FOSSIL ENERGY

DOE/BC/14202-6
(DE92001007)

MICROBIAL ENHANCEMENT OF OIL PRODUCTION FROM CARBONATE RESERVOIRS

Final Report for the Period
January 1990 - May 1991

By
Ralph S. Tanner
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Michael J. McInerney
Roy M. Knapp
Jon P. Adkins

November 1991

Performed Under Contract No. DE-AC22-90BC14202

University of Oklahoma
Norman, Oklahoma

National Energy Technology Laboratory
National Petroleum Technology Office
U.S. DEPARTMENT OF ENERGY
Tulsa, Oklahoma

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DOE/BC/14202-6
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SUMMARY

About one half of the world's oil is produced from carbonate reservoirs. Microbially enhanced oil recovery (MEOR) is recognized as a potentially important technology for recovering the remaining oil-in-place after primary and secondary production, especially in carbonate reservoirs where it was recognized over 40 years ago that microbial acid production could have a direct effect on the petrophysical properties of carbonate rocks. However, only a small amount of research has been done on MEOR in carbonate systems. Most of the research in MEOR has taken place in sandstone systems; this is understandable since sandstones are often more permeable than carbonates, are easier to manipulate and model in the laboratory, and are free of the complicating factor of the property of dual flow regimes found in many carbonate rocks.

The objective of this research contract was to examine MEOR in carbonate reservoirs by reviewing the literature pertinent to MEOR in carbonate systems, determining the microbial ecology of carbonate reservoirs, examining the effect of microorganisms on carbonate matrices, documenting MEOR in model carbonate core systems, and constructing a mathematical model for MEOR in carbonates.

While there has been relatively little research on MEOR in carbonate petroleum reservoirs, review of the literature indicated that two common microbial activities, acid production and gas production, would provide useful mechanisms for MEOR, especially in carbonate reservoirs. The available field trial data supported this indication, showed that microbial activity can occur to an

extent to affect the properties of an entire reservoir, and that MEOR has been successful in carbonaceous reservoirs. Analysis of the reservoir data from 10 states showed that 7,794 of the 19,297 carbonate reservoirs in the dataset had physical parameters which would allow MEOR. Essentially, a carbonate reservoir which has undergone waterflood would also be considered a candidate for MEOR.

Study of the microbial ecology of four carbonate reservoirs showed that each contained a small but metabolically diverse population of mesophilic microorganisms. The low population numbers were due to lack of available nitrogen, phosphate, and, probably, suitable energy sources. However, these microorganisms would likely be sufficient to support in situ MEOR without the injection of cells into the reservoir.

Microorganisms had predictable effects on the permeability and porosity of sandstone and limestone cores. Microbial growth and biomass production resulted in a lowering of permeability and porosity of sandstones. Polymer-producing microorganisms also lowered the permeability and porosity of limestone cores. However, microorganisms which carried out a vigorous acid- and gas-producing fermentation of carbohydrate increased the permeability and porosity of limestone cores due to dissolution of the carbonate matrix. In one case, a core into which fluids could not be pumped was penetrated and made permeable by an acid-producing microorganism.

MEOR was demonstrated in four carbonate chip packed cores, each of which had a different type of microorganism. MEOR was apparent only after microbial growth and metabolism. Some enhanced

oil recovery may have occurred even as the result of the activity of a microorganism that did not ferment carbohydrate or produce acid.

A first generation mathematical model was constructed for MEOR in carbonates which incorporated the effect of organic acid dissolution of the matrix in addition to balanced substrate utilization and end-product formation, biomass production, and transport features utilized from a model developed for MEOR in sandstone systems.

This research has resulted in presentations at three scientific meetings and five manuscripts for publication (submitted or in preparation).

Some specific future research was indicated from the work presented here. The microbiology of halophilic thermophilic microorganisms, which would be important for many MEOR applications, needs further attention. The exact mechanisms of MEOR in carbonate reservoirs needs further study in aseptically prepared and maintained cores. MEOR in consolidate limestone rock will probably have to be studied at a scale larger than a laboratory core in order to capture the dual porosity characteristics of carbonate reservoirs.

Sufficient knowledge does exist for planning and execution of a MEOR field trial in a carbonate reservoir. The candidate reservoir should be under waterflood and the available data indicate that stimulation of acid- and gas-producing microorganisms would be the first appropriate MEOR treatment.

PRESENTATIONS AND MANUSCRIPTS

Presentations:

Udegbunam, E. O., R. M. Knapp, M. J. McInerney, and R. S. Tanner. March, 1991. Potential of Microbial Enhanced Oil Recovery (MEOR) in the Petroleum Reservoirs of the Mid-Continent Region. Petroleum-Reservoir Geology in the Southern Midcontinent. Oklahoma Geological Survey.

Adkins, J. P., L. C. Cornell, and R. S. Tanner. May, 1991. Microbial Ecology of Carbonate Reservoirs. Abstr. Annu. Meet. Amer. Soc. Microbiol., I3, p. 191.

Udegbunam, E. O., J. P. Adkins, R. M. Knapp, M. J. McInerney, and R. S. Tanner. October, 1991. Assessing the Effects of Microbial Enhanced Oil Recovery on Pore Structures of Rocks. Soc. Petrol. Engin., 66th Annual Technical Conference & Exhibition.

Manuscripts (Submitted and in Preparation):

Udegbunam, E. O., R. M. Knapp, M. J. McInerney, and R. S. Tanner. Potential of Microbial Enhanced Oil Recovery (MEOR) in the Petroleum Reservoirs of the Mid-Continent Region. Oklahoma Geological Survey.

Tanner, R. S., E. O. Udegbunam, M. J. McInerney, and R. M. Knapp. Microbially Enhanced Oil Recovery in Carbonate Reservoirs. Geomicrobiology Journal.

Adkins, J. P., and R. S. Tanner. Microbial Ecology of Carbonate Reservoirs. Geomicrobiology Journal.

Adkins, J. P., E. O. Udegbunam, R. M. Knapp, M. J. McInerney, and R. S. Tanner. Microbially Enhanced Oil Recovery in Packed Carbonate Cores. Environmental Science & Technology.

Udegbunam, E. O., J. P. Adkins, and R. S. Tanner. Effects of Adsorption on Substrate Availability in Carbonate Reservoirs. Energy Sources.

Microbially Enhanced Oil Recovery: Carbonate Reservoirs

Abstract

About half of the world's oil production is from carbonate formations; there is also significant production from carbonaceous sandstone formations. However, most of the research in microbially enhanced oil recovery (MEOR), a potentially important tertiary recovery technology, has focused on sandstone reservoirs. In general, sandstone formations are geologically simpler than carbonate formations and easier to model in the laboratory. Carbonate formations have a wide range of pore geometries and distributions, resulting in complex flow dynamics. The low matrix permeabilities and the dual porosity characteristics of most carbonates can result in low oil recovery efficiencies by waterflooding and, coupled with the chemistry of carbonates, slow the implementation of enhanced oil recovery methods. A review of the data on carbonate reservoirs in Dwight's Energydata TOTL system indicated that about 7,800 oil-producing carbonate reservoirs in the ten states surveyed have environmental, geological and petrophysical conditions that would make them suitable candidates for MEOR. A review of a number of MEOR field trials showed that rates of oil production could be increased by as much as 200%. Microbial activity in these trials was probably due to that of indigenous populations rather than those injected for the trials. Detrimental effects such as loss of injectivity and increased souring were not reported. Based on analysis of the geology and petrophysical characteristics of carbonates, two common mechanisms of MEOR, microbial acid production and microbial gas production, are especially suitable for use in carbonate reservoirs.

I. INTRODUCTION

There is a great potential for the use of microorganisms to enhance oil recovery, and microbially enhanced oil recovery (MEOR) has been the subject of a number of recent reviews (Donaldson et al., 1989; McInerney and Westlake, 1990; Moses, 1990; Updegraff, 1990; Yen, 1990). Unfortunately, this potential has not yet been transformed into widely used technologies in the petroleum industry. One reason for this is the misconception that MEOR is a single technology which can be used indiscriminately in any reservoir. This ignores the fundamental principles upon which enhanced oil recovery is based. There is no average oil reservoir; the chemical and physical properties of reservoirs are quite variable, as are the factors which entrap oil. Thus, it is no more likely that a single microbial species or product will solve all of the problems in the petroleum industry than it is likely that a single drug will cure all known diseases. This has not stopped the discovery of cures to most diseases and should not stop us from developing novel and economical technologies to recover additional oil. The specific factor(s) respnsible for the entrapment of oil in reservoirs must be identified and then appropriate technologies developed to recover that oil.

The basic scientific understanding of mechanisms of MEOR is incomplete. This is particularly true for MEOR in carbonate reservoirs. The pore structures and hydrodynamics of carbonate reservoirs are more complex than those of sand/sandstone reservoirs, and, understandably, most of the research in MEOR has

been conducted in sand/sandstone systems. In addition, much of the information available on MEOR in carbonate reservoirs or carbonaceous systems is difficult to interpret when evaluated against our current understanding of microbial ecology and microbial physiology. However, there are a large number of microorganisms with different physiologies which offer a number of possible approaches for developing useful microbial technologies to enhance oil recovery. Thus, MEOR should not be considered as a single potential technology but as a collection of potential technologies, applied as appropriate to specific problems. Once the reservoir characteristics contributing to oil entrapment are understood, then a specific microbial process can be used to help recover that oil. Implementation of MEOR must also be based on sound engineering and ecological principles in order for it to be successful.

