstracts resulting from cooperative research with the Montana State University Center for Interfacial Microbial Process Engineering, and the New Mexico Petroleum Recovery Research Center may be found in sections 4 and 5 respectively.

1.5 Future Research and Direction

Future research at the INEL will continue to focus on defining microbial mechanisms for oil recovery, mitigation of formation and product souring, and interfacial microbial processes. Continued success of this research will be manifested as a demonstratable field technology researched and engineered on a site specific basis. Clearly the fundamental understanding of mechanisms contributing to MEOR processes will be applicable in a multitude of areas such as bioremediation and bioprocessing technologies.

Efforts to cultivate environmental isolates with enhanced phenotypic traits will be continued. Evaluation of MEOR in conjunction with or following other EOR technologies will begin. Evaluation efforts will be inclusive of synergistic microbial effects on oil recovery. Efforts will be initiated to identify potential environmental effects of pilot and field demonstration. Required controls, permits, and approvals will be initiated beginning with the selection of a target reservoir.

It is expected that application of microsensor technology developed jointly with the Montana State University Center for Interfacial Microbial Process Engineering will be brought online at the INEL in laboratory scale (1 x 6 in) core experiments. The technology will be evaluated for use in biotic and abiotic souring experiments to further the knowledge base of mitigation, prevention, and abatement. The potential for expanding the technology to field applications will be determined.

Continued success of the wettability program will be manifested by the development of methods for controlling and altering wettability for improved waterflooding and enhanced oil recovery. Identifying and understanding conditions for optimum oil recovery by waterflooding is a major goal of the program.

2. MICROBIAL (Bacillus licheniformis JF-2) MEDIATED OIL RECOVERY

Although microbial enhanced oil recovery (MEOR) was first conceived in 1926¹ and has been actively pursued by numerous researchers since, a broad forum of investigation is still available today. Several historical reviews have been written on MEOR^{2,3,4,5} and recently the technology has been recognized as a "potentially significant process" by the United States Department of Energy (DOE).⁶ MEOR processes are inclusive of two technologies: the injection and maintenance of desirable organisms into reservoir formations, and the stimulation of indigenous reservoir microbial populations. The application of MEOR technologies has resulted in three dominant areas of application: (a) single well treatments for well-bore maintenance and near-well oil production,⁷ (b) use of microbially produced polymers and biomass for increased waterflood sweep efficiency,^{8,9} and (c) the use of bacteria for in situ generation of acids, gasses, surfactants, and alcohols for enhanced oil recovery (EOR).^{10,11} An additional application of MEOR could be in conjunction with existing EOR technologies by providing an economic source of sacrificial agents such as polymers or surfactants.

The attraction of MEOR is ostensibly the potential cost benefit in comparison to other EOR methods. Because MEOR processes are more complex than other EOR processes, it is necessary to understand and control bacterial growth and metabolism in addition to the complexities associated with all EOR applications. Therefore, the economic viability of MEOR in comparison to established EOR technologies should be determined.

This section contains the results from oil recovery experiments performed with Bacillus licheniformis JF-2, obtained from the American Type Culture Collection (ATCC 39307).¹² Bacillus licheniformis JF-2 is a surfactant producing organism originally isolated from an oilfield environment. The surfactant produced by Bacillus licheniformis JF-2 has been isolated and characterized by other laboratories.^{13,14}

Surfactants enhance the recovery of oil through a reduction of the interfacial tension (IFT) between the organic (oil) and aqueous (brine) interfaces. IFT reductions between these interfaces causes oil displacement by reducing capillary forces and allowing oil to be displaced from the rock pores. Microbially mediated reductions of IFTs have been previously reviewed.^{15,16,17} The surfactant produced by Bacillus licheniformis JF-2 is thought to be physiochemically and structurally similar to that produced by Bacillus subtilis.¹⁸ 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 possesses a complex structure. The structure of an anionic petroleum sulfonate and the proposed structure of the surfactant produced by Bacillus subtilis are given in Figure 2-1. Aliphatic (propyl in this case) group(s) attached to the aromatic ring of the sulfonate surfactant may vary in number, length, and composition. Inactivation of commonly used anionic petroleum sulfonate type surfactants by constituents of crude oil has been reported.¹⁹ The effects of various oils on the efficacy of Bacillus licheniformis JF-2 biosurfactant has not been thoroughly evaluated.

(a)

Figure 2-1. Anionic petroleum sulfonate type surfactant (a), and the biosurfactant produced by *Bacillus subtilis* (b). Biosurfactant structure after Horowitz and Griffin.¹⁸

(b)

Surfactant produced by *Bacillus licheniformis* JF-2 (lichenysin) has been reported to lower the IFT of Octane/NaCl Brine (5%) to <0.1 mN/m.¹³

Bacillus licheniformis JF-2 also produces a polymer, levan, which is synthesized from sucrose by the enzyme levan sucrase. Levan is a non-water soluble polysaccharide biopolymer produced when the organism is grown on sucrose under certain conditions. Because of the water insolubility, levan production represents a potential plugging problem. *Bacillus licheniformis* JF-2 is capable of producing a significant amount of levan biopolymer (*i.e.*, 67 mg/10⁵ CFU or roughly 10 g/L). Therefore, consideration of the conditions under which this material is produced is warranted.

2.1 Experimental Procedures and Apparatus

2.1.1 Oil Selection and Analysis

Medium to heavy crude oils (17.5 to 38.1°API [0.9459 to 0.8343 g/cm³ at 15.6°C]) were selected for research. Liquid chromatography (LC) was performed on the oils for class separation (aliphatic, aromatic, pentane precipitable asphaltics, and nonpentane precipitable resins). Gas chromatography (GC) was performed on the LC effluents and deasphaltnated (pentane precipitable) oil samples. Elemental analysis (carbon, nitrogen, hydrogen, and sulfur) was performed on deasphaltnated (pentane precipitable) oil and viscosities were determined on whole oil. General characteristics of the oils are given in Table 2-1. Extensive oil characterization has been previously reported.²⁰

Table 2-1. General characteristics of the crude oils selected for MEOR research.

Oil	Sand	County	State	°API	Density at 15.6°C (g/cm ³)	Relative Viscosity at 23°C (Pa·s)
Burbank (BUR)	Burbank	Osage	OK	38.1	0.8343	0.006
Schuricht (SCH)	Minnelusa	Crook	WY	25.4	0.9081	0.054
Moorcroft (MOO)	Minnelusa	Crook	WY	22.3	0.9200	0.142
Alworth (ALW)	Cole	Jim Hogg	TX	19.1	0.9396	0.134
Lick Creek (LCR)	Meakin	Union	AR	17.5	0.9459	0.288

2.1.2 Microbial Analysis and Growth for Coreflood Experimentation

Organisms were grown for coreflood experiments as previously reported^{20,21,22} in minimal Medium E 1502 for *Bacillus*²³ with 1% sucrose as the sole carbon source and 2.5% NaCl. Sucrose was chosen as the carbon source because it represents the major carbohydrate constituent of beet molasses (molasses has historically been the feedstock of choice for MEOR applications in the field²⁴). Sucrose represents 63.5% of total solids in beet molasses and 95.4% of all carbohydrate present.²⁵

Bacillus licheniformis JF-2 was grown in minimal Medium E 1502 for *Bacillus* with varying concentrations of sucrose (0 to 10%), sucrose + glucose (0 to 10% total carbohydrate), and nitrate (1% as NaNO₃) for biopolymer studies.

Washed cells were prepared by centrifuging (10 min x 8,000 rpm, GS-3 rotor) the culture and resuspending the resulting pellet in fresh Medium E. Adherence to this protocol ensured that oil production was the result of in situ metabolism. Cell-free supernatants were also prepared by centrifugation. Analysis of the cell-free supernatants for biological activity (viable cells and spores) and potential nutrients (total carbohydrate, polymeric carbohydrate, and protein) was performed.

2.1.3 Preparation of Cores for Flooding

Nonfired Berea sandstone cores (permeability 85 to 510 md) used for experimentation measured 25.4 mm in diameter and 152.4 mm in length. The cores were coated with epoxy, fitted with brass end plates, evacuated, and then saturated with 0.45 µm filtered, vacuum degassed brine (2.5 wt% NaCl, 0.5 wt% CaCl₂).^{20,22}

Brine permeabilities were determined using Darcy's law. Porosity and pore volume (PV) were determined using dry and brine saturated core weights, brine density, and core dimensions. All saturations were determined volumetrically and verified by material balance.

Initial oil saturation was achieved by pumping crude oil through each core until brine production ceased. Initial water (S_{wi}) and oil (S_{oi}) saturations were determined and the cores were flooded with brine to waterflood residual oil saturation (S_{orwf}). All coreflooding procedures were performed at room

temperature. Oil saturation flow rates were 20 mL/h. Waterflood flow rates were held at 5 ft/d. Microbial flood flow rates were held between 1 to 2 ft/d (0.3 to 0.6 m/d) frontal advance rate.

2.1.4 Coreflooding and Effluent Quantification

The equipment used for all coreflood experiments and the quantification of coreflood effluents have been previously reported.^{20,22}

Viable bacterial preparations (60% PV) and cell-free supernatants were injected at a rate of 0.3 m/d or less to maintain injection pressures <1379 kPa (200 psi). Floods performed with viable organisms were incubated for 2 wk at 37°C following injection. During incubation, internal core pressures were monitored to ascertain the production of gas. Viable bacterial floods were produced to microbial flood residual oil saturation (S_{ormf}) with brine (2.5% NaCl). Cell-free supernatant floods were injected with 1 PV of supernatant, shut in for 24 h at room temperature, and then injected with 2 PV of brine. The sequence was repeated a total of 5 times (15 PV) to determine the biosurfactant residual oil saturation (S_{orbf}).

Oil recovery is reported as a percent of the original oil in place (OOIP) and as recovery efficiency (E_r , % of waterflood residual oil). E_r was calculated as previously reported.²²

2.1.5 Biopolymer Assay

Carbohydrates were assayed as reducing sugars by 3,5-dinitrosalicylic acid according to Chaplin and Kennedy.²⁶

2.1.6 Bacillus licheniformis JF-2 Metabolism of Crude Oil

Metabolic effects of Bacillus licheniformis JF-2 on crude oil compositions were determined by comparing crude oil constituents (as volume % from LC analysis) of oil extracted from aerobic bacterial emulsifications with oil extracted from abiotic shake flasks incubated under identical conditions. Gas chromatographs were also obtained for aerobic and anaerobic emulsification studies (Schuricht crude only). Comparisons of GC data were made by comparing pristane/ C_{17} and phytane/ C_{18} ratios of biotic and abiotic culture flasks and GC profiles (data not shown).

2.1.7 Measurement of Interfacial Tensions

IFTs were measured by real-time video image analysis of inverted pendant drops using an instrument designed and constructed at the INEL.^{20,21,22,27} Detailed theoretical derivations for the determination of the IFTs from pendant/sessile drops have been previously discussed.^{28,29,30} Instrumentation, algorithms, data acquisition, and techniques used at the INEL have been previously reported.^{20,21,22,27}

IFTs were measured on systems with published IFT values for standardization. Data were obtained for all crude oils using sterile growth medium and cell-free supernatants. All measured values were obtained between 22 and 24.5°C. Literature values are reported at 20°C. Measured IFTs were obtained with stock reagents using density values given on the material safety data sheets (MSDS), no temperature correction was applied.

2.2 Results

2.2.1 Microbial Analysis and Growth

Bacillus licheniformis is a facultatively anaerobic, spore-forming gram positive rod that is $\approx 0.7 \times 2.0 \mu\text{m}$ in size.³¹ The organism has thermotolerance to 50°C, halotolerance (NaCl) to 10% (highest value tested in our laboratory), and a pH tolerance from 4.5 to at least 8.5 when grown on Medium E. The organism does not reduce sulfate (indicated by no production of H₂S on Triple Sugar Iron Agar) and does not adhere to hydrocarbon water interfaces.²¹ Bacillus licheniformis JF-2 does produce a surfactant (previously discussed) and a polymer, levan.