The physical, chemical and geological factors limiting oil production from carbonate reservoirs are identified in this review. The current state-of-art of MEOR in carbonate reservoirs is then reviewed to assess its potential and feasibility in these reservoirs.

II. GEOLOGY OF CARBONATE RESERVOIRS

The importance of carbonate reservoirs is underscored by the fact that perhaps over half of the world's oil production is from carbonate formations. In many significant geological regions of the world, such as the Middle East, Canada, and Mexico, almost all oil and gas production is from carbonate reservoirs. In the United States, all of the major oil-producing areas, except Pennsylvania, contain oil-bearing carbonate formations (Craze, 1950; Harbaugh, 1967; Landes, 1970).

The geology and petrophysical characteristics of a reservoir are important factors controlling oil recovery. The greater part of the existing literature on the mechanics and performance of reservoirs is on silicio-clastic lithologies. There is a relative lack of information on the mechanics and performance of carbonate reservoirs, primarily because of their great genetic and geological complexity (Chilingar et al., 1972). The ability of a carbonate rock mass to act as a reservoir depends on whether the rock has a favorable internal architecture imparted to it by primary and secondary diagenetic processes. The particular geology and petrophysics of a carbonate reservoir impacts reservoir performance and merits a brief review.

A. Mineralogy

Carbonates are defined as sedimentary rocks with over 50% carbonate content. True limestone rocks contain over 90% calcium carbonate (Benson, 1984; Krumbein and Sloss, 1963). The most

common clastic components of carbonates are clays and quartz. Important reservoir rocks which contain more than 50% limestone are shaly limestone (argillaceous carbonate) and sandy limestone; that containing less than 50% limestone is limy sandstone (carbonaceous sandstone or calcareous sandstone) (Amyx et al., 1960; Benson, 1984; Krumbein and Sloss, 1963).

The mineralogy of carbonate rocks is relatively simple. Carbonate rocks primarily consist of calcium and/or magnesium cations with the carbonate anion. The most common rock-forming carbonate minerals are aragonite, calcite and dolomite (Klein and Hurlbut, 1977; La Moreaux et al., 1984; Scoffin, 1987). Aragonite is a relatively pure orthorhombic calcium carbonate containing very small amounts of magnesium and traces of strontium, lead, barium and potassium. It is a high temperature polymorph of calcium carbonate, chemically identical to but with a different crystal structure than calcite. Aragonite is unstable under surface conditions. Calcite has a hexagonal-rhombohedral crystal structure. Most calcites are relatively pure calcium carbonate. Manganese, iron and magnesium substitute for calcium ions more readily in calcite than aragonite. Dolomite is calcium-magnesium carbonate, structurally similar to calcite but with alternate layers of calcium ions replaced by magnesium ions. As a rock mass, dolomite is thought to be secondary in origin, formed from ordinary limestone as magnesium ions replace some of the calcium ions by a process called dolomitization. Dolomitization may increase, reduce or not change the original porosity of the limestone (Amthor et al., 1988).

B. Classification and Porosity

Carbonate rock and porosity classifications are numerous. Classifications emphasizing geology and genesis (Dunham, 1962; Folk, 1959; Folk, 1962) are the most commonly used; these were reviewed by Benson (1984). Other workers have focused on the petrophysical attributes of carbonate pore systems, especially as they relate to the performance of oil and gas formations, for classification (Amthor et al., 1988; Archie, 1952; Harbaugh, 1967; Rieke et al., 1972; Roehl and Choquette, 1985; Stout, 1964; Thomeer, 1960). No method of classification of carbonates and their porosity is entirely satisfactory, and the various classifications are not readily interchangeable. According to Felsenthal and Ferrell (1972), carbonate rocks are so complex that many approaches and analytical methods are needed for proper evaluation of oil content and optimum application of recovery methods. However, a simple classification of carbonate reservoir pore systems having at least a generalized correlation with reservoir performance is needed, particularly for the purpose of engineering studies.

The geometry and reservoir properties of carbonate rocks often show a relationship with the original depositional conditions. Previous studies on carbonates showed that the reservoir potential of a carbonate rock is determined by its sedimentary fabric framework, which is of one or both of two basic types: grain-supported or mud-supported (Dunham, 1962; Folk, 1959; Folk, 1962). Reservoir potential is modified by post-depositional processes such as recrystallization, precipitation, etc., which may

either preserve, enhance or destroy reservoir properties such as porosity and permeability.

Folk (1959; 1962) showed that all pore systems in carbonates are made up of only three principle end-members: grains, carbonate mud, and sparry calcite cement. Each end-member and combinations thereof are formed in either high, transitional or low energy marine environments.

Grains, also called allochemical constituents or allochems, provide the framework constituents of carbonate rocks. The four dominant grain categories are called skeletial grains or bioclasts, ooids, peliods or pellets, and intraclasts; these are formed in high energy environments. Skeletial grains are whole or fragmented fossils of organisms that inhabited the depositional environment. These can provide information on the original ecologies of the depositional environment. Ooids are spherical grains whose nuclei are surrounded by a number of concentric layers of aragonite or calcite crystals, which formed as the grains were moved about in warm, high energy marine environments saturated with calcium carbonate. Pellets are fecal particles generated by sediment-ingesting organisms. Intraclasts are large (greater than two mm), re-worked particles of carbonate mud and grains that were re-deposited within the depositional environment.

Microcrystalline carbonate mud, also called micrite, usually formed in low energy environments from the disarticulation of calcareous algae (Benson, 1984). The presence or absence of mud in carbonate rocks has a dominant role in determining pore systems in reservoirs. All other things being equal, the presence of mud in carbonates seems to reduce their potential as reservoirs.

Sparry calcite cement is common is rocks deposited in high energy environments, forming as calcium carbonate precipitated in pores in the depositional environment.

Carbonate rocks are also often classified according to their depositional character (Dunham, 1962). Boundstones are rocks whose original components were bound together during deposition; examples include stromatolites and fossilized reefs. Other rock types are grainstone, packstone, wackestone and mudstone; in these, the original components were not bound together during deposition. Grainstones are grain-supported and do not contain mud. Packstones are also grain-supported but contain some mud. The mud-supported rocks are wackestone, which contains more than 10% grains, or mudstone, which contains less than 10% grains.

The main difference between carbonates and sandstones, aside from their chemical composition, is in the origin and geometry of the porous systems in the two kinds of rock. In sandstones, the porous system is the result of openings between individual sand grains. Sandstone porous systems are tortuous and irregular but, in a macroscopic sense, may have fairly consistent properties of porosity and permeability within a given rock (Craze, 1950). A wide variety of pore geometry and distribution exists in carbonates. Voids in limestones and dolomites range in size from less than one micron to meters in diameter and may be: within carbonate crystals (fluid inclusions); between carbonate crystals (intercrystalline); between grains (intergranular); within bedded or massive units (microvugs and cracks); within formations (fissures, large-scale dissolution features, etc.) (Choquette and Pray, 1970; Scoffin, 1987).

In carbonate reservoir rocks, pore space may have both primary and secondary origins. Primary porosity originated during deposition of carbonate sediments. Some intercrystalline or intergranular limestones have retained their primary porosity and may have porous systems geometrically similar to those of sandstones. Primary porosity is most affected by the degree of initial cementation and compaction.

Secondary porosity is formed after deposition and results from processes such as dissolution, recrystallization, dolomitization, fracturing and other physical/chemical changes (Amthor et al., 1988; Harbaugh, 1967). Carbonate sedimentary rock can be dissolved by organics acids or meteoric water, resulting in the formation of micropores, vugs, caverns, etc., that dramatically affect its porosity. Calcareous rocks dissolve in the presence of dissolved carbon dioxide if water movement is sufficient to keep the formation water undersaturated with calcium carbonate. If not, calcite precipitation occurs, resulting in decreased porosity. The effect of dolomitization was discussed above.

Physiochemical processes often lead to partial or complete obliteration of matrix porosity in carbonates. However, oil can be produced from interconnected micropores, vugs, fractures and related openings in these rocks. A great number of carbonate reservoirs in Oklahoma are of this type.

Carbonate reservoirs are generally of three porosity types: intergranular/intercrystalline, vugular/solution and fracture/matrix (Bulnes and Fitting, 1945; Rieke et al., 1972). Intergranular/intercrystalline pore systems consist mainly of primary voids with little or no secondary modification. These are

like sandstones in that their pore geometry is determined by the size, shape and packing of grains. Examples include the Lansing-Kansas City formation in Finney, Kearney and Haskell Counties, Kansas, and the San Andres formation in the Permian basin in Texas (Chilingar et al., 1972). The performance of this type of carbonate pore system is essentially the same as that of sandstone, with comparable fluid content and drive mechanisms.

Vugular/solution porosity in carbonate reservoirs is found in grain-supported carbonate rock which has undergone significant post-depositional changes. The pore system is more complex than that of intergranular/intercrystalline systems and may exist as fissures, micropores and solution caverns. Generally, fractures may exist but do not dominate the flow mechanics. Examples of this type of carbonate pore system include the Viola formation in Oklahoma County, Oklahoma, and Nemaha County, Kansas. These reservoirs can show dual porosity characteristics.

In a fracture/matrix porosity system, the property of dual porosity is well developed. The matrix is often of low permeability but is thought to contain most of the oil. The fractures may contain only a small portion of the hydrocarbon pore space but dominate reservoir drainage. An example of this type of carbonate pore system is found in the Bois d'Arc sequence of the Hunton formation in Oklahoma County, Oklahoma. This reservoir contains an extensive, interconnected system of fractures and solution channels which have about 10% of the total reservoir pore space in a rock of otherwise very low permeability (Elkins, 1969). This resulted in severe channeling of naturally encroaching water as well as injected fluids with little or no benefit to oil

recovery. An extensive, interconnected fracture system oriented basically in southwesterly and northeasterly directions was reported in the chalky carbonate formations of the Eko field, Norway (Northrop and Teufel, 1990). Here, the matrix porosity was high, ranging from 25-48% in the productive region of the reservoir. However, the effective permeability of the natural fracture system was 100 times greater than the matrix absolute permeability, and pilot waterflooding yielded poor results. Waterflooding might have proved more effective if the drive had followed the preferred orientations of the fractures (Northrop and Teufel, 1990).

Oil recovery from carbonates is also influenced by pore geometry (size and sorting) and wettability. Pore throat size and pore sorting are held to be important to the efficiency of oil recovery from a reservoir, but the actual effect of these variables may vary from reservoir to reservoir. Relevant literature includes Amthor et al. (1988), Friedman et al. (1980), Jodry (1972), Melrose and Brandner (1974), Pickell et al. (1966), Ruzyla and Friedman (1964), Stout (1964) and Yu and Wardlaw (1986). Distribution of fluids within a given pore structure is partly determined by wettability. Rocks can be oil-wet, water-wet or neutral; wettability and its effects on other rock properties have been reviewed by Anderson (1986; 1987a; 1987b). In oil-wet rocks, waterflooding will generally displace oil only from the flow channels, leaving residual oil trapped in smaller pores and around the surface of larger pores (Etris et al., 1988; McInerney and Westlake, 1990). Further oil recovery at this stage often entails

wettability reversal as a mechanism. Many carbonates tend to be oil-wet (Chilingar and Yen, 1983).

Overall, carbonate reservoirs are both different from and more complicated than sandstone reservoirs. This has been a major reason for the relative lack of information regarding MEOR in carbonate systems; sandstone systems are more amenable to laboratory study and modeling. The specific problems of very low permeability of many oil-bearing carbonate rocks, contributing to poor primary recovery, and dual porosity in many carbonate reservoirs, contributing to poor secondary recovery, can make application of most enhanced oil recovery (EOR) processes in carbonates challenging.

III. EOR MECHANISMS

Experience in sandstone reservoirs has shown that selection of applicable EOR techniques depends on the properties of the reservoir's rock, the reservoir's fluids, and their interaction (Lake, 1989; National Petroleum Council, 1976; National Petroleum Council, 1984). In carbonate reservoirs, the carbonate pore structure and pore size distribution must also be considered (Amthor et al., 1988; Etris et al., 1988; Friedman et al., 1980; Ruzyla and Friedman, 1982; Wardlaw, 1976). Study of pore structure and geometry should indicate carbonate reservoirs in which EOR would be considered, i.e., those with low initial displacement efficiency and high post-primary recovery residual oil saturation. Knowledge of the pore structure and fluid properties of a reservoir is important for selection of appropriate EOR techniques. Understanding of the conventional EOR processes currently applicable to carbonate reservoirs, together with the operating mechanisms and their relative successes, can guide research on and selection of the most promising MEOR mechanisms for the different pore systems of carbonates.

A. Conventional EOR Methods

There are about seven major EOR techniques which can be classified into three broad areas of application (Lake, 1989; National Petroleum Council, 1976; National Petroleum Council, 1984). Under thermal flooding are steam flooding and in situ combustion. Classified under chemical flooding are

surfactant-polymer injection, polymer injection and alkali injection. Under miscible solvent flooding is CO_2 injection and miscible light hydrocarbon or alcohol injection.

1. Thermal flooding

The dominant mechanism in thermal flooding is viscosity reduction. Thermal flooding is often used to recover heavy oils. The effectiveness of steam flooding in recovering heavy oil from fractured carbonate samples was studied (Briggs et al., 1989); recoveries in excess of 50% of the original oil-in-place were reported. There is no MEOR mechanism directly analogous to thermal flooding. However, recovery of heavy oil after bacterial degradation has been suggested in the literature (ZoBell, 1946).

2. Chemical flooding

The common chemical flooding processes, surfactant, polymer and alkali injection, have been traditionally restricted to use in sandstone reservoirs (National Petroleum Council, 1976; National Petroleum Council, 1984). Chemical flooding in carbonate reservoirs has not been reported in the literature. The feasibility of chemical flooding was evaluated for a limestone reservoir with favorable characteristics: oil-wet rock, low salinity, low temperature, moderate oil viscosity and high residual oil saturation (Olsen et al., 1990). Surfactant flooding of core samples was considered successful, recovering 45.3% of the oil-in-place after waterflooding, but recoveries using alkaline or polymer flooding were poor.

The mechanisms of oil recovery in surfactant flooding are to reduce the interfacial tension between fluids in the reservoir and to increase the water wettability of formation rock. Commercially available surfactants are inactivated by divalent cations; consequently, carbonates are not considered suitable candidates for surfactant EOR (National Petroleum Council, 1976; National Petroleum Council, 1984; Schneider and Owens, 1976).

In alkaline flooding, chemicals such as sodium hydroxide, sodium orthosilicate or sodium bicarbonate are injected into a reservoir. The injected alkali interacts with crude oil in situ to form a surfactant. The crude oil is then mobilized as the surfactant reduces interfacial tension, reverses rock wettability and forms emulsions (Lake, 1989). As in surfactant flooding, chemical/rock interactions can significantly impact the effectiveness of alkaline flooding and this process has not been applied in carbonate formations (Lake, 1989; National Petroleum Council, 1976; National Petroleum Council, 1984).

In polymer flooding, polymers are added to injection water to increase its viscosity and to decrease the aqueous phase mobility. This should increase waterflood sweep efficiency. The general types of polymers are polyacrylamides and biologically produced polysaccharides. Factors that limit the application of polymer flooding in carbonate reservoirs include the effect of divalent cations and brine on polyacrylamide and the low permeability of many carbonate reservoirs (Chang, 1978; Gogarty, 1983; Lake, 1989; National Petroleum Council, 1976; National Petroleum Council, 1984). Polymer flooding in carbonate reservoirs has not been

reported in the literature, although use of this technique has been reported in calcareous reservoirs (Townsend et al., 1977).

3. Solvent flooding

The primary mechanism proposed for solvent flooding, also called miscible flooding, is extraction (Lake, 1989). Solvents used include alcohols, refined hydrocarbons, petroleum gas, natural gas and carbon dioxide. The cost of solvents other than carbon dioxide is often prohibitive for economical oil recovery. The use of a solvent consisting primarily of light hydrocarbons was reported in a dolomitized carbonate reservoir (Bilozir and Frydl, 1989). This field project was partially successful, but only about half of the projected oil production was recovered. In the same report, it was suggested that microbially produced compounds such as alcohols and methane could be used for solvent flooding (Bilozir and Frydl, 1989).

The most common EOR process applied to carbonate reservoirs is carbon dioxide flooding (National Petroleum Council, 1976; National Petroleum Council, 1984). The important potential mechanisms of oil recovery for carbon dioxide flooding include: oil swelling, oil viscosity reduction, oil density decrease and the acidic effect of carbon dioxide on reservoir rock (Henry et al., 1981; National Petroleum Council, 1976; National Petroleum Council, 1984). The effect of carbon dioxide on carbonate rock was documented indirectly during a pilot trial in a west Texas field (Henry et al., 1981). During carbon dioxide flooding, the levels of carbonate and bicarbonate ions in produced waters increased. The

proposed mechanism for this increase was through the acidic dissolution of rock: $CO_2 + H_2O + CaCO_3 \text{-----}> Ca^{++} + 2\ HCO_3^-$ (Henry et al., 1981). Though the extent of dissolution was considered minimal, it appeared that improvement of porosity and permeability in the reservoir was possible. Carbon dioxide flooding has been reported in other carbonate reservoirs, with varying degrees of success (Kane, 1979; National Petroleum Council, 1976; National Petroleum Council, 1984; Rowe et al., 1982). These reports suggest that the in situ production of carbon dioxide by microorganisms may be an important mechanism for oil recovery and has been part of several MEOR proposals.

B. Mechanisms of MEOR in Carbonate Reservoirs

The basic mechanisms for MEOR remain essentially unchanged from those originally proposed by ZoBell (1946; 1947). The specific mechanisms by which microorganisms could enhance oil recovery include: acid production leading to dissolution of rock matrix and increases in pore size and permeability; gas production resulting in decreased oil viscosity, repressurization of the reservoir and dissolution of reservoir matrix; solvent production acting directly as an extractant or as a cosurfactant to decrease interfacial tension and increase oil mobility; surfactant production to decrease interfacial tension and improve microscopic displacement efficiency; polymer production to increase the viscosity of the aqueous phase, improving the mobility ratio and sweep efficiency; selective plugging to block the dominant flow channels and improve

sweep efficiency; biotransformation of crude oil to remove selectively the heavy asphaltic fractions, decreasing oil viscosity and improving its mobility. These mechanisms will be discussed separately, primarily in the context of applicability to in situ MEOR in carbonate reservoirs.