Analysis of culture supernatants used for core injections is presented in Table 2-2.

Table 2-2. Characterization of Bacillus licheniformis JF-2 cell-free supernatant fluid.

Viable cells (plate count)	13 CFU/mL
Total particles (AODC ^a)	$2.8 \times 10^3/\text{mL}$
Total protein (dye binding)	27.1 mg/mL
Total carbohydrate (DNSA ^b)	3.9 mg/mL
Polymer carbohydrate (DNSA)	TDTM ^c
pH	6.28

a. Acridine orange direct count.

b. Dinitrosalicylic acid assay.

c. Too dilute to measure.

Indigenous microbial populations from Berea sandstone have been cultured in Trypticase Soy Broth (TSB). The indigenous organisms do not grow in Medium E aerobically or anaerobically. Indigenous organisms grown in TSB do not alter IFT or emulsify oil in flask test experimentation and therefore do not contribute to oil recovery in these experiments.

2.2.2 Coreflooding and Effluent Quantification

All coreflood experiments were conducted with cultures grown and injected in Medium E as previously described. Bacillus licheniformis JF-2 washed cells and cell-free supernatants recovered water-flood residual oil from Berea sandstone cores for all oils tested. There appears to be no significant recovery trend with respect to oil composition. In general, the most notable difference between the washed cell injections and the injections of cell-free supernatants was the number of pore volumes injected during the production flood before oil was produced. Washed cell preparations produced oil following ≈ 2.5 PV total fluid injection. Cell-free supernatants required ≈ 8 PV total fluid to achieve production.

Recoveries facilitated with Bacillus licheniformis JF-2 washed cells are presented in Table 2-3. Recoveries facilitated using cell-free supernatants are presented in Table 2-4. Graphical presentation of data is provided in Appendix A.

Table 2-3. Corefloods utilizing Bacillus licheniformis JF-2 washed cell injections.^a

CORE	TEST	OIL	VIS (Pa·s)	PERM (μm^2)	S_{oi} (%PV)	S_{orwf} (%PV)	S_{ormf} (%PV)	MEOR ^b REC (%OOIP)	MEOR ^c REC (%OOIP)	E_r (% S_{orwf})
22	BL ^d	BUR	0.006	0.211	65.8	32.6	29.6	4.6	4.6	9.2
20	CTRL ^e	BUR	0.006	0.319	70.7	34.0	34.0	0.0	0.0	0.0
25	BL	SCH	0.054	0.084	72.3	39.5	37.9	1.4	2.2	4.1
10	BL	SCH	0.054	0.264	76.7	33.4	29.9	2.7	4.6	10.5
19	CTRL	SCH	0.054	0.426	64.1	27.0	27.0	0.0	0.0	0.0
12	BL	ALW	0.134	0.117	72.9	18.6	15.5	4.3	4.3	16.6
23	BL	ALW	0.134	0.307	53.3	11.9	7.7	7.1	7.9	35.3
18	CTRL	ALW	0.134	0.132	66.6	25.5	25.5	0.0	0.0	0.0
24	BL	MOO	0.142	0.113	72.3	39.4	38.3	1.3	1.4	2.8
13	BL	MOO	0.142	0.121	78.8	25.6	14.7	9.9	13.8	42.6
17	CTRL	MOO	0.142	0.188	58.9	26.1	23.2	---	4.9	11.1

a. For all corefloods, temperature = 23°C and brine = 2.5 wt% NaCl.

b. Oil recovery at 6 pore volumes of brine injected.

c. Oil recovery with brine injection until oil production ceases.

d. Washed Bacillus licheniformis JF-2 cells resuspended in Medium E.

e. Nutrient control, Medium E.

The heaviest oil tested in this study (Lick Creek) gave varied results in coreflood experiments. Although oil was recovered in microbial flood experiments, variability between experiments was high. Control cores resulted in oil recoveries comparable to those experienced with Moorcroft West. Procedural complications have prevented reliable data acquisition.

Scanning electron microscopy (SEM) has been used to analyze post-microbial flood cores (permeability = 110 md) to ascertain the spatial distribution of the microbial cells.^{20,21,22} Data indicate complete distribution with a predominance of organisms located distal to the point of injection. SEM analysis of control cores also indicates the presence of indigenous organisms. These indigenous organisms do not appear to increase in number during incubation.

2.2.3 Production of Levan by Bacillus licheniformis JF-2

Cultures of Bacillus licheniformis JF-2 were grown under various sucrose, sucrose + glucose, and nitrate concentrations and incubation times to determine optimal levan production. At the end of incubation, cell free supernatant samples were hydrolyzed by oxalic acid. The resultant reducing

Table 2-4. Corefloods utilizing Bacillus licheniformis JF-2 cell-free supernatant injections.^{a,b}

CORE	TEST	OIL	VIS (Pa·s)	PERM (μm^2)	S _{oi} (%PV)	S _{orwf} (%PV)	S _{orbf} (%PV)	OIL ^c REC (%OOIP)	E _r (%S _{orwf})
33	CFS ^d	BUR	0.006	0.345	75.5	45.3	40.9	5.8	9.7
27	CFS	SCH	0.054	0.113	69.6	37.6	33.9	5.3	9.8
29	CFS	SCH	0.054	0.385	67.5	38.0	32.2	8.6	15.3
31	CFS	ALW	0.134	0.345	72.5	30.1	28.0	2.8	7.0
28	CFS	MOO	0.142	0.464	73.9	41.9	37.4	6.1	10.7
35	CFS	MOO	0.142	0.493	79.6	49.9	41.7	10.3	16.4
36	CTRL ^e	MOO	0.142	0.740	83.1	41.6	41.6	0.0	0.0
37	CTRL ^f	MOO	0.142	0.642	87.2	46.2	46.2	0.0	0.0

a. For all corefloods, temperature = 23°C and brine = 2.5 wt% NaCl.

b. Metabolites of Bacillus licheniformis JF-2 injected into core without dilution. Minimal viable organisms injected (Table 2-2).

c. Oil recovery at ≈ 15 PV of fluid injected (5 biosurfactant / brine sequences each consisting of 1 PV biosurfactant followed by 2 PV brine).

d. Cell-free supernatant.

e. Brine control (2.5% NaCl).

f. Nutrient control (Medium E).

monosaccharide (fructose) was quantified to give a relative measure of the original levan content. Parallel experiments were performed using cell-free crude extracellular enzyme extracts containing levan sucrase activity. Biotic cultures and crude enzyme extracts in each experiment were accompanied by corresponding abiotic controls.

Crude enzyme extract experiments demonstrate an optimum sucrose concentration of 5.0 mg/mL. In vitro levan production dropped markedly at concentrations below 5.0 mg/mL and above 10 mg/mL. The decrease in levan sucrase activity seen at concentrations higher than 5.0 mg/mL is most likely the

result of feedback inhibition by glucose, a product of the enzymatic hydrolysis of sucrose. Such a sharp decrease is unexpected but may be explained by the fact that many polyfructan/glucan synthesizing enzymes can exhibit hydrolysis of sucrose without the corresponding polymerization reaction under certain conditions. This situation would result in a larger than equimolar ratio of glucose to levan polymer production and may serve to inhibit levan sucrase activity to the extent suggested by the results.

Results from whole culture experiments demonstrate an increasing trend in *in vivo* levan production in proportion to sucrose concentration. These results are contrary to experiments suggesting constitutive rather than catabolite linked expression of the enzyme. The absence of any inhibitory effect is most likely because of the rapid removal of glucose by-product from the system by way of intracellular glucose transport.

2.2.4 Bacillus licheniformis JF-2 Metabolism of Crude Oil

Bacillus licheniformis JF-2 has no metabolic capacity for the degradation of crude oil. This is indicated by both compositional analysis based on LC fractionation and GC analysis of fractional components and whole oil. All results for Burbank, Moorcroft West, Alworth, and Lick Creek were consistent with Schuricht. Volumetric differences in the components of pre- and post-microbial flood oil samples are experimental artifacts. The apparent loss of the aliphatic constituents is because of the extensive manipulation necessary to process the post-microbial flood samples. This artifact is manifested by the apparent gain in aromatic, nonpentane precipitable, and pentane precipitable components. Because data are presented as a percentage of total volume, all losses in the aliphatics from manipulation result in a proportionate increase in all other components. This is further substantiated by GC data obtained from the LC fractions. Although vol% of the aliphatic class components decreased, the pre- and post-flood GC chromatographs were similar.

2.2.5 Measurement of Interfacial Tensions

Systems with known IFT values ranging from 0.05 to 72.7 mN/m were measured (Table 2-5). The IFTs of all crude oils and common bacteriological growth media (TSB, Medium E) were also measured (Table 2-6). The effects of surfactant(s) on the interface of crude oils and culture supernatants of Bacillus licheniformis JF-2 grown aerobically in TSB were also quantified (Table 2-6). IFTs between Lick Creek (the heaviest oil) and cell free supernatants were reduced 94.6%, while IFTs between Burbank (the lightest oil) and the same supernatant were reduced 84.9%.

2.3 Discussion

In this study, moderate weight crude oils were recovered in laboratory systems to the same extent as light oils by Bacillus licheniformis JF-2 grown and injected in Medium E. Also, Bacillus licheniformis JF-2 surfactant(s) lowered the IFT of all oils tested regardless of composition. Even the heaviest oils were effected to at least the same extent as the lightest.

Although IFTs below 1 mN/m were not observed (even when cells were grown in complete media), oil was recovered from waterflooded cores. IFTs of at least one or two orders of magnitude lower than those measured are thought to be necessary for an effective recovery process. The IFTs obtained in the core may be substantially less than those obtained during IFT measurements because of localized, close proximity production of surfactants and cosurfactants in the core. If reductions in IFTs

Table 2-5. Measurement of interfacial tension standards.

Phase "Aqueous"/"Organic"	Published IFT (mN/m)	Measured IFT (mN/m) ^a	Replicates
Ternary System ^b	0.05 ³²	0.046 ± 0.002	n = 10
Detergent ^c /Toluene	0.25 ³²	0.29 ± 0.02	n = 10
Water/n-Butanol	1.8 ²⁸	1.87 ± 0.06	n = 6
Water/n-Octyl alcohol	8.5 ³³	8.17 ± 0.03	n = 7
Air/Methanol	22.6 ³³	21.36 ± 0.10	n = 5
Water/Benzene	35.0 ³³	32.46 ± 0.21	n = 6
Water/Carbon Tet.	45.0 ³³	41.94 ± 0.25	n = 6
Water/n-Hexane	51.1 ³³	48.22 ± 0.21	n = 7
Air/Water	72.7 ³³	72.63 ± 0.19	n = 10

a. Interfacial tensions ± standard deviation by method of least squares.

b. Components (w/w): 48% brine (4% w/v NaCl), 46% toluene, 2% SDS, 4% n-butanol. Bottom phase taken as "aqueous", top phase taken as "organic"; data interpolated from Birdi and Stenby, Figure 3.³²

c. Components (w/v): 2% NaCl, 2% SDS, 8% n-butanol; data interpolated from Birdi and Stenby, Figure 4a.³²

Table 2-6. Interfacial tension of crude oils, common bacteriological growth media, and trypticase soy broth cell-free supernatant (metabolites).