1. Acid production

Microbial acid production has always been viewed as one of the basic mechanisms for MEOR, and this mechanism has been specifically targeted for application in carbonate reservoirs (Davis, 1967). Microbial fermentation of carbohydrate results in the production of organic acids which then interact with the carbonate rock matrix: $2\ H^{+} + CaCO_3 \text{-----}> Ca^{++} + H_2O + CO_2$. The utility of this MEOR mechanism as opposed to the simple injection of acid itself into a reservoir is the in situ, reservoir-wide production of organic acid (Davis, 1967), preferably at the carbonate-water interface, followed by dissolution of the carbonate matrix. Local acid production by microorganisms would likely have little impact on highly permeable portions of a reservoir, but any dissolution in the low permeability portions of a reservoir might significantly increase local permeability. The importance of this mechanism is supported by both laboratory and field trial experience in MEOR. In many MEOR field trials the pH of produced water was lowered 1-2 units during MEOR, demonstrating that acids can be produced microbially in quantities sufficient to affect an entire reservoir (Davis, 1967; Johnson, 1979; Lazar, 1987; Lazar et al., 1988;

Yarbrough, 1987; Yarbrough and Coty, 1983). A large information base exists on the physiology of microbial acid production from carbohydrates which can be used to optimize the process (Gottschalk, 1986).

A potential problem with the application of this process is that calcium ions produced by acid dissolution of the rock matrix may themselves precipitate elsewhere in the reservoir, causing plugging. However, if this were a likely possibility it would also been observed in carbonate reservoirs undergoing carbon dioxide flooding.

Microbial acid and gas production are probably the most important mechanisms for MEOR in carbonate reservoirs. However, a common phenomenon in the microbial fermentation of carbohydrate is that acid production leads to a decrease of culture pH, first causing a reduction in microbial metabolism and, if the pH becomes low enough (generally 5.0-5.5), then a cessation of microbial metabolism. An attractive feature of carbonates for MEOR is that as the acidic end-products are produced, they react with and are neutralized by the carbonate matrix (Lazar et al., 1988). This would allow a more rapid and complete fermentation of injected carbohydrate during in situ MEOR.

2. Gas production

Production of gaseous end-products (carbon dioxide, hydrogen and methane) during the anaerobic fermentation of carbohydrate has been implicated as another important mechanism for MEOR. Of these three gases, the production of carbon dioxide is probably the most

important. In an active microbial ecosystem, hydrogen produced during fermentation will be used as an energy source supporting anaerobic respiration (Zehnder, 1988): nitrate reduction, sulfate reduction, methanogenesis or acetogenesis. The production of methane has not been the subject of a laboratory or field study in MEOR. The available literature indicates that methanogenesis may not be a significant activity during carbohydrate fermentation in highly saline environments. However, it has been suggested that microbial methane production could result in partial repressurization of a reservoir, leading to improved oil recovery (Finnerty and Singer, 1983).

Carbon dioxide flooding is a recognized EOR technology (Orr and Tabor, 1984). A proposed mechanism for EOR by carbon dioxide flooding is the dissolution of rock matrix by the gas in the form of carbonic acid, leading to improved reservoir properties (see solvent flooding above, also): $H_2CO_3 + CaCO_3 \text{-----}> Ca^{++} + 2\ HCO_3^-$. The solution of carbon dioxide into crude oil also results in swelling the oil, lowering its viscosity and improving its flow characteristics. Many of the field trials in carbonaceous reservoirs provide ample evidence that microbial activity resulted in an increase of carbon dioxide production from the reservoir and a decrease in the viscosity of the oil (Davis, 1967; Hitzman, 1983; Hitzman, 1988; Yarbrough and Coty, 1983).

3. Solvent production

The microbial production of solvents (butanol, acetone and isopropanol) is a desired activity in MEOR since these compounds

are miscible in crude oil, decreasing its viscosity and improving its mobility (Ulmer et al., 1983). The production of solvents by clostridia is usually dependent both on the presence of acetate and butyrate in the environment and on a relatively low pH of 4.5-5.0 (Jones and Woods, 1986). Given the acid neutralizing capability of carbonates, it is unlikely that a low enough pH could be established during an in situ fermentation for solvent production to be a major microbial activity. Some localized exceptions to this generalization might occur, such as in well bore stimulation technologies.

4. Surfactant production

The microbial production of surfactants reduces the interfacial tension between oil and water and releases crude oil trapped by capillary pressure. The production of surfactants has long been recognized as a potential mechanism for MEOR (LaRiviere, 1955). As with acid production, the reservoir-wide in situ production of a biosurfactant would probably enhance oil recovery more than the injection of a surfactant (Davis, 1967). For carbonate reservoirs, the surfactant-producing bacterium should be able to grow anaerobically in a saline medium. A bacterium that produces surfactant and can grow under these conditions, Bacillus licheniformis strain JF-2, has been described (Javaheri et al., 1985; Jenneman et al., 1983; McInerney et al., 1989).

5. Selective plugging

In many waterfloods in clastic reservoirs, the ultimate recovery of oil is dominated by the pore sweep efficiency of the flowing phase. Laboratory research in sandstone systems (Jenneman et al., 1984; Raiders et al., 1988) has shown that microbial selective plugging is technically feasible and can divert fluid flow from more permeable regions to less permeable regions even under conditions which favor crossflow. However, due to the low porosity and permeability of most carbonate matrices, sweep efficiency is not as important of a concern as whether one will be able to inject any water at all into a formation. In carbonate reservoirs, fractures and vugs are the main channels for fluid flow. Plugging these channels may result in the complete loss of injectivity in a carbonate reservoir.

The utility of selective plugging in carbonates may be limited to reservoirs that are highly fractured and have many interconnected flow channels so that plugging off one channel will not result in complete loss of flow in the entire reservoir. However, it remains to be determined whether a microbial process will be able to effectively plug large macropores and flow channels. In sandstone, the largest pore channels generally do not exceed 50 um. However, fractures and vugs in carbonates can be much larger than this. Selective plugging might be useful to improve waterflooding by diverting flow from watered-out, highly permeable regions of a reservoir to less permeable regions. Given the difficulties which can be encountered in waterflooding carbonate reservoirs in general, it might be prudent to first

conduct MEOR using an acid and gas producing process, and then use selective plugging as a further MEOR treatment after completion of the acid and gas producing phase.

The above discussion illustrates that MEOR is a collection of processes which can be carried out separately in the same reservoir. Acid- and gas-producing fermentations and surfactant production are probably the most important mechanisms for MEOR in carbonate reservoirs, but there can be roles for other mechanisms. These mechanisms should be rigorously tested in the laboratory. However, there is some field trial evidence supporting the utility of MEOR in carbonate reservoirs (below).

IV. MEOR RESEARCH IN CARBONATE RESERVOIRS

A. Laboratory Studies

There are few laboratory studies on MEOR in carbonate systems, primarily because these are inherently more complex and less amenable to study than sandstone systems. Early work included study of the release of oil by sulfate-reducing bacteria (SRB) in packed cores of crushed and sized carbonaceous materials (marble or oyster shells), in addition to sand-packed columns and sandstone cores (Udegraff and Wren, 1954). The activity of SRB did not result in the release of significant amounts of oil from carbonaceous materials. However, the authors pointed out that the SRB used did not produce large amounts of acid or carbon dioxide as end-products of metabolism nor did they produce surfactants. These activities are generally recognized as important mechanisms for MEOR (ZoBell, 1947). This study by Updegraff and Wren (1954) effectively ended examination of SRB for MEOR in the United States, although further research on the use of SRB for MEOR, including field trials, continued elsewhere (Dostalek and Spurny, 1957a; Dostalek and Spurny, 1957b; LaRiviere, 1955).

The ability of microorganisms to penetrate or plug limestone cores was examined (Myers and McCready, 1965; Myers and Samiroden, 1967; Raleigh and Flock, 1965); this work, along with studies in sandstones, was summarized by Updegraff (1983). The basic conclusions from these studies were that microorganisms can penetrate limestone in the presence of an aqueous phase, even cores with permeabilities less than 0.1 mD, and that the presence of oil

did not inhibit microbial penetration. The rate of penetration or degree of plugging did not directly correlate with the measured permeability or porosity of the core. The observation that microorganisms can penetrate cores with very low permeabilities (less than 0.1 mD) is contrary to the notion that these cores would not have pores large enough to allow microbial transport. This observation merits further investigation.

Chang and Yen (1985) studied the effect of Clostridium acetobutylicum on the recovery of oil from a Kansas (Osawatomie) limestone core. The core had a permeability of 25 mD and was 25% oil saturated. A nutrient mixture of 4% molasses, 0.5% ammonium phosphate and 1.5 mM sodium pyrophosphate was injected into the core along with about a tenth pore volume of a culture of C. acetobutylicum. After incubating the core for an unspecified period of time, the process of injecting nutrients and cells was repeated. Approximately 33% of the oil in place was recovered by repeating this "huff and puff" method. The authors proposed that the mechanism of oil recovery was a large pressure drop across the core which might have acted to "push" the oil out of the core. Whether this pressure drop was the result of the low permeability of the core or the result of in situ gas production was not determined. Analysis of the data in this study clearly shows that indigenous bacteria rather than the injected clostridial species were active in the core. The core was flooded with a brine containing 10% sodium chloride, which is inhibitory for C. acetobutylicum. Analysis of the sugar and organic acid concentrations in the influent and effluent showed the loss of malonic, lactic and acetic acids, and the production of maltose;

none of these activities are known properties of C. acetobutylicum. The fact that butyric acid was not detected in the effluent indicates that a clostridial fermentation was probably not involved.