Oil	Medium E Control IFT (mN/m) ^a	TSB Control IFT (mN/m) ^b	TSB Cell-Free Supernatants IFT (mN/m) ^b	Percent Reduction in IFT (mN/m)
BUR	25.12 ± 0.62	24.33 ± 0.44	3.67 ± 0.35	84.9
SCH	32.13 ± 0.40	22.57 ± 0.52	2.20 ± 0.20	90.2
MOO	34.74 ± 0.56	28.83 ± 0.88	2.96 ± 0.06	89.7
ALW	25.36 ± 0.61	20.00 ± 0.17	1.55 ± 0.11	92.2
LCR	29.60 ± 1.19	18.83 ± 0.77	1.01 ± 0.04	94.6

a. Interfacial tensions ± standard deviations by method of least squares. Number of replicates = 10.

b. Interfacial tensions ± standard deviations by method of least squares. Number of replicates = 7.

correlated directly with oil recovery, heavy oils would be recovered better than light oils. However, coreflood data does not entirely support this trend in all experiments. Therefore, it is believed that the same reduction in IFT for any given oil may not facilitate the same extent of recovery. This may be explained by differences in wettability, differences in the viscous forces needed to overcome capillary forces in the porous media, or discrete charge/charge interactions of the oil with the rock. Capillary number theory indicates that large differences in IFTs will have a small effect on oil recovery unless the capillary number is at or near a critical point for the rock/fluid system being studied. Therefore, a 10% difference in IFT reduction (84.9% versus 94.6%) might be inconsequential in this system. These small effects on oil recovery may also be masked by experimental error. Polymers produced by Bacillus licheniformis JF-2 have no impact on oil recovery in these experiments.

Bacillus licheniformis JF-2 biosurfactant is not inactivated by any of the oils tested. Some petroleum sulfonates have been reported to be inactivated by some components of crude oil.¹⁹ Further, the composition (and therefore the economic fidelity) of the crude oil is not compromised during microbially mediated oil recovery facilitated by Bacillus licheniformis JF-2.

The amount of oil mobilized was about the same for both washed cells and cell-free supernatants for all oils tested. Data from the bacterial adherence to hydrocarbon assay indicate that oil mobilization results from produced metabolic end products and not the physical presence of cells. Assays for polymer were negative indicating no relative contribution for sweep improvement. This is further substantiated by oil recovery obtained using cell-free injections. However, floods utilizing cell injections required a smaller total injection volume ($\approx 1/3$) to mobilize the same amount of oil as that resulting from cell-free injections. This indicates that in situ cell metabolism is effective in producing sufficient quantities of surfactants to mobilize oil. SEM data indicate a distribution of cells in the core that follows flow direction.

Cell-free injections contained only nominal numbers of Bacillus licheniformis JF-2 but did contain 3.9 mg/mL carbohydrate and 27.1 mg/mL of protein (Table 2-2), both of which represent a potential source of nutrients for the indigenous population. Organisms indigenous to the Berea sandstone, however, do not contribute to oil recovery.

Although specific mechanism(s) of surfactant mediated oil recovery are still not fully understood, it is clear that oil recovery in laboratory core floods is increased by microbial surfactants. Further research is underway to determine if the surfactants have equal efficacy on different components of crude oil and to determine if solubilization or emulsification of the crude oil into the aqueous phase is sufficient to account for the observed oil recovery without the need for ultra-low IFTs.

The recovery of residual crude oil from known reserves will continue to be an important aspect of current energy policy. Clearly, novel EOR technologies are and will be employed to facilitate continued oil production. Although further research is required, this and previous work indicate the application of MEOR may be a viable EOR technology. The greatest utilization of MEOR potential may be realized by combining MEOR with other EOR technologies.

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Appendix A

**Graphical Presentation of Coreflood Data
Found in Tables 2-3 and 2-4.**

Figure A-1. Core 10. Coreflood utilizing Bacillus licheniformis JF-2 washed cell injection.

Figure A-2. Core 12. Coreflood utilizing Bacillus licheniformis JF-2 washed cell injection.

Figure A-3. Core 13. Coreflood utilizing Bacillus licheniformis JF-2 washed cell injection.

Figure A-4. Core 22. Coreflood utilizing Bacillus licheniformis JF-2 washed cell injection.

Figure A-5. Core 23. Coreflood utilizing Bacillus licheniformis JF-2 washed cell injection.

Figure A-6. Core 24. Coreflood utilizing Bacillus licheniformis JF-2 washed cell injection.

Figure A-7. Core 25. Coreflood utilizing Bacillus licheniformis JF-2 washed cell injection.

Figure A-8. Core 27. Coreflood utilizing Bacillus licheniformis JF-2 cell-free supernatant injections.

Figure A-9. Core 28. Coreflood utilizing Bacillus licheniformis JF-2 cell-free supernatant injections.

Figure A-10. Core 29. Coreflood utilizing Bacillus licheniformis JF-2 cell-free supernatant injections.

Figure A-11. Core 31. Coreflood utilizing Bacillus licheniformis JF-2 cell-free supernatant injections.

Figure A-12. Core 33. Coreflood utilizing Bacillus licheniformis JF-2 cell-free supernatant injections.

Figure A-13. Core 35. Coreflood utilizing Bacillus licheniformis JF-2 cell-free supernatant injections.

Figure A-14. Core 36. Control coreflood utilizing brine injections.

Figure A-15. Core 37. Control coreflood utilizing nutrient (Medium E) injections.

3. MICROBIAL ISOLATION AND CHARACTERIZATION

Ultimate application of MEOR processes in the field will rely intimately on adaptation of microbial physiology to specific reservoir environments. Three options are available to collect and/or adapt organisms: (a) organisms displaying a desirable MEOR phenotype may be acclimated to grow at reservoir conditions, (b) reservoir and reservoir-like environments may be screened for desirable organisms, or (c) the genotype responsible for desirable phenotypic traits may be genetically engineered into organisms capable of thriving at reservoir conditions. Adaptation can be an arduous process and phenotypic traits considered desirable may be lost. Genetic engineering is a plausible approach offering eloquence and ultimate applicability, but is also an expensive and tedious process (the legal ability to release genetically engineered organisms into the environment is also uncertain). Collecting organisms from oil-field operations and other similar environments is believed to be the method of choice. Microbial isolates selected for analysis in the MEOR program were screened for phenotypic and genotypic traits that allow them to function in oil reservoir environments such as those found in the Minnelusa sands of the Powder River Basin, Wyoming. These properties are facultative anaerobicity, thermotolerance to 45°C, halotolerance (in the form of NaCl) to 2.5%, and a desirable MEOR phenotype. Desirable phenotypes for MEOR include organisms capable of producing: (a) acids, (b) gasses, (c) solvents, (d) polymers, (e) surfactants, or (f) any combination of a to d and no capacity for sulfate reduction. Also desirable would be the ability to degrade asphaltic oil components or significantly perturb the oil/water interface through direct physical interactions. For application of MEOR technologies, the organisms should have no significant detrimental effect on the economically valuable constituents of crude oil.

3.1 Isolation Techniques for Field Isolates

Samples were collected aseptically in screw cap containers from the noted locations (section 3.1.1). All isolates were screened for thermotolerance to 45°C, halotolerance to 2.5% NaCl, and growth on minimal medium E (ATCC 1502) for *Bacillus*¹ (all constituents per liter: 1 g (NH₄)₂SO₄, 0.51 g MgSO₄, 25.0 g NaCl, 5.3 g KH₂PO₄, 10.6 g K₂PO₄, 0.01 g Na₂EDTA, 0.03 g MnSO₄, 0.001 g CaCl₂, 0.001 g FeSO₄, 0.001 g ZnSO₄, 0.001 g CoCl₂, 0.0001 g CuSO₄, 0.0001 g Na₂MoO₄, 0.0001 g AlK(SO₄)₂) with 1% sucrose as the sole carbon source. All selections were conducted under aerobic conditions. Halotolerance and minimal media experiments were conducted at 30°C. Sucrose was chosen as the carbon source because it represents the major carbohydrate constituent of beet molasses (molasses has historically been the feedstock of choice for MEOR applications in the field²). Sucrose represents 63.5% of total solids in beet molasses and 95.4% of all carbohydrate present.³

Samples were streaked onto Trypticase Soy Agar (TSA) containing 2.5% NaCl and incubated under anaerobic conditions at room temperature. Colonies were picked and re-streaked for secondary anaerobic incubation. Isolated colonies were picked and streaked again onto TSA for primary aerobic incubation at 30°C. Facultative anaerobes (organisms growing under anaerobic and aerobic conditions) were picked from the aerobic plates and re-streaked. Well isolated colonies were picked and inoculated into 3 mL of Trypticase Soy Broth (TSB) with 2.5% NaCl and grown at 30°C overnight, gram stained to check homology and gram reaction and held at 4°C as stock cultures.

3.1.1 Collection Environments

Isolates were collected from several oil field environments including well head gravel pads at the Moorcroft West, Schuricht, and Bracken Minnelusa leases in the Powder River Basin (Wyoming); heater treater effluent pit (Moorcroft West); and a metals plating/stripping waste stream at Kelly Air Force Base.

3.2 Identification and Characterization of Field Isolates

Organisms collected in FY 1990 have been tentatively identified by gas liquid chromatography of fatty acid methyl esters (FAME). Biochemical and morphological characteristics have been previously discussed.⁴ Of the nine isolates having plausible MEOR phenotypes, one strain of Pseudomonas stutzeri, one strain of Enterococcus malodoratus, three strains of Bacillus pumilus, two strains of Bacillus subtilis, and two strains of Bacillus firmus were collected. Discrete biochemical analysis for definitive identification is ongoing. Tentative identification is important to the species level to aid in determining pathogenicity.

All isolates were tested for their capability to lower the interfacial tension between Schuricht crude oil and cell free supernatants (aerobic overnight TSB cultures). Data are given in Table 3-1.

Table 3-1. IFTs between Schuricht crude and cell-free supernatants of field isolates grown in TSB.

<u>Isolate</u>	<u>IFT (mN/M)^a</u>	<u>N^b</u>	<u>AODC^c</u>
MED E Control	20.66 ± 0.52	10	-----
HB101 (<u>E. coli</u>)	19.44 ± 0.18	5	2.5 x 10 ¹⁰
<u>B. licheniformis</u>	3.34 ± 0.30	5	2.2 x 10 ¹⁰
SEW BL	3.48 ± 0.74	5	2.6 x 10 ¹⁰
COW BL	14.52 ± 1.80	5	1.4 x 10 ¹⁰
SPCDW	21.12 ± 0.22	5	2.5 x 10 ¹⁰
SPCDY	20.51 ± 0.59	5	3.0 x 10 ¹⁰
COWPY1	19.80 ± 0.15	5	3.5 x 10 ¹⁰
FSC6DW	20.55 ± 0.26	5	-----
YELDY	20.62 ± 0.44	5	3.7 x 10 ¹⁰
TNK3PY	21.11 ± 0.18	5	5.6 x 10 ⁹
MWWH	20.04 ± 0.31	5	1.5 x 10 ¹⁰

a. Standard deviation by method of least squares.

b. N = number of replicates.

c. Total cell count by acridine orange direct count for sample.

IFT measurements were made by video image analysis of inverted pendant drops as previously described.^{5,6} Data indicate emulsification by IFT reduction as a result of surfactant production is the most likely mechanism for Bacillus licheniformis JF-2, SEW BL, and COW BL (both tentatively identified as strains of Bacillus subtilis). The mechanism of oil emulsification for other strains (except

FSC6DW and SPCDW - acid producers) is unknown. It is known that all are negative for adherence to hydrocarbon water interfaces and therefore emulsification may be the result of solvent or lipophilic protein production.

The effect of oil composition on Bacillus licheniformis JF-2 surfactant(s) was also investigated. Organisms were grown in TSB aerobically for 48 h at 37°C. Oils used are delineated in Table 2-1. Complete oil characterization has been previously reported.⁴ All oils were affected to at least the same extent with a general trend toward greater efficacy for heavier oils.

Further IFT testing of Bacillus licheniformis JF-2, SEW BL, and COW BL was performed (Table 3-2). Schuricht (API gravity 25.6 at 60°F) and Lick Creek (API gravity 17.5 at 60°F) crude oils were selected as representative of medium and heavy crude oils.

Table 3-2. IFTs for cell-free supernatants (Medium E) measured with Schuricht and Lick Creek crudes.

<u>Isolate</u>	<u>O₂ Status</u>	<u>Crude Oil</u>	<u>Interfacial Tension^a (mN/m)^b</u>
Blank	-----	Schuricht	34.26 ± 0.54
Blank	-----	Lick Creek	23.28 ± 1.20
<u>B. licheniformis</u>	Aerobic	Schuricht	27.54 ± 1.06
COW BL	Aerobic	Schuricht	32.25 ± 1.27
SEW BL	Aerobic	Schuricht	30.31 ± 1.60
<u>B. licheniformis</u>	Aerobic	Lick Creek	14.13 ± 0.64
COW BL	Aerobic	Lick Creek	17.10 ± 0.49
SEW BL	Aerobic	Lick Creek	10.08 ± 1.81
<u>B. licheniformis</u>	Anaerobic	Schuricht	34.52 ± 0.46
COW BL	Anaerobic	Schuricht	33.39 ± 1.65
SEW BL	Anaerobic	Schuricht	32.66 ± 1.49
<u>B. licheniformis</u>	Anaerobic	Lick Creek	19.27 ± 0.31
COW BL	Anaerobic	Lick Creek	16.26 ± 1.08
SEW BL	Anaerobic	Lick Creek	20.49 ± 1.27

a. Interfacial tensions taken for incubation time of 16 h.

b. Interfacial tension ± 99.95 % confidence interval, number of analysis = 10.

The cells were grown both aerobically and anaerobically in Medium E for 16 h at 30°C, and centrifuged to remove the bacterial cells. Supernatant IFTs of SEW BL and COW BL were compared to supernatant IFTs determined for Bacillus licheniformis JF-2 (a known producer of biosurfactant) grown

under the same conditions. In no case was the IFT lowered to levels generally accepted as needed for increased oil recovery.

Evaluations of crude biosurfactant preparations from Bacillus licheniformis JF-2 were also performed. The organism was grown in aerobic 5 L cultures for biosurfactant production. Biosurfactants were isolated using modified procedures of McInerney et. al.⁷ Cells were removed by centrifugation. Biosurfactant(s) was isolated by acidifying the supernatants to pH 2 with HCl and allowing them to stand at 4°C until a precipitate formed. The precipitate was collected by centrifugation, dissolved in water, and the pH adjusted to 7.0. This solution was lyophilized and designated as crude biosurfactant. Crude biosurfactant was dissolved in distilled water and the IFT (surface tension) between air and the solution measured. Figure 3-1 is a plot of the interfacial tension of air as a function of the crude biosurfactant concentration. The lowest IFT obtained was 46 mN/m with a crude biosurfactant concentration of 150 mg/mL. No change in the slope was detected, indicating the highest concentration of crude biosurfactant used was below the critical micelle concentration (CMC).

Figure 3-1. IFT of air and an aqueous solution of crude biosurfactant from Bacillus licheniformis JF-2.

A plot of the interfacial tensions of crude biosurfactant solutions against two crude oils is shown in Figure 3-2. Crude biosurfactant solutions were varied in salt concentration to study the effect of salinity on the interfacial tension. The crude biosurfactant concentration was 150 mg/mL. Schuricht and Lick Creek oils were selected as the second phase for the interfacial tension measurements. Figure 3-2 shows that increasing the salinity of the precipitate solution resulted in increasing IFTs with both oils. The IFTs measured for Lick Creek oil were consistently lower than for Schuricht oil as expected from previous observations.

Microbial collection efforts during FY 1991 resulted in 31 isolates with MEOR phenotypes. Of

Figure 3-2. IFT of aqueous crude biosurfactant as a function of salinity and crude oil.

these, 14 demonstrated enhanced capability to emulsify oil. Identification of these 14 isolates indicated all were opportunistic pathogens or pathogens. All 14 isolates were cultured from a waste holding pit at the Moorcroft West lease. The collection consisted of the following genera; *Salmonella*, *Citrobacter*, *Vibrio*, *Enterobacter*, and *Proteus*. The most promising isolates were typed as *Salmonella diarizonae*, *Citrobacter diversus*, and *Proteus mirabilis*. Application of the most promising isolates from this collection may be ultimately realized in laboratory experiments to better define mechanisms of MEOR. It is doubtful these isolates would be applicable in the field.

Isolates (total of 46) from Kelly Air Force Base obtained as part of another INEL research program (Demonstration of Noncyanide Metal Strippers) were screened for MEOR phenotypes. The isolates had been tentatively identified by FAME. Five of the isolates were identified as *Bacillus licheniformis* (it is unknown if any are strain JF-2). Of these five however, only four emulsified oil aerobically (one emulsified oil anaerobically). In addition, one isolate emulsified oil aerobically (*Agrobacterium tumefaciens*) and three emulsified oil anaerobically (*Bacillus licheniformis*, *Agrobacterium tumefaciens*, and *Cellulomonas fimi*). Seven of the isolates produce gas, 22 tolerate 2.5% NaCl, 12 tolerate 5% NaCl (one of the *Bacillus licheniformis* isolates failed to grow at 5% NaCl), and 18 are facultatively anaerobic in Trypticase Soy Media (all *Bacillus licheniformis* isolates were facultatively anaerobic). The organisms were isolated from activated sludge, an oil skimmer, and a runway tank (holding tank for runway effluents). All *Bacillus licheniformis* isolates came from the activated sludge. Four of the isolates degrade (slightly) oil aerobically while none are capable of anaerobic degradation (as expected). Of the four isolates that degrade oil (Schuricht crude), three are *Bacillus licheniformis*.

Research continues with the FY 1990 collection and other isolates collected during FY 1991. As a general trend, the isolates emulsified oil better anaerobically than they did aerobically, one was positive for adherence to hydrocarbon water interfaces, none had metabolic capacity for crude oil, and all grew extremely well under anaerobic conditions.

3.3 Polymer Production by Bacillus licheniformis JF-2

See "MICROBIAL (Bacillus licheniformis JF-2) MEDIATED OIL RECOVERY" Section 2.

3.4 Sulfate Reducing Bacteria and the Mitigation of Biogenic Souring

It is commonly assumed that oil reservoirs are anaerobic environments. Although oxygen concentrations may be high in injection waters, production waters are usually anaerobic. The problem of product souring (increased concentrations of sulfide in the produced gas, oil, or water) during production of oil reservoirs is ostensibly because of the activity of anaerobic sulfate reducing bacteria. These bacteria cause increased sulfide concentrations in oils, gasses, and production waters. As well as microbial mechanisms of sulfate reduction, abiotic mechanisms such as electrochemical and geochemical reactions may be important. High sulfide concentrations in production fluids are toxic, cause corrosion, and may plug the formation because of metal sulfide precipitates. Crude oil often lacks sulfide during primary production with sulfide first occurring during secondary waterflood operations. If sulfate reducing bacteria contribute to product souring, they are either inactive or not present in unproduced reservoirs. Thus, waterflooding is either responsible for the introduction of sulfate reducing bacteria or the stimulation of sulfate reducers already present.

Contamination of petroleum reservoirs with H_2S poses severe economic and ecological problems. Most oil production operations attempt to inhibit the activity of sulfate reducing bacteria by the addition of antimicrobials to injection waters. Control of sulfate reducing bacteria has been sporadically successful at best and sulfide production eventually occurs. This is due in part to the lack of a realistic method to evaluate the efficacy of antimicrobials in vitro before application. It is clear from a recent literature review⁸ that little is known regarding selective inhibition of sulfate reducing bacteria.

3.4.1 Isolation, Growth, and Initial Experimentation on Sulfate Reducing Bacteria.

Research to establish multivariant analysis techniques for biocide efficacy were initiated in FY 1991. Select biocides are being tested for efficacy under a variety of conditions including the presence of sandstone, oil, bacterial concentrations, and biocide concentration. A consortium of sulfate reducing bacteria isolated from the heater treater at the Moorcroft West Unit and Desulfovibrio desulfuricans (ATCC 13541) were selected for experimentation. Cultures are grown and maintained in a modified Postgate B medium⁹ consisting of (all components/L): 2.1 g MOPS, 0.5 g KCl, 1.0 g NH_4Cl , 25.0 g NaCl, 4.0 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2$, 4.08 g Na Lactate (60% V/V), 0.1 g yeast extract, 0.15 g KH_2PO_4 , 1.0 mg resazurine, 6 μg $Na_2SeO_3 \cdot 5H_2O$, 8 μg $Na_2WO_4 \cdot H_2O$, 0.1 g Na thioglycolate, 0.1 g L-ascorbic acid, and 0.01 g $FeSO_4 \cdot 7H_2O$. The media is adjusted to pH 7.5 with NaOH. Boiling medium is dispensed under a nitrogen purge into nitrogen purged tubes, sealed with teflon lined silicone septa and autoclaved immediately (Na thioglycolate, L-ascorbic acid, and $FeSO_4 \cdot 7H_2O$ are added as filter sterilized stocks after autoclaving).

Metabolism is being measured by GC headspace analysis of active sulfate reducing bacteria and acidified culture samples. This results in total sulfide analysis (including decomposition of thioglycolate reductants and biocides) for aqueous samples.

Gas chromatography is performed on a Hewlett Packard Series II 5890A GC equipped for data acquisition and reduction with a Hewlett Packard Chemstation. A GS-Q 30 m x 0.53 mm megabore capillary column is used under isothermal conditions (80°C oven, 130°C injection, and 130°C detection) resulting in a retention time for sulfide of 1.4 min during a 3.0 min run. Detection of sulfide dimers is by flame photometry. Split sample ratio is 50:50. Standard samples from 0.0165 to 0.268 ng/ μ L have been analyzed. Data acquisition results in the generation of a nonlinear power curve that must be reduced by squaring the mass concentration for a semilog plot. Subsequent data are linear with a regression coefficient of 0.996.

3.5 Discussion

A new collection of isolates obtained during FY 1991 appeared more promising than Bacillus licheniformis JF-2 or any of the isolates collected during FY 1990. However, the application of these isolates in field environments is questionable, and limited time and resources will be applied to these in the future. Isolates collected during FY 1990 were typed by FAME analysis. Of these isolates, two appear to be as effective as Bacillus licheniformis JF-2 in oil emulsification. Both of these isolates are Bacillus subtilis species that could potentially produce the surfactant *surfactin*. IFTs mediated by these cultures grown on a complete medium (TSB) demonstrated IFT values between 3 and 14 mN/m. Clearly, these values are higher than even conservative estimates of the IFTs necessary for effective oil recovery by low tension flooding (10^{-2} mN/M). When grown under minimal conditions (Medium E) higher IFTs are noted. These values are most likely because of the concentration of biosurfactants being below the CMC. At surfactant concentrations below the CMC, interfacial tension values increase rapidly. Both isolates appear to affect the IFT of a heavier (Lick Creek) oil to a greater extent (as does Bacillus licheniformis JF-2) both aerobically and anaerobically.

Bacillus licheniformis JF-2 surfactants affected the IFTs of all oils tested regardless of composition. Further, Bacillus licheniformis JF-2 biosurfactant is not inactivated by any of the crude oils tested. Even the heaviest oils tested were affected to at least the same extent as the lightest. In fact, IFTs between Lick Creek (the heaviest oil) and cell free supernatants were reduced 94.6%, while IFTs between Burbank (the lightest oil) and the same supernatant were reduced 84.9%. All other oils fell between these values with a general trend from lightest to heaviest. If reductions in interfacial tensions correlated directly with oil recovery, heavy oils would be recovered better than light oils. Coreflood data however, do not entirely support this trend in all experiments (see Section 2). Therefore, it is believed that the same reduction in IFT for any given oil may not facilitate the same extent of recovery. This may be explained by differences in wettability, differences in the energy needed to overcome capillary forces in the porous media, or discrete charge-charge interactions of the oil with the media. Capillary number theory indicates that apparently large differences in interfacial tensions will have a small effect on oil recovery unless the capillary number is at a critical point. Therefore, a 10% difference in IFT reduction (84.9% versus 94.6%) might be inconsequential in this system. These small effects on oil recovery may also be masked by experimental error.