In order to understand the mechanism of oil recovery observed in the field trial conducted in a reservoir in Union County, Arkansas (Yarbrough and Coty, 1983), Yarbrough (1987) studied the effect of the presence of carbonate in sand-packed columns on oil recovery. The conclusion of these experiments was that the enhancement of oil recovery by microorganisms was dependent on the presence of carbonate in the sandpack and on microbial acid production, which would react with the carbonate component. Microbial gas production had little effect on the recovery of residual oil. As mentioned by the author, this was an interesting result which was not predicted based on earlier studies.

The study of MEOR in carbonates in the laboratory has not been extensive enough to determine the feasibility of microbial processes nor is there enough information available to determine the most effective method to enhance oil recovery. Although microbial acid and gas production has long been proposed to be the most important mechanisms whereby microorganisms can enhance oil recovery, well controlled experiments to support these generalizations are not available. Clearly, further laboratory research is required to determine if MEOR is feasible in carbonate systems and to determine the mechanisms of oil recovery.

B. Field Trials

Probably the best documented MEOR field study in the United States was that done in a sandstone reservoir in Union County, Arkansas (Davis, 1967; Hitzman, 1988; Yarbrough and Coty, 1983). The reservoir was essentially an unconsolidated sand containing 1-16% carbonate (8% in the trial zone) with a depth of 610 meters and a temperature of 32-40° C. It had been waterflooded. The MEOR treatment consisted of the injection of beet molasses along with the solvent and gas producing species, C. acetobutylicum, as the inoculum. After microbial treatment, production of carbon dioxide in the test well greatly increased and about 20% of this carbon dioxide was derived from the carbonate in the reservoir. Volatile acid end-products of metabolism matching those of a Clostridium kluyveri fermentation were detected in the produced water and C. kluyveri was isolated from this water. There was no direct evidence to show that the injected bacterium, C. acetobutylicum, was active in the formation. The field trial was a success, with oil production increasing by 250%. The mechanism proposed for the enhanced oil recovery was in situ production of organic acids, which subsequently reacted with carbonates in the reservoir, leading to an improvement in reservoir characteristics.

MEOR has been conducted for over 15 years in Romania, primarily in carbonaceous sandstone reservoirs rather than carbonate reservoirs per se (Hitzman, 1988; Lazar, 1987; Lazar et al., 1988). The process generally entailed the injection of 4% molasses and a population of anaerobic microorganisms selected for the ability to ferment molasses vigorously with the production of acids and gases

into a reservoir. Increased oil recovery and evidence of in situ microbial metabolism, i.e., a decrease in the pH of produced water and an increase in the carbon dioxide concentration of produced gas, was observed when suitable reservoirs were chosen. Continued MEOR was dependent on the continued injection of nutrients into the reservoir. In some cases, it was clear not only that microbial activity resulted in increased oil recovery, but also that the process was economical, with the incremental costs less than three dollars per barrel of oil recovered. Reservoir characteristics deemed suitable for MEOR included a permeability greater than 300 mD, a temperature less than 55° C, pH near neutrality, moderate or low salinity, a residual oil saturation greater than 60% and at least 25% carbonate in the reservoir matrix. The presence of carbonate was regarded as essential by Lazar and his colleagues, who proposed that the carbonate buffered the reservoir against the organic acid end-products of metabolism produced from the fermentation of molasses. This maintains the pH high enough (greater than 5.5) so that continued microbial activity is not inhibited. Microbial acid and gas production were believed to be the important mechanisms for oil recovery (Lazar et al., 1988), consistent with the observation that the pH of the produced water decreased and that the concentration of carbon dioxide in the produced gases increased during the treatment (Hitzman, 1988).

Field trials in carbonate reservoirs in other eastern European countries have been reported, including three from Hungary. One trial was conducted in a limestone reservoir with a depth of 700 meters and a temperature of 50° C (Forbes, 1980; Hitzman, 1983; Lazar, 1978). Molasses, nitrate and phosphate, followed by a

bacterial inoculum, were injected into the reservoir, which was then shut in for six months. After production was resumed, it was observed that the pH of the produced water decreased from 7 to 6, the concentration of carbon dioxide in the produced gas increased, the viscosity of the produced oil decreased and oil production from the reservoir increased by 60%. In a second reported trial, the injection of a nutrient mixture (molasses, nitrate and phosphate) along with an inoculum enriched from sewage sludge into a carbonaceous sandstone reservoir with a depth of 30-100 meters and a temperature of 25-30° C also resulted in a lowering of the pH and viscosity of the produced brine and oil, respectively, and an 126% increase in oil production. A third trial was reported in a limestone reservoir with a depth of 2,460 meters and a temperature of 97° C where molasses supplemented with sucrose and nitrate was injected along with an inoculum consisting of microorganisms enriched from sewage sludge and Desulfovibrio desulfuricans (Hitzman, 1983). Again, a decrease in the pH of the produced brine (9 to 6) and in the oil viscosity was noted, along with increased numbers of D. desulfuricans in the produced water. Oil production increased by 60%. This trial is interesting since it contends that a mesophilic bacterium, D. desulfuricans, survived and perhaps grew in this hyperthermophilic environment. Regardless of the source of the microorganisms involved, it does show that MEOR processes can be effective at high temperatures.

One of the criticisms of these studies is that it is not clear how the shut in period affected oil recovery. The major question asked is whether the amount of additional oil recovered is more

than that which would have recovered if the well had remained in continuous production. Such information is vital in assessing the technological performance and economics of MEOR.

A successful field trial in a carbonate reservoir in the former German Democratic Republic was recently reported (O. M. J. Wagner, MEOR from carbonate reservoirs with complex formation characteristics, 1990 MEOR International Conference). The formation was a fissured-porous carbonate reservoir with a permeability of 10-50 mD, a depth of 1,240 meters and a temperature of 53° C. The produced brine contained 33% dissolved solids, reported as being mainly potassium chloride. Microorganisms were enriched using 4% beet molasses with ammonium chloride and polyphosphate as the nutrient source. The microbial enrichment consisted mainly of clostridia that fermented carbohydrate to carbon dioxide, hydrogen and organic acids, with the production of some ethanol and butanol. Laboratory studies showed that MEOR could recover residual oil, but few details of these studies were given. The injection of the nutrient mixture and enriched inoculum into the reservoir resulted in a dramatic 600% increase in oil production. Plans were described to apply this process in a carbonate reservoir with a temperature of 82° C.

Microbial inoculants have been used to control paraffin plugging in limestone reservoirs (Oppenheimer and Hiebert, 1988). A paraffin-degrading inoculum producing carbon dioxide, organic acids and detergents is used. This method was used in reservoirs which had been waterflooded; however, further data were not available.

Another well bore clean out process using microorganisms has been reported (Johnson, 1979). In this process, a mixed culture of Bacillus and Clostridium strains along with cattle feed molasses was placed in the well bore for a period of 10-14 days. Gases (carbon dioxide, methane and nitrogen) and solvents were reported as the end-products of microbial metabolism during the process. Again, these products indicate the involvement of microorganisms other than those present in the inoculum. This well bore clean out process was reported to be successful in carbonate or carbonaceous sandstone reservoirs. However, the criteria for success were not rigorously defined.

Analysis of the results of these field trials shows that additional oil has been recovered after the implementation of a MEOR process. Although the mechanisms for the recovery are not clear and, in some cases, oil recovery could be due to factors other than the microbial process itself, it is clear that the detrimental effects often associated with microbial activity in petroleum reservoirs, such as formation plugging and souring, were not documented. Much evidence exists to support the contention that in situ microbial activity did occur and, thus, could be the cause of the increased oil production. The pH of the produced brines decreased as a result of the production of microbial end-products of metabolism, showing that microbial activities can affect an entire reservoir. The produced oil had a lowered viscosity, probably due to the solution of carbon dioxide into the oil and possibly due to other microbial products (solvents, etc.). It is apparent that MEOR can be useful for enhancing oil recovery from carbonate and carbonaceous reservoirs.

V. MICROBIOLOGY OF CARBONATE RESERVOIRS

The common biological perception of a petroleum reservoir has been that it is a hostile environment essentially devoid of microbial life. However, recent research on the microbiology of thermophilic environments and halophilic environments would suggest that a great many carbonate reservoirs have conditions that make them suitable habitats for microorganisms; specifics will be discussed below (Reservoir Environment and MEOR Potential). Thus, one cannot a priori exclude the possibilty that an active microbial population exists in a petroleum reservoir. Direct evidence for the existence of active and diverse microbial populations in unconsolidated subsurface reservoirs to a depth of 300 meters was obtained through the use of specialized drilling and sampling procedures for the collection of uncontaminated aquifer material (Ghiorse and Wilson, 1988; Jones et al., 1989). The direct study of deeper, consolidated reservoirs is more difficult due to the problems in obtaining uncontaminated samples. However, in reviewing the information on microbial activity in deep, consolidated reservoirs, McInerney and Westlake (1990) concluded that several lines of evidence support the contention that microbial activity can and does occur in reservoirs which are in hydrogeological communication with aquifers or have been subjected to waterflooding or an EOR process.