The efficacy of the biosurfactants tested here in respect to increased interfacial tension reductions of heavier oils may be explained by charge-charge interactions. More detailed analysis of the

biosurfactant is needed to allow for elucidation of specific mechanistic interactions of the surfactant across the oil/water interface.

IFT data obtained on crude preparations of Bacillus licheniformis JF-2 biosurfactant are unexpectedly high. It is thought that the protocols used for isolation of the biosurfactants is inefficient or incomplete. This has recently been proposed in a DOE quarterly report.¹⁰ The biosurfactant used was below the critical micelle concentration. When used to test the effect of oil gravity on IFT, lower IFTs were observed with the heavier oil. This data is consistent with previous observations made on cell free supernatants of TSB cultures. No inflection point was noted for the surfactants as an effect of salt concentration. Other researchers have noted inflection points at $\approx 5\%$ NaCl. This would represent the optimal salt concentration for the surfactant

The failure of biocides in the field is most likely because of not fully understanding the interaction of the antimicrobials within the reservoir environment. These interactions may be chemical or physical interactions with other chemicals such as surfactants, adsorptive losses onto mineral surfaces, loss of aqueous bulk phase concentrations through partitioning the agent into the organic (oil) phase, inactivation because of temperature, or oxidation. These and other factors must be understood before solving problems of delivering the appropriate concentration to the area of microbial activity.

3.6 References

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4. MICROSENSOR PROBE TECHNOLOGY FOR THE DIRECT MEASUREMENT OF SULFIDE^a

4.1 Statement of the Problem

Microbially-induced formation souring occurs by sulfate reducing bacteria (SRB) converting sulfate to hydrogen sulfide gas (H_2S) in the formation. Other forms of souring include both electrochemical and geochemical reactions. If souring is sufficiently severe, it is necessary to remove H_2S from the produced gas and oil to meet product specifications, environmental constraints, and to prevent corrosion of the product handling system. Souring can also contribute to plugging in the form of iron precipitates. It is generally believed that SRB are not present in the formation from the genesis of the hydrocarbons but that they are introduced during drilling or injection operations.¹ Regardless of the bacterial source, injection of nutrients into the formation to enhance oil recovery could aggravate souring by increasing the carbon source for SRB either directly or through metabolic products from other organisms that metabolize the primary substrates.

The fundamental process in souring is the production of H_2S . To adequately study the souring phenomenon and monitor possible methods of control, it is essential to be able to measure the activity of SRB spatially within porous medium, i.e., as a function of distance from the injection point that is generally accepted to be the source of substrate and nutrients. To accurately determine the spatial distribution of microbial activity it is necessary to detect differential changes in sulfate conversion, which is more easily performed through product (sulfide) detection.

Sampling of product streams for offline analysis involves several problems that are difficult to overcome. H_2S is readily adsorbed on most surfaces making it difficult to handle quantitatively in a batch mode. Furthermore, it is easily oxidized or lost to any gas or liquid phase present. Consequently, in situ/online measurements are preferred.

Several sulfide detectors are available that can be used for online measurements. Notably, all detect sulfide ion (S^{2-}) rather than total sulfide ($H_2S + HS^- + S^{2-}$), 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^{2-}) 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 utilizes the detector development expertise, SRB experience, and process analysis capabilities of the Montana State University Center for Interfacial Microbial Process Engineering (CIMPE).

^aAdapted from the original report "Sulfide Detector for Monitoring Microbially-Induced Formation Souring, Idaho National Engineering Laboratory Subcontract C85-110754," P. Conn, Z. Lewandowski, F. Roe, M. Ellerd, U. Rogers, P. van Hout, J. Inma and D. Goeres, The Center for Interfacial Microbial Process Engineering, Montana State University, February 1992

4.2 Program Development

A program was implemented February 1991 to address the role of souring in microbially enhanced oil recovery (MEOR) processes as part of the INEL MEOR research program. The program goal was to develop microsensor technology amenable to laboratory (and ultimately field scale) applications for the online/in-situ detection of total sulfide. The objectives were to (a) develop a detector that could measure total sulfide concentrations independent of the aqueous medium pH, (b) use the detector technology to develop a probe, and (c) thoroughly test the probe for monitoring the total sulfide concentration in core experiments containing oil, brine, and microorganisms. To this end, a program was developed to (a) design and construct a dual pS/pH detector that had a polarization capability allowing for pH adjustment by consumption of H^+ in the electrode measurement area, (b) calibrate and optimize the probe in an anaerobic flow system using aqueous solutions of varying pH, ionic strength, and temperature, and (c) determine the sensitivity of the probe to hydrocarbon/substrate/nutrient/microbial environment of produced water by testing it in laboratory core reactors containing oil coated sandstone.

4.3 Approach

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 dominant 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 would also require 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.

4.4 Electrode Construction Techniques

4.4.1 Sulfide Electrode

Solid state silver sulfide (AgS) membrane electrodes gained wide use on potentiometric measurements of sulfide ions in solution in the late 1960's.^{2,3} Microelectrodes analogous to these macro-scale counterparts have been used in ecological research for many years.⁴

4.4.2 Sulfide Electrode Construction

Construction of the electrode was performed by tapering a 4 cm piece of platinum wire ($d=0.1$ mm) by immersing it repeatedly in saturated KCN solution while applying 3 Vac between it and a graphite counter electrode. The desired taper was obtained with a 15 min treatment. The tapered wire was inserted into a previously pulled lead glass capillary tube (5 cm in length, 2 cm in diameter). This was further pulled to a tip diameter of 50 μm (Stoelting Pipet Puller No. 51217), and ground flat on a diamond impregnated wheel (Narishige model EG-4). The platinum wire was recessed ≈ 3 μm by immersing it in 0.1 M KCN with an anodic potential for 30 s.

The recessed platinum tip was plated with silver by periodic reverse plating (PRP).⁵ Acceptable plating was achieved using a reduction potential of -1.0 V for 1.0 sec and an oxidation potential of 1.0

V for 0.75 s. Cycling was repeated for 1 h to complete plating. The plating solution consisted of 0.53 g AgCN, 0.68 g KCN, 0.45 g KHCO₃, and 0.1 g KOH in a total volume of 10 mL distilled water (pH = 11.5 to 12). The electrode was rinsed in distilled water for 2 min, immersed in 0.001 M NaS for 30 min, and rinsed again in distilled water. Final probe diameter = 50 μ m.

4.4.3 Sulfide Electrode Calibration

Calibration was performed according to Hseu and Rechnitz.² The sulfide probe and a commercial pH probe were immersed in 100 mL of a 0.01 M NaS solution (pH = 11.9) and sulfide potentials were measured versus a Ag/AgCl reference electrode. The pH was decreased 1 increment at a time to a final pH of 3.0. The pH and sulfide potential were recorded at each increment. Based on the pH (measured) and total sulfide (calculated from the original solution), the concentration of S²⁻ and the pS (-log₁₀[S²⁻]) were calculated.

Calculations of sulfide species were based on the following equations:

$$[TS] = [S^{2-}] \left[\frac{(10^{-pH})^2}{K_1 K_2} + \frac{(10^{-pH})}{K_2} + 1 \right] \quad (1)$$

$$[HS^-] = [S^{2-}] \frac{(10^{-pH})}{K_2} \quad (2)$$

$$[H_2S] = [S^{2-}] \frac{(10^{-pH})^2}{K_1 K_2} \quad (3)$$

where

TS = total sulfide

K₁ = H₂S dissociation constant 1 (pK₁ = 7.0)

K₂ = H₂S dissociation constant 2 (pK₂ = 13.7).

Data is presented graphically in Figure 4-1. The curve was linear to pS $\approx$ 17 to 18 with a slope of 29.6 mV/pS.

Intrinsic in this method is the assumption that the electrode is only sensitive to sulfide concentration and does not respond to pH. To validate this assumption, the electrode was tested in sulfide-free solutions of varying pH. The results (Figure 4-2) indicate no pH dependance.

4.4.4 pH Electrode

For the measurement of pH distributions across biofilms or at a surface, microelectrodes are the most convenient. Microelectrodes with either glass or liquid ion selective membranes have been applied

Figure 4-1. Calibration curve (pS versus mV) of a sulfide probe.

Figure 4-2. Response of a sulfide probe to pH.

in physiology, medicine, and microbial ecology. Both constructions are very fragile which hinders their application in more rigorous environments. A relatively sturdy, thick (200 μm tip diameter) probe construction has been proposed by Lewendowski in the past.⁶ Since then, new developments in solid state iridium oxide pH microelectrodes have permitted further progress in near surface pH measurements. Iridium oxide electrodes can be prepared by sputtering,^{7,8} thermal,^{9,10,11} or electrochemical oxidation of iridium wire^{12,13,14,15} Electrochemical oxidation of Iridium substrate is the most frequently used technique and involves cycling iridium in sulfuric acid at potentials between -0.25 and +1.25 versus a standard calomel electrode. The procedure forms an iridium oxide that is pH sensitive.¹⁶ Electrochemically oxidized iridium produces predominantly hydrated oxide.¹⁷ Electrochemical oxidation was selected for construction primarily because of the good Nernstian responses offered by hydrated iridium oxides. The Nernstian responses of iridium oxides are explained by mechanisms predicting 1.5 electrons transferred/ H^+ with a slope of ≈ 90 mV/pH unit [Equation (4)].¹⁷

4.4.5 pH Electrode Construction

Anodic polarization in 0.5 M H_2SO_4 produced the best results. Ten Vdc (versus graphite) was applied to iridium wire (99% 5 cm in length, 75 μm in diameter, Englehard) for 5 to 10 min. This produced a taper to a 2 to 10 μm tip. The wire was enclosed in lead glass (Houde Glass Co., NJ) using the procedure of Revsbech and Jorgensen.¹⁸ The iridium wire was exposed at the tip by grinding (Narishige EG-4 diamond wheel) off the excess glass. Grinding was monitored with an Infinity model CFM microscope.

Iridium oxide wires prepared by potential cycling can display poor film adhesion of the oxide layer to the metal surface. This adhesion can be mechanically reinforced and protected by recessing the wire into the glass. The wire was recessed by immersing it in 0.5 M H_2SO_4 and applying +2.0 Vdc (versus graphite). Stirring was required to remove bubbles. The electrode was then rinsed in distilled water. The electrode tip was cleaned by immersion in 0.05 M Na_3PO_4 with -5.0 Vdc applied for 10 min, and rinsed in distilled water. The oxide layer was formed onto the cleaned wire surface with a programmable potentiostat (EG&G PARC 273, Princeton, NJ).

Neither thermally nor electrochemically prepared electrodes are exclusively coated with either anhydrous or hydrous oxides (although electrochemically prepared electrodes are predominantly hydrous). The pH sensitive oxide is composed of both hydrous and anhydrous oxides. The degree of hydration changes with time causing a drift in calibration. Furthermore, there is no consistency in the measured slope (mV/pH) for electrodes prepared by identical processes. Electrochemically prepared electrodes have been reported to have slopes between 69.7 and 81.9 mV/pH unit. Electrodes prepared here demonstrated an initial slope of 77.3 mV/pH unit that decreased to 65.8 mV/pH unit after 1 wk. Because the greatest change in oxide hydration occurs during the first 12 h of equilibrium,¹⁷ electrodes were aged in distilled water for 12 h and in air for 2 h. Electrodes were stored in air.

4.4.6 pH Electrode Calibration

A World Precision Instruments model FD 223 electrometer (impedance = 10^{15} Ω) was used to measure pH from 1 to 12. The Nernstian slope for operation of 1 wk was 77.3 mV/pH unit with an E_o

of 751 mV. Slopes of 70 to 80 mV/pH unit were typical of freshly cycled electrodes. Slopes usually settled to ≈ 65 mV/pH unit after several calibrations and uses. Response was immediate for most pH buffers.

4.4.7 Reference Electrode

A silver/silver chloride reference electrode was constructed by first sealing a piece of 24 gauge silver wire (99.95%) in a piece of 3 mm lead glass tubing. HCl (0.091 M) was placed in the open end of the tube in contact with the silver wire, and an oxidizing potential of ≈ 2 V was applied (carbon rod counter electrode) to the wire. After a grayish coating of AgCl formed, the HCl was replaced by saturated KCl (also silver saturated). A 3 cm length of Glass Ultrawick (World Precision Instruments, Cat. No. UW200-6) was sealed to the lead glass tube with epoxy cement.

4.4.8 Combination Electrode

A combination probe was constructed from an IrO_2 pH electrode, a Ag/AgS sulfide electrode, and a Ag/AgCl reference electrode. The electrodes were placed into 5 cm length of 19.5 mm glass tubing with the active ends protruding slightly. The terminal ends, protruding from the other end of the glass tube, were sealed with Parafilm. Epoxy was injected into the open end of the tube to seal the electrodes in place (Figure 4-3).

Figure 4-3. Diagram of the combination (pH/pS/reference) electrode.

4.5 Electrode Testing

4.5.1 Stability Testing of a Dual pH/pS Electrode

To screen the stability of the pH and pS probes, a recirculating tubular flow reactor system was assembled. A buffered (pH 7.0) sulfide solution (0.25 g $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$, 3.4 g KH_2PO_4 in 1000 g air free water) was pumped at 180 mL/min through packed, crushed sandstone (Berea, 232 g) contained in a tubular (2.28 x 40 cm) reactor. A combination pH/pS dual electrode was installed in the side arm of a nylon swagelok "T" downstream from the packed bed. The reactive surfaces of the electrodes were flush with the tube wall to mitigate dead volume and time. The detector output voltage (pH electrode/pS electrode voltage difference) was continuously monitored to ascertain sulfide breakthrough. On sulfide breakthrough, stability measurements were initiated. As expected, the probe signal drifted for several days (explained by the uptake of sulfide by the sandstone, see subsection 4.3.2) and then began to stabilize.

4.5.2 Sulfide Reactions with Berea Sandstone

The formation rock can be an important sink for uptake of sulfide when souring occurs. This reaction can also be an important factor in laboratory studies where Berea sandstone is used in core or packed bed experiments. Accordingly, the reaction of sulfide with Berea sandstone was studied in a flow reactor system.

A 2 x 40 cm, tubular reactor was packed with crushed Berea sandstone. A deaerated sulfide solution (prepared by adding NaS and HCl to O_2 free synthetic sea water) was pumped at 60 mL/h through a seawater flooded flow reactor into a nitrogen flooded collection flask. Sulfide uptake was measured by collecting samples of the reactor effluent and analyzing them by a standard colorimetric method.¹⁹ Sulfide breakthrough was delayed because of displacement of sea water from the void volume and uptake by the sandstone. The sulfide solution was replaced with sulfide free water following breakthrough (≈ 150 min) to provide a reference curve for measuring the void volume displacement (also measured independently). The nature and magnitude of the uptake was determined by the difference between the breakthrough and the reference curves (frontal analysis).

Frontal analysis suggests the sulfide reaction is rapid and irreversible (within the time scale of this experiment and detection limits - ≈ 0.1 ppmwS). The sulfide uptake was independent of the sulfide concentration, but strongly dependent on the pH of the aqueous medium. At pH 10, the uptake of sulfide by sandstone was 62 ppmwS; at pH 8 it was 78 ppmwS; and at pH 6 it was ≈ 1600 ppmwS. Thus, sandstone uptake of sulfide is a significant factor that must be addressed when designing and executing souring experiments in sandstone environments.

4.5.3 Stability Testing of a Combination (pH/pS/Reference) Electrode

The sealing technique described in subsection 4.3.1 was used to construct a total sulfide electrode (described in subsection 4.2.8). Excellent calibration stability was observed in a 5 d test using a recirculating system containing no sandstone (sulfide solution described in subsection 4.3.1). Electrode (pH and pS) drift (relative to the reference electrode) was <0.1 pH or pS unit with low noise levels. Signal variation range of the two electrodes is ≈ 0.003 units (pH or pS).

4.6 Discussion

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. The preferred approach to the measurement of total sulfide was to convert all of the sulfide species to the sulfide ion by adjusting the pH immediately surrounding the electrode to the strongly basic range. This approach would have permitted the determination of total sulfide from a single sulfide electrode measurement under conditions where the detection limit of the sulfide electrode is not a problem. The alternate approach (ultimately used here) of measuring both sulfide ion (pS) and hydrogen ion (pH) involves two measurements and the uncertainty of detection sensitivity of the sulfide ions. The exact value of the equilibrium constant for the HS^- dissociation reaction is extremely critical in this regard. From the equilibrium expression [Equation (5)],

when $\text{pH} = \text{pK}_2$, $[\text{S}^{2-}]/[\text{HS}^-] = 1$. Therefore, the pH must be significantly $> \text{pK}_2$ for the sulfide ion to be a quantitative measure of the total sulfide.

There is uncertainty over the value of K_2 in the literature. Values have been collected that range from 12.44 to 17.1.²⁰ The Handbook of Chemistry and Physics lists a value of 12.0.²¹ During the calibration of the sulfide electrode it was found that a value for pK_2 of ≈ 13.7 was consistent with the detector response. This value suggests that the pH adjustment approach to measuring total sulfide is impractical if not impossible. The same calibration studies indicated that the sulfide electrode sensitivity is sufficient for souring studies at pH values normally found in formations.

The detection limit of the probe is $\approx 10^{-18}$ molar in the pH range normally encountered in formations. At a pH of 7, this sulfide ion concentration would correspond to a total sulfide concentration of 10^{-11} molar using Equation 1 (subsection 4.2.3) and a $\text{pK}_2 = 13.7$. Therefore, sulfide ion detection is not limiting. Future research will include testing the probe under a variety of conditions normally encountered in laboratory coreflood experimentation.

4.7 References

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5. EVALUATION OF RESERVOIR WETTABILITY AND ITS EFFECT ON OIL RECOVERY

Fluid distribution in oil reservoirs and the displacement of oil by water or other recovery fluids is governed by interfacial phenomena. This interaction is commonly known as wettability. While the importance of saturation history is recognized, the once common expectation that all reservoirs should be water wet (because water was the first fluid to occupy the pore space) has given way to the realization that a wide spectrum of wetting conditions occur. This includes strongly and weakly wetted systems (by both oil and water) as well as mixed wet systems.

Research is being conducted 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. To this end, investigations have focused on surface phenomena, wetting changes induced by crude oils on solid surfaces, factors causing wettability changes, and the influence of these changes in wettability on displacement efficiency. Identifying and understanding conditions for optimum oil recovery by waterflooding is a primary objective.

5.1 Fiscal Year 1991 Progress

Significant progress has been made toward understanding the relationship between wettability and oil recovery in natural systems. A list of publications,¹⁻¹³ manuscripts prepared,¹⁴⁻²⁴ and presentations,²⁵⁻⁴² for the period October 1990 through December 1991, is included. Some highlights are briefly summarized here.

Key to recent advances was the use of crude oils to alter wettability. In comparison to other systems, the use of crude oils to alter wettability is an arduous and complex process. Often, crude oils are not well-characterized and vary from sample to sample for the same reservoir and each reservoir yields a unique oil. Nevertheless, the oils studied to date appear to have some common characteristics with regard to wetting behaviors. Selected crude oils have been used to prepare cores with a wide range of initial wetting conditions for use in systematic reservoir wettability studies.^{5,7,10,11,16,18,19}

In one such study, wettability of Berea sandstone (assessed by an Amott-style imbibition test) was observed before and after exposure to individual drilling mud components and filtrates of typical formulations.¹⁷ Wettability was most affected when initially water-wet samples were exposed to any of the individual mud components in concentrations spanning the ranges typical of their actual application. These same components were used to prepare drilling muds. Filtrates from these muds were used to simulate invasion of the formation through a mud filter cake. The filtrates showed much less tendency to alter wettability. Cores used in these studies had initial wettabilities ranging from strongly water-wet, through neutral samples, to fairly strongly oil-wet. One particular formulation, suitable for a wide range of drilling conditions, gave especially small changes in wetting over the entire range of initial conditions tested. Further study is needed to determine what special properties this formulation may possess that might make it or similar water-based muds applicable in cases where wettability preservation is one of the coring objectives.

The wettability produced in a core sample by exposure to crude oil can be varied systematically by control of key variables such as oil and brine chemistries, temperature and duration of exposure, and water saturation.^{7,11,13,18,19} Oil chemistry, water saturation, temperature, and duration of crude oil exposure are of primary importance. Brine chemistry may play a secondary role. The wetting states produced are fairly reproducible and persist for some time, although not indefinitely. Extremes of oil-wetting required more strenuous treatment and a method using asphalt dissolved in diesel was developed.

An extensive study has been made of the effect of wettability on oil recovery. A peak in waterflood displacement efficiency was found at very weakly water-wet conditions where the Amott number is only slightly greater than zero.¹¹ Cores that are either more water-wet or more oil-wet have decreased recovery at breakthrough and at all further stages of injection.

In conjunction with the crude oil/brine/rock studies, adsorption of crude oil components onto glass surfaces pre-equilibrated with various brines is under investigation.¹⁰ Adsorption is judged by measurement of contact angles with pure oil and water phases after removal of the bulk crude oil. This work has shown that crude oils alter wetting after very short periods of exposure, the extent of change varies with the crude oil, brine pH, and to a lesser extent, brine ionic strength. Large hysteresis has been observed between advancing and receding conditions under this test protocol, but less hysteresis is seen for surfaces which remain under oil for the duration of the test procedure.

During the coming year, attention will be given to imbibition rate phenomena in order to better quantify various wettability states induced by crude oil. Continued success of this program will be manifested by the development of methods for controlling and altering wettability for improved waterflooding and enhanced oil recovery. Identifying and understanding conditions for optimum oil recovery by waterflooding is a major goal of the research.

5.2 Recent Publications

1. Morrow, N.R.: "Introduction to Interfacial Phenomena in Oil Recovery" in *Interfacial Phenomena in Oil Recovery*, Ed. N.R. Morrow, Marcel Dekker, October 1990.
2. Morrow, N.R. and Melrose, I.C.: "Applications of Capillary Pressure Data to the Determination of Connate Water Saturation" in *Interfacial Phenomena in Oil Recovery*, Ed. N.R. Morrow, Marcel Dekker, October 1990.
3. Buckley, J.S., "Multiphase Displacements in Micromodels," in *Interfacial Phenomena in Oil Recovery*, Ed. N.R. Morrow, Marcel Dekker, October 1990, pp. 157-199.
4. Cather, M.E., Morrow, N.R., and Klich, I.: "Characterization of Porosity and Pore Quality in Sedimentary Rocks," in *Characterization of Porous Solids, II*, Elsevier, Amsterdam (1991) pp. 727-36.
5. Morrow, N.R.: "Wettability and Its Effect on Oil Recovery," SPE Distinguished Author Series JPT, December 1990.