Probably the greatest effort to describe the microbiology of oil reservoirs was attempted in the Soviet Union (Eksertsev and Kuznetsov, 1954; Ginsburg-Karagicheva, 1933; Kuznetsov et al., 1963). However, many of the earlier studies lacked the

thoroughness required to determine the applicability of MEOR in carbonate reservoirs. Often, these studies did not include a detailed description of the geology and geochemistry of the reservoir, state whether connate or injected waters were examined or state whether the reservoir had been waterflooded. The microbiological methods used allowed the isolation of the easily cultivated microorganisms rather than using methods that simulated the habitat to isolate and/or enumerate the numerically dominant and ecologically most important microorganisms. The study by Bailey et al. (1973a) provides the best evidence for microbial activity in a carbonate reservoir, the Williston basin formation in southeastern Saskatchewan. Isotopic fractionation and gas chromatographic analysis indicated that crude oil produced from a portion of the reservoir which had been waterflooded with a low salinity brine containing oxygen and sulfate had been biodegraded. Subsequent laboratory core experiments using an aerobic oil-degrading microbial population showed that unaltered oil from the basin could be converted into an oil which had characteristics similar to the oil biodegraded in situ (Bailey et al., 1973b).

Most researchers agree that SRB are universally associated with reservoir water, physical conditions permitting (Postgate, 1984). This likely accounted for much of the early interest in using SRB as agents for MEOR (Davis, 1967; Updegraff and Wren, 1954). However, there is considerable disagreement as to the source of these bacteria in a reservoir; are they native to the formation or were SRB introduced during development of the reservoir? SRB were found in aseptically sampled aquifer material at depths down to about 300 meters (Jones et al., 1989) and in groundwaters sampled

from depths of 10 to 260 meters (Dockins, 1980). The latter study found that the lighter isotope of sulfur was selected during sulfate reduction, indicating a biological origin of the produced hydrogen sulfide. ZoBell (1958) found viable SRB in brine and core samples taken from strata down to depths of 3,700 meters near petroleum and sulfur deposits. This well was drilled using biocide-treated alkaline (pH 11.5) drilling mud to reduce the chance of contamination, and SRB were not recovered from this drilling mud. These studies show that subsurface formations can contain active populations of SRB, and that the presence of these bacteria in hydrocarbon-bearing carbonate reservoirs should be expected.

Many MEOR processes are based on the injection of one or many microbial species into a reservoir. These technologies usually are based on the assumptions that a reservoir does not contain microorganisms, an unlikely possibility given the discussions presented here (see also MEOR Reseach in Carbonate Reservoirs and Reservoir Environment and MEOR Potential), and that the desired physiological type(s) of microorganism is not present in a reservoir. While the latter may be true in certain reservoirs, there have not been rigorously controlled field trials that prove that an inoculum was needed for MEOR or that, in the case of carbonate or carbonaceous reservoirs, introduced microorganisms were those predominantly active in a reservoir. The injection of a microorganism into a reservoir does not ensure its subsequent survival and activity. After waterflooding, most reservoirs would have an established community of microorganisms that had been selected for growth in that particular environment. The normal

homeostatic processes of this community will act to prevent the establishment of transient species unless the prevailing environmental conditions are changed or perturbed. Thus, the addition of a small number of microorganisms alone is unlikely to alter the reservoir ecosystem. However, the addition of large amounts of a readily utilizable nutrient source would temporarily change the environment. This is the basis for most MEOR processes, in which a reservoir or well is treated with both microorganisms and a suitable nutrient, usually molasses. This procedure does not guarantee that the injected microorganisms will be active in situ; the evidence suggests that the growth of indigenous microorganisms will prevail. In the best documented field trial, C. acetobutylicum, a solvent-producing species, was injected along with nutrients into a reservoir (Davis, 1967; Hitzman, 1983; Yarbrough and Coty, 1983). Neither this bacterium nor its end-products of metabolism were detected in produced water during the field trial. The microbial activity that did result was most probably due to other species indigenous to the formation. The literature on sewage treatment and agricultural practices conclude that microbial inoculants are often not required. Usually, the desired physiological type of microorganism(s) is already present, and all that is required is that suitable conditions be generated to stimulate its metabolism and growth. For MEOR, when one considers the volume of a petroleum reservoir, it is doubtful whether even relatively large scale inoculations would quantitatively change population levels.

Although the microbiology of carbonate reservoirs has not been studied specifically, certain generalizations about the

microbiology of oil fields can be made. It is reasonable to assume that if a reservoir has conditions favorable for microbial life, then it probably contains a microbial population that reflects that particular subterranean environment. The size of this population is relatively small and the activity is low, based on data obtained from aseptically sampled unconsolidated aquifer sediments (Jones et al., 1989) and on recent bacteriological analyses of oil field brines (McInerney et al., unpublished data; Tanner et al., unpublished data). The activity and size of an indigenous population can be stimulated by the injection of additional nutrients such as carbon, nitrogen, sulfur, phosphorous and, sometimes, oxygen sources into a reservoir. The selective injection of nutrients or other compounds provides a mechanism to control the activities of these microorganisms in situ (McInerney, 1983).

VI. RESERVOIR ENVIRONMENT AND MEOR POTENTIAL

The activity of microorganisms will be governed by the environmental conditions that exist in a reservoir. The factors that affect microbial activity in petroleum reservoirs were recently reviewed (Jenneman, 1989). The more important factors which might limit microbial activity in carbonate reservoirs are permeability, temperature, pressure and salinity; these will be discussed below. As the potential limits to in situ microbial activity are discussed, these will be assessed against the data on carbonate reservoirs in Dwight's Energydata TOTL system managed by the Natural Resources Information System at the University of Oklahoma in order to estimate the applicability of MEOR in carbonate reservoirs. Reservoir data from the top 11 oil-producing states (Oil and Gas J., 1990) were examined: Texas, Alaska, Louisiana, California, Oklahoma, Wyoming, New Mexico, Kansas, North Dakota, Colorado and Mississippi. Arkansas was included in the survey to represent a low-volume oil-producing state. Dwight's Energydata TOTL system was last updated in February, 1986, and a larger data set will be examined than compared to previous surveys (Clark et al., 1981; Jenneman, 1989). While data on the permeability, temperature, etc. are not totally complete for all of the reservoirs in the survey, the data available are adequate for a general feasibility study of MEOR in carbonate reservoirs. As with any EOR technology, actual application must be evaluated on a case-by-case basis.

A. Occurrence of Carbonate Petroleum Reservoirs

Three categories of carbonate reservoirs are identified in Dwight's Energydata TOTL system: limestones, dolomites and carbonate-sandstone mixtures. The distribution of carbonate reservoirs and the ratio of carbonate to sandstone reservoirs in the database for the 12 states examined is given in Table 1. It contained only one carbonaceous reservoir from California and only two from Alaska. These states were excluded from further examination their number of carbonate reservoirs was too small to impact this study.

B. Permeability

For in situ applications to be economical, a large portion of the reservoir must be affected by the microbial treatment. This generally means that the microorganisms involved must be able to penetrate and propagate throughout the reservoir; i.e., the reservoir matrix must have sufficient permeability so that penetration and growth of microorganisms will not be restricted. Based on an analysis of the relationship of bacterial cell size and the pore size of different permeability classes of oil-bearing sandstones, Clark et al. (1981) and Jenneman (1989) concluded that a permeability of 75 mD would represent the lower limit for the transport of microorganisms through the reservoir. Studies of the penetration of a Bacillus species through Berea sandstone cores showed that little or no penetration occurred in cores with a permeability less than 100 mD (Jenneman et al., 1985). However,

Table 1. Distribution of Carbonate Reservoirs in Dwight's Energydata TOTL System.

State	Number of Petroleum Reservoirs: Limestones and Dolomites	Number of Petroleum Reservoirs: Carbonate-Sandstone Mixtures	Ratio of Carbonate/Sandstone Reservoirs
AK	1	1	1:1
AR	182	14	1:4
CA	0	1	0:1
CO	108	50	1:9
KS	5,334	2,170	6:1
LA	190	16	1:14
MS	126	3	1:8
ND	258	9	21:1
NM	662	58	1:1
OK	2,073	176	1:3
TX	10,199	56	1:3
WY	165	50	10:1

the transport of microorganisms through a core with a permeability of less than 0.1 mD has been reported (Meyers and McCready, 1965). Thus, it may be possible for microorganisms to penetrate even very tight formations.

Many carbonate rocks have very low matrix permeabilites; measurements less than 0.1 mD are not unusual (Table 2). However, fluid flow through carbonate rocks with low matrix permeabilities can occur through fissures and/or vugs, which may have local permeabilities greater than 1 D. For efficient transport of microorganisms into a reservoir, Updegraff (1983) stated that the pore throat size must be at least twice the diameter of the microorganism. Given that porous rock with a permeability of 10 mD can have an average pore throat diameter of about 1 um and that a diameter of 0.5 um is not unusual for a bacterial cell, we have used a permeability of 15 mD as the lower limit for the application of MEOR in carbonate reservoirs to estimate the feasibility of MEOR in these reservoirs in the United States. Simplistically, one may decide that any reservoir that has undergone waterflooding is a candidate for MEOR since the waterflood has introduced microorganisms into the reservoir and/or stimulated the growth and activity of in situ populations.

The distribution of the average absolute permeability of carbonate reservoirs in the states selected for analysis is given in Table 2. The percentage of reservoirs with permabilities greater than 15 mD and greater than 75 mD was determined. More than twice as many carbonate reservoirs can be considered as candidates for MEOR when 15 mD compared to 75 mD is used as a potential limit.