6. Mason, G. and Morrow, N.R.: Capillary Behavior of a Wetting Liquid In Irregular Triangular Tubes," *J. Coll. Inter. Sci.*, January 1991 vol 141, pp. 262-74.
7. Jia, D., Buckley, J.S., and Morrow, N.R.: "Control of Core Wettability with Crude Oil," SPE paper 21041 proceeding of the SPE International Symposium on Oilfield Chemistry, Anaheim, CA, February 20-22, 1991.
8. Ma, S. and Morrow, N.R.: "Effect of Firing on Petrophysical properties of Berea Sandstone," SPE paper 20495 proceedings of the SPE International Symposium on Oilfield Chemistry, Anaheim, CA, February 20-22, 1991.
9. Morrow, N.R., Cather, M.E., Buckley, J. S., and Dandge, V.: "Effects of Drying on the Petrophysical Properties of Low-Permeability Gas Sands," SPE 21880 presented at the 1991 SPE-Joint Rocky Mountain Regional Meeting, Denver, CO, April 15-17.
10. Buckley, J.S. and Morrow, N.R., "An Overview of Crude Oil Adhesion Phenomena," 6th IFP Research Conference on Exploration-Production, Saint-Raphael, France, September 4-6, 1991.
11. Jadhunandan, P. and Morrow, N.R.: "Effect of Wettability on Waterflood Recovery for Crude Oil/ Rock Systems,- paper SPE 22597 proceedings of the 66th Annual Technical Conference and Exhibition, Dallas, October 6-9, 1991.
12. Jiang, M., Ma, S., and Morrow, N.R.: "Correlation of Capillary Pressure Relationships and Calculation of Permeability," paper SPE 22685 proceedings of the 66th Annual Technical Conference and Exhibition, Dallas, October 6-9, 1991.
13. Jadhunandan, P. and Morrow, N.R.: "Spontaneous imbibition in Crude Oil/Brine Systems," *In Situ (1991) 15*, No. 4, 319-45.

5.3 Other Recent Manuscripts and Reports

14. Wei, M., Wilson, J.L., Morrow, N.R., and Bowman, R.S.: "Effect of Wetting on Capillarity and Non-Aqueous Phase Liquid Saturations in Homogeneous Porous Media presented at the 65th Colloid and Surface Science Symposium, Norman, OK, June 17-19, 1991.
15. Wei, M., Bowman, R.S., Wilson, J.L., and Morrow, N.R.: "Stability of Wetting Properties of Silane-Treated Glass Exposed to Water, Air, and Oil." Manuscript to be submitted.
16. Jia, D., Buckley, J.S. and Morrow, N.R.: "Oil Wet Cores Prepared with Asphalt in Diesel Solutions," Supplement PRRC Quarterly Report, "Evaluation of Reservoir Wettability and its Effect on Oil Recovery," July 1 -Sept. 30, 1991 (Oct. 1991).

17. Jia, D., Buckley, J.S., and Morrow, N.R.: "Effect of Water-Based Drilling Fluids on the Wetting Properties of Core Samples," PRRC Quarterly Report, "Evaluation of Reservoir Wettability and its Effect on Oil Recovery," Supplement No. 2 (Oct. 1991).
18. Zhou, X.M. and Morrow, N.R.: "Experimental Investigation of the Effect of Rock Wettability on Slow-Rate Waterflood Behavior for Crude Oil/Brine/Rock Systems," PRRC Report No. 91-55, November 1991.
19. Zhou, X.M., Torsaeter, O., and Morrow, N.R.: "The Effect of Crude Oil Aging Time and Temperature on the Rate of Water Imbibition for Crude Oil/Brine/Sandstone Systems," PRRC Quarterly Report, "Evaluation of Reservoir Wettability and its Effect on Oil Recovery," Supplement No. 1 January 1992.
20. Morrow, N.R. and Buckley, J.S.: "Evaluation of Reservoir Wettability and its Effect on Oil Recovery," PRRC Quarterly Report for October 1 to December 31, 1990.
21. Morrow, N.R. and Buckley, J.S.: "Evaluation of Reservoir Wettability and ill Effect on Oil Recovery," PRRC Quarterly Report for January 1 to March 31, 1991.
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23. Morrow, N.R. and Buckley, J.S.: "Evaluation of Reservoir Wettability and its Effect on Oil Recovery," PRRC Quarterly Report for July 1 to September 30, 1991.
24. Morrow, N.R. and Buckley, J.S.: "Evaluation of Reservoir Wettability and its Effect on Oil Recovery," PRRC Quarterly Report for October 1 to December 31, 1991.

5.5 Presentations

25.	November 5, 1990	Institute Francais du Petrole, Paris, France
26.	November 6, 1990	Institute Francais du Petrole, Paris, France
27.	January 23, 1991	Phillips Petroleum, Bartlesville Oklahoma
28.	January 24, 1991	Department of Energy, Bartlesville Project Office,
Bartlesville, Oklahoma
29.	February 25, 1991	UNOCAL Research, Brea, California
30.	April 3, 1991	Department of Petroleum Engineering, New Mexico
Institute of Mining and Technology, Socorro, New
Mexico

31.	May 29, 1991	Mobile R&D Corp. Advanced Core Analysis Workshop,
Dallas, Texas
32.	May 29, 1991	Department of Petroleum Engineering, Herriot Watt
University, Edinburgh, Scotland
33.	June 3, 1991	Statoil Research Laboratories, Stavanger, Norway
34.	June 4, 1991	Statoil Research Laboratories, Stavanger, Norway
35.	June 6, 1991	Wettability of chalk Reservoirs Symposium, Trondheim,
Norway
36.	June 10, 1991	Statoil, Bergen, Norway
37.	June 11, 1991	Norskhydro/University of Bergen, Joint Seminar,
Bergen, Norway
38.	September 6, 1991	Panel Discussion, IFP Conf. on Physical Chemistry of
Colloids & Interfaces in Oil Production, St. Raphael,
France
39.	September 9, 1991	Elf Aquitaine, Pau, France
40.	October 6, 1991	Jim Melrose Symposium, Mobil Research & Develop-
ment, Dallas, TX
41.	October 8, 1991	SPE Annual Fall Meeting, Dallas, Texas
42.	October 16, 1991	Chevron Reservoir Fluid Mechanics Research Sympo-
sium, La Habre, California

6. TARGET RESERVOIR SELECTION AND FIELD APPLICATION

Field application of MEOR requires selection of appropriate sites as well as specifically designing, engineering, and applying an MEOR system for the selected field. Analysis of select small Minnelusa Sand formations in the Powder River Basin in Wyoming was conducted in FY 1990. Further information has been gathered and experiments performed indicating the applicability of MEOR in these fields. The reserves in the Powder River Basin amenable to MEOR have been assessed, EOR technology currently in place has been evaluated, and historical perspectives of the Schuricht lease and Moorcroft West Minnelusa Sand Unit have been performed. The completion of this task would not have been possible without collaborative effort between the Idaho National Engineering Laboratory, an independent field services supply company (TIORCO, Inc.), and an independent owner/operator (KSL Enterprises).

6.1 Assessment of Powder River Basin Reserves Amenable to MEOR

It is estimated that there are 2.2 to 3.6 billion barrels of oil (BBO) remaining in the Powder River Basin in reservoirs that are potentially feasible for MEOR applications. This estimate is based on the information obtained from the Tertiary Oil Recovery Information System (TORIS) reservoir database maintained by the Bartlesville Project Office (BPO); the Wyoming Oil and Gas Conservation Commission (WOGCC), Oil and Gas Statistics, 1990; Wyoming Oil and Gas Fields, Powder River Basin, Wyoming Geological Association, 1981, and Dwight's Energydata Inc., Vision data gathering system. The criteria selected to screen for potential MEOR reservoirs were temperature $< 70^{\circ}\text{C}$ (extrapolated from a depth of 9500 ft), permeability > 15 md, salinity $< 10\%$, and pH between 4 and 9.¹ In many cases, only the temperature criterion could be applied because the other criteria (permeability, salinity, and pH) were not generally available. If permeability information was available, the permeability cut-off was also applied.

The WOGCC lists 1294 reservoirs in the Powder River Basin. Of these, 1069 met the reservoir screening criteria. The WOGCC does not compile estimates for the original oil in place (OOIP) but does track cumulative production. The 1069 reservoirs remaining after the screening have produced a total of 2.4 BBO (production data obtained from Dwight's Energydata, 1990 statistics). The TORIS database contains data for only 71 of the 1069 reservoirs that met the temperature criteria. Most of these reservoirs are classified as Eolian in TORIS. The OOIP of the 71 TORIS reservoirs is 3.7 BBO and the cumulative production is 1.5 BBO for a remaining oil in place (ROIP) of 2.2 BBO. Thus, $\approx 40\%$ of the OOIP for these 71 reservoirs has been produced. Even though only 6.6% of the reservoirs that pass the screening criteria in the Powder River Basin are included in the TORIS database, the cumulative production from the TORIS subset of reservoirs accounts for 62.5% of the total cumulative production. Thus, the remaining 93.4% of the reservoirs passing the screening criteria have produced only 37.5% of the cumulative production in the Powder River Basin. Many of the smaller reservoirs have been more recently discovered and may have produced considerably $< 40\%$ of the OOIP.

An upper bound on the OOIP of 6.0 BBO in the potential MEOR target reservoirs in the Powder River Basin can be estimated by assuming that the reservoirs in TORIS account for 62.5% of the OOIP as well as 62.5% of the cumulative production. If it is assumed that the cumulative production is 40%

of the OOIP for all the reservoirs as it is for the TORIS subset, the total ROIP is 3.6 BBO. Thus, the ROIP for these reservoirs is estimated to be between 2.2 and 3.6 BBO. A more accurate estimation of the ROIP and the potential reserves for EOR processes in the target reservoirs would require an extensive data gathering exercise and a reservoir-by-reservoir analysis.

6.2 Overview, Historical Perspective and Current EOR Practices of the Moorcroft West Unit and the Schuricht Field

Waterflooding is a common and long-standing practice for successfully increasing oil recovery in the Powder River Basin of Wyoming, especially in the Minnelusa formation. Polymer-augmented waterflooding is the most common enhanced oil recovery (EOR) technology used in the Minnelusa. Primary recovery in these reservoirs is generally low because of formation heterogeneity, high oil viscosity, and low gas/oil ratios. Operators (mostly independents) in the area are familiar with the application of polymer technology in conjunction with or following waterflooding and are, therefore, receptive to EOR technology.^{2,3} One possible EOR application in the Schuricht field or Moorcroft West Unit is microbial enhanced oil recovery (MEOR). The application of either polymer or surfactant based MEOR in the Schuricht field would allow comparison to the similar Moorcroft West chemical floods.

6.2.1 General Reservoir Characteristics

Primary recovery from Minnelusa reservoirs averages 11% of OOIP. The primary production mechanism is fluid expansion. Recovery by solution gas drive is limited because of the low gas/oil ratios.² Original oil in place is usually difficult to estimate and experience in determining this value shows the estimate to be low in many cases.³ A more detailed characterization of the Minnelusa formation has been previously reported.⁴

Secondary recovery operations are usually initiated early in exploitation life of the reservoir and are very successful because reservoir fill-up is small because of low primary recoveries. Well response usually occurs soon after the initiation of water injection. Because of reservoir heterogeneity and fairly viscous crude oils, polymer-augmented waterflooding is a viable technology for improving ultimate recoveries from these reservoirs through sweep improvement. Polymer-augmented waterflooding has been accepted and incorporated in the exploitation profile of most Minnelusa reservoirs to maximize ultimate recoveries.³

6.2.2 The Moorcroft West Unit

The Moorcroft West Minnelusa Sand Unit is located in the northeastern corner of the Powder River Basin $\approx$ 15 mi northwest of Moorcroft, Wyoming. The unit produces from a confined reservoir providing an ideal evaluation site for EOR technologies. There is little possibility for off-pattern operations at this site to influence interpretation of results. The Moorcroft West Minnelusa Sand Unit has been estimated to contain 686,000 stock tank barrels of oil (STBO). The primary producing mechanism is rock and fluid expansion (there is very little associated gas and no apparent active water drive in this reservoir). The porosity and water saturation of the Moorcroft West reservoir as determined from log calculations is 15.2% and 28.1%, respectively.⁵ Estimated permeability for the field averages 114 md (range = 6 to 150 md), average reservoir temperature is 120°F, and average sand thickness is 8.4 ft.⁴ Exploration of the field began with the drilling of the Goodstein No.1 in December 1961. The field was subsequently abandoned in January 1962. In February 1983, the Texas Trail No. 1 well (NW

NE 12-51N-68W) was completed in the same area (280 ft southwest down-dip). This well pumped 32 barrels of oil per day (BOPD) from two relatively thin pays at 5855 ft to 5858 ft and 5866 ft to 5869 ft. Also completed in 1983 was the Evans No. 1-12 (pumping 54 BOPD from two pays at 5916 ft to 5926 ft and 5930 ft to 5932 ft) 1140 ft southwest and down-dip from the Texas Trail No. 1. No water/oil contact is evident at either well. Total field production (both wells) fell to < 4 BOPD by 1988. Reservoir engineering and fluid analysis indicated that the two wells were producing from a common 805 acre-ft reservoir.

An engineered oil recovery program was initiated at the Moorcroft West Minnelusa Sand Unit with the initial objective of determining if the two existing wells in the field were in communication. Although geological analysis did not provide conclusive evidence of communication between the Evans No. 1-12 and the Texas Trail No. 1 wells, similar decline curves for both were noted. Further, early laboratory work indicated that the oils produced from the two wells were the same. In April 1989, the Evans well was converted for injection using a temporary water injection system. Water for injection was obtained using unburied piping (≈ 600 ft) connected from an existing livestock supply well to a 400 bbl holding tank (filled on meter with filtration). Injection was initiated on vacuum from the tank into the injection well. In August 1989, after 12,000 bbl [1.2% pore volume (PV)] of water were injected, production at the Texas Trails No. 1 increased from 3 to 6 BOPD with no increase in produced water volume indicating communication between the wells.

Following response to water injection, a skid-mounted water injection plant containing a dry solids feed system, liquid chemical storage and proportioning equipment, and a small triplex pump was installed. In October 1989, a volumetric sweep improvement program based on cationic and anionic polymers to treat reservoir heterogeneities and improve volumetric sweep efficiency was initiated. Relatively high molecular weight polyacrylamides were chosen based on the estimated permeability variation of the reservoir. The Dykstra-Parsons permeability variation coefficient for Moorcroft West is estimated at 0.65. Cationic polymer (dry granular cationic polyacrylamide) was injected under vacuum at 25 lb/d in 120 bbl (≈ 600 mg/L) injection water. Injection ended in November 1989. Cationic polymer was followed by anionic polymer (dry granular anionic polyacrylamide) in April 1990 at 10 lb/d in 100 bbls (≈ 286 mg/L) injection water. Targeted polymer injection volume equaled 6% PV. The purpose of the anionic polymer was to improve the adverse mobility ratio (7.0) experienced at Moorcroft West. Based on the increased pressures experienced (1,156 psig), cross-linking of the polymers was deemed unnecessary. The production well had soured by June 1990 (ostensibly biogenic in origin).

In September 1991, an alkaline/polymer (A/P) process designed to reduce residual oil saturation by reducing the interfacial tension between the oil and the brine was initiated. The alkaline/polymer process is based on the injection of a 0.2 wt% addition of potassium hydroxide (KOH, 45 wt%) and 225 mg/L of anionic polymer for $\approx 20\%$ PV. This design was developed through laboratory analysis indicating interfacial tension (IFT) reduction 100-fold or greater by the addition of KOH. Biocide (1,2-dibromo-2,4-dicyanobutane) is being injected as a slug once a month at 2 lb in 45 bbls water (127 mg/mL). Biocide application is designed to prevent microbial contamination of injection facilities and tubulars.

As of June 1991, production was 38 BOPD (Figure 6-1) from the Minnelusa "A" sand. Injection is averaging 45 barrels per day (BPD) water/polymer at 1100 psig surface pressure. Primary production from this reservoir was low ($\approx 5\%$ OOIP) and field production had dropped to 4 BOPD before initiation of water injection. Small volumes of water have been produced throughout the life of the reservoir, but there is no evidence of injection fluid breakthrough to June 1991.

Figure 6-1. Production history of the Moorcroft West Unit.

Cumulative injection through June 1991 was 56,947 barrels of water (BW) or 5.8% PV. A total of 4500 lb of cationic polymer at an average concentration of 606 mg/L and 1700 lb of anionic polymer at an average concentration of 284 mg/L have been injected. Chemical cost is \$1.46/bbl of oil produced since the initiation of water injection. Recovery through June 1991 is 49,950 BO (7.3% OOIP). Oil recovery since initiation of water injection through June 1991 is 15,978 BO (2.3% OOIP).

6.2.3 The Schuricht Field

A similar Minnelusa field containing a single well (Schuricht Well 21-24) $\approx$ 5 mi north of the Moorcroft West Unit was also completed in 1983. The well is producing from the Minnelusa "A" sand at 6500 to 6508 ft subsurface by fluid expansion (no associated gas or water drive) at a rate (June 1991) of 90 barrels of oil per month (BOPM). The well has not soured. Although existing reservoir data are limited, potential exists for additional well locations. It is believed no reservoir is too small to benefit from the application of EOR technology and that the development of the Schuricht field may be best served by following examples of successful technologies applied in the Moorcroft West Unit. Clearly, the time frame in which to institute planning for future EOR activities is during primary production, therefore allowing greater flexibility for field development.

Currently, the feasibility of applying an MEOR process to the Schuricht field or Moorcroft West Unit is being studied at the Idaho National Engineering Laboratory (INEL). From available reservoir, field, and fluid data, both the Moorcroft West Unit and the Schuricht field fit the published criteria of reservoir selection for MEOR processes.^{1,6} MEOR technology offers the potential of being cost-effective in the later stages of engineered oil recovery programs.

6.3 Fluid Analysis

Produced (oil and brine) and injected (water) fluids from the Moorcroft West Unit and Schuricht well were analyzed. The methods used to analyze and characterize the oils include liquid chromatography fractionation (LC), gas chromatography (GC), elemental analysis, viscosity determination (temperature variant), and interfacial tension. Oil analysis has been previously reported.⁴ Waters were analyzed for anions (Cl^- and SO_4^{2-}), cations (Na^+ , Ca^{2+} , Mg^{2+} , and Fe^{total}), pH, total dissolved solids, and hardness (as CaCO_3).

6.3.1 Interfacial Tension Analysis

Interfacial tensions (Figure 6-2) of oil samples and solutions of phosphate buffered (pH 7) salt (NaCl at 2.5%) with trace minerals were measured by video image analysis of inverted pendant drops.⁷ Interfacial tensions of oil samples and alkaline (KOH) solutions, cationic quaternary amine surfactant, sodium carbonate (Na_2CO_3), and surfactant/soda ash at a 50:50 ratio were measured by De Noüy ring at 23°C. Solutions were prepared as active chemical weight percent in deionized water. IFTs between Schuricht and Moorcroft West oils and an aqueous phase of phosphate buffered (pH 7) salts (NaCl , 2.5%) with trace minerals are 32.13 ± 0.40 mN/m ($n=10$) and 34.74 ± 0.56 mN/m ($n=10$), respectively (video image analysis). IFTs were also measured for Moorcroft West oil and solutions of KOH , a cationic quaternary amine surfactant, sodium carbonate (Na_2CO_3), and surfactant/soda ash (50:50) (Figure 6-2). IFTs ≈ 100 fold lower were attained by adding KOH to a final concentration of 0.2 to 3.0 wt%, surfactant/soda ash blends (50:50) at 0.2 to 3.0 wt%, or Na_2CO_3 at 1.5 to 3.0 wt%. The greatest efficacy was mediated by KOH at 0.2 wt%.

Figure 6-2. Interfacial tension analysis of Moorcroft West crude oil (De Noüy ring) and solutions of alkalies, surfactants, and alkaline/surfactant blends.

6.3.2 Water Analysis

Cations (Na^+ , Ca^{2+} , Mg^{2+} , Fe^{total}) were analyzed by atomic adsorption (flame) spectroscopy (Perkin-Elmer 2280). Chlorides, hardness (as CaCO_3), and alkalinity (pH) were analyzed by titration.⁸ Sulfates were analyzed turbidimetrically as barium salts at 415 nm.⁸ Total dissolved solids (TDS) were estimated by mathematical summation. Analysis of produced and injected waters from the Moorcroft West Unit are presented in Table 6-1. No water is produced or injected at the Schuricht Well. Reported data are average values compiled from August 1989 to September 1991.

Table 6-1. Water analysis.

Field	Moorcroft West ^a	Moorcroft West ^b
Water	Production	Injection
Location	Section 12-51N-68W	Section 12-51N-68W
Formation	Minnelusa "A"	Unknown shallow sand
Depth (ft)	5900 ft subsurface	200 ft subsurface
Sample Point	Texas Trail #1 Treater	Wellhead
pH	7.34 $\pm$ 0.47	7.3
TDS	42,370 $\pm$ 15,313 mg/mL	366 mg/mL
Hardness (as CaCO_3)	4,522 $\pm$ 1,234 mg/mL	21 mg/mL
Na^+	16,837 $\pm$ 3,044 ppm	93.0 ppm
Ca^{2+}	999 $\pm$ 95 ppm	31.3 ppm
Mg^{2+}	536 $\pm$ 76 ppm	14.6 ppm
Fe^{total}	5.8 $\pm$ 4.2 ppm	0
Cl^-	26,895 $\pm$ 3,256 ppm	1.94 ppm
SO_4^{2-}	1,763 $\pm$ 519 ppm	101 ppm

a. Standard deviation by method of least squares, deviations based on the following measurements: pH - 24, TDS - 12, hardness - 9, Na^+ , Ca^{2+} , Mg^{2+} , and SO_4^{2-} - 8, Fe^{total} - 7, and Cl^- - 22.

b. No standard deviation, discrete sample analysis only.

6.4 Discussion

Communication between the two wells of the Moorcroft West Field (Texas Trail No. 1 and the Evans No. 1-12) was demonstrated. Pressure increases at the Evans No. 1-12 well indicate that the injection of polymer is affecting the reservoir heterogeneities as desired. Oil production from the Texas Trail No. 1 well has increased significantly with no indication of fluid breakthrough. Although ultimate recovery from the applied EOR technologies is predicted to be 274,400 STBO or 40% OOIP, it is too early in the EOR program to determine incremental oil resulting from the sweep improvement technology or the A/P process. Therefore, production to date is most likely the result of water injection only. The benefits of the applied EOR technology will be evidenced by maintaining high recoveries throughout the life of the reservoir. The A/P process currently in place was designed primarily from laboratory results

indicating the efficacy of KOH. KOH is as effective as surfactant/soda ash blends (50:50) but has an associated cost six times less. Equivalent efficacy mediated by Na_2CO_3 (3.0 wt%) results in costs three times greater than KOH.

Oil analysis from the Moorcroft West Unit and the Schuricht well indicate that although the Moorcroft West oil is heavier, commonalities between the oils are evident. Further, based on physical parameters, both fields would be amenable to the application of MEOR technology. It is believed that the chemical flooding technologies implemented in the Moorcroft West Unit combined with the fluid and reservoir similarities between the Moorcroft West Unit and the Schuricht field results in a unique opportunity to study the application of MEOR in field situations.

Monitoring of the Moorcroft West Unit will continue for performance and economic viability. Baseline data (oil composition, oil production, and well response) will be useful to interpret relative flood performance. This data will be useful not only for technologies currently in place, but also for assessing relative performance of comparable technologies instituted in the future in similar fields.

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