Table 2. Distribution of Absolute Permeabilities (mD) of Carbonate Reservoirs in Dwight's Energydata TOTL System.

State	Reservoirs Sampled	Reported Permeabilities Minimum	Reported Permeabilities Maximum	Percent with Permeabilities: >15 mD	Percent with Permeabilities: >75 mD
AR	115	0.1	4,800	80.0	55.6
CO	13	2	122	30.8	7.7
KS	36	0.5	4,690	77.8	38.9
LA	43	0.3	665	62.8	27.9
MS	51	0.7	3,000	76.5	43.0
ND	59	0.1	517	33.9	5.0
NM	152	0.1	890	45.4	17.8
OK	34	0.03	1,740	32.4	8.8
TX	1,138	0.01	6,000	53.2	19.2
WY	55	0.06	500	25.5	5.5

C. Temperature and Pressure

The upper limit at which microorganisms can grow is continuously being increased as further research is conducted on natural environments where the temperatures exceed 70° C (extremely thermophilic) or 90° C (hyperthermophilic). There is a growing list of microorganisms that have optimal temperatures of 100° C or higher (Fiala and Stetter, 1986; Huber et al., 1987; Huber et al., 1989; Stetter et al., 1983; Zillig et al., 1990). The mounting evidence indicates that deep, and therefore hot, oil-bearing formations will contain microorganisms that can grow under extreme temperature conditions.

Based on review of the known thermophiles capable of metabolizing carbohydrates (Brock, 1986), Jenneman (1989) determined that a practical temperature limit for in situ MEOR was 75° C. Given our increased understanding of thermophilic microorganisms, most recently those from hyperthermophilic deep sea vents, we have selected a temperature of 82° C for the purpose of estimating the number of potential carbonate reservoirs suitable for MEOR. This is probably not an absolute temperature limit but is a limit that lends itself to manipulation in the database (many reservoir temperatures are entered in tens of degrees Fahrenheit; 180° F equals 82° C). The distribution of temepratures of carbonate reservoirs in Dwight's Energydata TOTL System is given in Table 3. It is interesting to note that a successful MEOR field trial was reported in a limestone reservoir in Hungary with a temperature of 97° C (Hitzman, 1983).

Table 3. Distribution of Temperatures in Carbonate Reservoirs.[a]

State	Reservoirs Sampled	Percent with Temperatures <82° C
AR	11	18.2
CO	10	60.0
KS	14	100
LA	16	37.5
MS	94	8.5
ND	287	22.4
NM	55	36.6
OK	26	92.8
TX	843	76.1
WY	17	31.8

[a]Temperature data from Dwight's Energydata TOTL System managed by the Natural Resources Information System at the University of Oklahoma.

Pressure as well as temperature increases with the depth of a formation. The effect of pressure and temperature on microbial activity may be synergistic. Bubela (1983) reported that increasing the pressure on a culture of an unidentified anaerobic microorganism from atmospheric to 20,000 kPa increased the optimal growth temperature from 55 to 65° C and simultaneously decreased the generation time from 17 to 12 hours; these are results that could bear repeating. In general, increases in pressure up to 20,000 kPa have been shown to have little or no effect on microbial activity (Marquis, 1983; Marquis and Matsumura, 1978). Pressures exceeding 50,000 kPa can be considered inhibitory to microbial activity.

D. Salinity

A large number of petroleum reservoirs in the United States have brines containing more than 10% sodium chloride, which emphasizes the importance of halophilic or halotolerant microorganisms for MEOR, with the latter group having the potential for wider application (Jenneman, 1989). Previously, salinities of 10% were considered the upper limit for the application of MEOR (Clark et al., 1981; Jenneman, 1989). However, our understanding of halophilic microorganisms and ecosystems has increased greatly in the last several years, and these topics have been the subject of several recent reviews (Javor, 1989; Rodriguez-Valera, 1988; Vreeland, 1987). It is evident from these studies that active microbial ecosystems can be found in environments with salinities as high as 25% sodium chloride. Previous research focused on the

halophilic archaebacteria and phototrophic eubacteria in saline environments. Recent studies of several halophilic environments, including hypersaline petroleum reservoirs, show the importance of heterotrophic eubacteria (V. Bhupathirajo, K. Fowler, W. Jenkins, T. Lui, M. McInerney and R. Knapp, submitted for publication; J. Adkins, D. Ahshapanek, D. Nagle, P. Sharma and R. Tanner, unpublished data). Thus, reservoirs with salinities up to 25% sodium chloride are potential targets for MEOR. The results of a survay of brine properties from carbonate reservoirs in Dwight's Energydata TOTL System are given in Table 4. The majority of reservoirs in each of the states examined have brines with salinities less than 25%. Average sodium chloride concentrations ranged from 0.99% and 2.02% in Wyoming and Colorado, respectively, to 19.2% and 20.9% in Arkansas and North Dakota, respectively.

E. pH

Although pH can be a factor limiting biological activity in several environments, it is often not limiting in petroleum reservoirs since most reservoirs have pH values within the range of growth of most known microorganisms (Jenneman, 1989). Carbonate and carbonaceous reservoirs would not have low pH values due to the buffering capacity of the carbonate ion. The natural buffering capacity of carbonate reservoirs would allow for a greater extent of metabolism compared to that in a sandstone reservoir since microbial acid production would not lower the pH to an inhibiting level (Lazar et al., 1988).

Table 4. Average Composition (ppm) of Brines from Carbonate Reservoirs.[a]

State	Element or Chemical						
	Chloride	Sulfate	Bicarbonate	Sodium	Potassium	Calcium	Magnesium
AR	135,000	298	130	57,100	1,780	25,500	2,920
CO	12,500	1,320	1,370	7,680	305	932	187
KS	59,900	1,300	293	31,000	232	5,290	1,530
LA	58,400	138	526	32,600	268	3,450	603
MS	93,200	143	201	44,600	1,300	11,600	1,250
ND	136,000	1,780	314	73,300	2,820	10,100	1,570
NM	49,600	1,940	801	23,900	935	4,070	1,440
OK	94,400	400	182	47,400	424	9,330	1,800
TX	50,800	1,070	524	24,800	466	4,450	1,080
WY	5,710	1,660	1,380	4,150	166	499	135

[a]Data for the major components of produced brines from Dwight's Energydata TOTL System manged by the Natural Resources Information System at the University of Oklahoma.

F. Petroleum

The presence of oil itself can be another factor limiting microbial activity and the feasibility of MEOR (Jenneman, 1989). Limitiations imposed upon MEOR by the petroleum phase include toxicity of the light volatile fractions and the difficulty in recovering heavy oils because of the unfavorable mobility ratios between the brine and the petroleum. Reservoirs whose crude oil has an API gravity of less than 17° are probably not good candidates for MEOR processes unless the process is specifically designed to improve the oil's mobility.

Biodegradation of the asphaltic and paraffinic fractions of petroleum, yielding a product with improved flow characteristics, has long been proposed as a viable mechanism for enhanced oil recovery (Kuznetsov et al., 1963). Several commercially available well bore stimulation MEOR processes are based on this mechanism (Johnson, 1979; Oppenheimer and Heibert, 1988). Basically, oxygen (or nitrate) is required for the biodegradation of hydrocarbons at an appreciable rate (Atlas, 1984). Therefore, the application of technologies based on the biodegradation of hydrocarbons will be limited to regions near a well bore where aerobic conditions can be established and maintained for a sufficient length of time. Such technologies may prove effective in stimulating oil production from individual wells but will not be effective deep within a reservoir. Although anaerobic biodegradation of crude oil has been reported (Davis and Yarbrough, 1966; Moses et al., 1982; Novelli and ZoBell, 1944), these results require reexamination. If anaerobic crude oil

degradation does occur, at best it would be at a very slow rate which would preclude it from being used economically.

G. EOR Criteria and Feasibility of MEOR in Carbonate Reservoirs

The limiting parameters for MEOR as discussed in this review and by Jenneman (1989), and those for other EOR technologies are given in Table 5. For most of these EOR processes, a further criterion is that at least 20% of the original oil-in-place remains in the reservoir; realistically, due to the low overall recovery after primary and secondary production, this is not a limiting parameter. The criteria limiting applicability of MEOR compare favorably to those limiting most conventional EOR processes. MEOR may be more advantageous compared to conventional EOR in reservoirs with higher salinities and oil viscosities.

Based on our discussion of the limiting criteria for MEOR, we have calculated a percentage of carbonate reservoirs treatable for the ten states examined, and this is given in Table 6. The result of this exercise indicates that MEOR should be applicable in about 40% of the 19,297 carbonate reservoirs in the United States surveyed in the database.

In the above discussion, each parameter was treated independently. However, in the field all of these factors act in combination with each other. Thus, before application of a MEOR process in the field, the effect of these parameters in combination, duplicating field conditions, should be tested to insure success. In general, suitable target reservoirs for the application of MEOR are those which have liquid water. In most

Table 5. Screening Criteria for Application of Enhanced Oil Recovery Technologies.

	Permeability (mD)	Temperature (° C)	Salinity (%)[a]	Oil Gravity (° API)
MEOR[b]	>15	<82	<25	>17
MEOR[c]	>75	<75	<10	>17
CO_2 Flooding[d]	>5	NR[e]	NR	>25
Surfactant Flooding	>40	<93	<10	>25
Polymer Flooding	>20	<93	<20	>20
Alkaline Flooding	>20	<93	<10	>30

[a]As sodium chloride.

[b]Limiting parameters as discussed in the text.

[c]Criteria from Jenneman (1989).

[d]Limiting parameters for conventional EOR technologies obtained from the National Petroleum Council (1976; 1984) and Goldburg and Price (1980).

[e]NR = not restrictive.

Table 6. Percent of Carbonate Reservoirs in Each State Potentially Treatable by MEOR.

State	Permeability >15 mD	Temperature <82° C	Salinity <25% NaCl	API Gravity >17°	Percentage Treatable[a]
AR	80.0	18.2	69.5	67.0[b]	6.8
CO	30.8	60.0	98.8	33.3	6.1
KS	77.8	100	98.5	99.7	76.4
LA	62.8	37.5	97.8	67.0[b]	15.4
MS	76.5	8.5	78.0	21.4	1.1
ND	33.9	22.4	66.7	95.3	4.8
NM	45.4	36.6	91.6[c]	24.0	3.7
OK	32.4	92.8	91.6[c]	99.7	27.5
TX	53.2	76.1	95.6	77.5	30.0
WY	25.5	31.8	100	31.4	2.5

[a]Percentage calculated by considering each parameter as an independent variable.

[b]An average for Arkansas and Louisiana.

[c]An average for New Mexico and Oklahoma.

cases, any reservoir that has undergone a successful waterflood should be a prime candidate for the application of a MEOR process since it contains the aqueous phase required for microbial activity and since waterflooding probably either introduced or stimulated the growth of microorganisms.

VII. CONCLUSIONS AND RECOMMENDATIONS

Carbonate formations have a wide range of pore geometries and distributions, ranging in size from less than one micron to meters in diameter, which results in complex porosity and flow characteristics. This is one of the most important factors affecting oil recovery from carbonate reservoirs. Some limestones have retained their primary porosity with little or no secondary modification; many of these formations have porous systems geometrically similar to those of sandstones and have comparable fluid content, flow mechanics and oil recovery efficiencies. However, most carbonate formations have undergone significant post-depositional modification and show dual porosity characteristics, where the matrix has low permeability, low porosity and most of the remaining original oil-in-place, and where fractures and/or vugs dominate the fluid flow mechanics.

The complex geology and fluid mechanics of most carbonate reservoirs has been problematic for oil recovery. Primary production recovers some of the oil present in interconnected solution channels, vugs and fractures, but little from the matrix. Due to the low permeability of the matrix and the dual porosity characteristic, waterflooding carbonate reservoirs often yields lower oil recovery efficiencies than that which result in most sandstone formations. These problems combined with the presence of divalent cations have slowed the implementation of EOR in carbonate reservoirs

Although many of the available EOR technologies have not been extensively studied in carbonate reservoirs, what little

information that exists can serve as the initial guide to identify potentially useful MEOR procedures. While surfactant flooding is not considered suitable in carbonate reservoirs because of detrimental effect of calcium and magnesium cations, several biologically produced surfactants have been shown to be active in relatively high concentrations of calcium suggesting that MEOR based on biosurfactant production may be beneficial. Miscible flooding techniques such as solvent and carbon dioxide flooding have had some degree of success in carbonate reservoirs. MEOR methods based on in situ carbon dioxide generation should be successful, and they may also have the added benefit of repressurizing the formation. Analysis of the geological and petrophysical properties of carbonate reservoirs suggests that mechanisms that improve matrix porosity and permeability without detrimentally affecting the injectivity of fluids should result in enhanced oil recovery. This would be most easily done by the in situ production of acids by bacteria.

Based on these analyses, we recommend that the following microbial processes be considered for MEOR in carbonate reservoirs: microbial acid and gas production to improve the matrix porosity and for carbon dioxide miscible flooding, and microbial surfactant production to alter matrix wettability and reduce interfacial tension-this process would probably use microorganisms that also produce acid and gas.

None of these potential microbial recovery mechanisms has been systematically tested in a controlled laboratory situation in carbonate systems. The effect of the microbial end-products of metabolism themselves independent of in situ activity on the

structure of carbonate rock and on oil recovery needs to be determined. The low permeability and porosity of the matrix of many carbonate rocks suggests that the transport of the required nutrients and the propagation and movement of the microorganisms themselves could be limited. However, reports that microorganisms can penetrate carbonates with permeabilites less than 0.1 mD indicate that nutrient and cell transport are possible. Given the dearth of information on MEOR in carbonate reservoirs, it would be presumptive to exclude a MEOR mechanism before it has been tested, but microbial acid, gas and surfactant production appear to offer the most promise in the near future.

Most of the information relevant to the microbiology of carbonate reservoirs is flawed since the source of the sampled brine was not specified and/or cultivation methods were used that did not simulate the environment, making it likely that the microorganisms isolated were ecologically neither numerous or important. The implementation of reservoir-wide microbial processes will often depend on the stimulation of indigenous microbial populations. Thus, it is imperative that an accurate assessment of the kinds and activities of the microorganisms present and the factors that govern their metabolism and growth be conducted.

We have shown in this review that the microbial activities observed in several laboratory studies and field trials in carbonates were not those of the injected microorganism. Given the volume of most carbonate reservoirs, it would be unlikely that one could inject enough microorganisms, if one could inject them at all, to have a significant impact on the population already present

in the reservoir. Immediate attention should be directed at studying the microbiology of a reservoir at the time of discovery to determine if microorganisms are already present in a reservoir prior to development or if the observed microbial populations are introduced into the reservoir during development. Also, there is no information in the literature on microorganisms that are both halophilic and thermophilic, conditions that are found in most oil-bearing formations.

Controlled field trials of MEOR in reservoirs where the geology and fluid dynamics in addition to the microbiology are well characterized are needed. While the available supporting laboratory data are incomplete, the review of the relevant field trial data suggests that MEOR in carbonate reservoirs is not only feasible, in several cases it successfully and economically recovered additional oil. Our understanding of the mechanisms of MEOR are adequate for planning and conducting a well controlled field experiment in a carbonate reservoir. The reservoir selected for this trial should be well characterized so that meaningful predictions can be made a priori and then tested in the field. Controls to account for the effects of shut in, etc. must also be included. Given the rapidity at which many carbonate reservoirs are nearing the end of their useful primary and secondary production in the United States, the time to initiate controlled trials of MEOR in carbonate reservoirs is currently at hand.

ACKNOWLEDGEMENTS

We wish to thank Anne Mycek-Memoli at the Oklahoma Geological Survey for her assistance in searching Dwight's Energydata TOTL System for data on carbonate reservoirs.

REFERENCES

Amthor, J. E., D. C. Kopaska-Merkel, and G. M. Friedman. 1988. Reservoir characterization, porosity and recovery efficiencies of deeply-buried Paleozoic carbonates-examples from Oklahoma, Texas and New Mexico. Carbonates & Evaporites 3:33-52.

Amyx, J. W., D. M. Bass, Jr., and R. L. Whiting. 1960. Petroleum Reservoir Engineering. McGraw-Hill Book Co., New York.

Anderson, W. G. 1986. Wettability literature survey-part 2: wettability measurement. J. Petrol. Technol. 38:1246-1262.

Anderson, W. G. 1987a. Wettability literature survey-part 4: effects of wettability on capillary pressure. J. Petrol. Technol. 39:1283-1300.

Anderson, W. G. 1987b. Wettability literature survey-part 6: the effects of wettability on waterflooding. J. Petrol. Technol. 39:1605-1622.

Anonymous. 1990. OGJ production report. Oil & Gas J. 88:57.

Archie, G. E. 1952. Classification of carbonate reservoir rocks and petrophysical considerations. Bullet. Amer. Assoc. Petrol. Geolog. 36:278-298.

Atlas, R. M. (ed.). 1984. Petroleum Microbiology. Macmillan Publishing Co., New York.

Bailey, N. J. L., A. M. Jobson, and M. A. Rogers. 1973a. Bacterial degradation of crude oil: comparison of field and experimental data. Chem. Geol. 11:203-221.

Bailey, N. J. L., H. R. Krouse, C. R. Evans, and M. A. Rogers. 1973b. Alteration of crude oils by waters and bacteria-evidence from geochemical and isotope studies. Amer. Assoc. Petrol. Geolog. Bullet. 57:1276-1290.

Benson, D. J. 1984. Lithology-carbonate rocks and geological processes, p. 21-30. In P. E. La Moreaux, B. M. Wilson, and B. A. Memon (ed.), Guide to the Hydrology of Carbonate Rocks. UNESCO, Paris.

Bilozir, D. E., and P. M. Frydl. 1989. Description and performance analysis of a mature miscible flood in Rainbow Field, Canada, p. 289-304. In Proceedings 1989 SPE Annu. Technical Conference and Exhibition. Soc. Petrol Engineers, Richardson, Texas.

Briggs, P. J., D. L. Beck, C. J. J. Black, and R. C. Bissell. 1989. Heavy oil from fractured carbonate reservoirs, p. 419-428. In Proceedings 1989 SPE Annu. Technical Conference and Exhibition. Soc. Petrol. Engineers, Richardson, Texas.

Brock, T. D. (ed.). 1986. Thermophiles: General, Molecular, and Applied Microbiology. John Wiley & Sons, New York.

Bubela, B. 1983. Combined effects of temperature and other environmental stresses on microbiologically enhanced oil recovery, p. 118-123. In E. C. Donaldson and J. B. Clark (ed.), Proceedings, 1982 International Conference Microbial Enhancement of Oil Recovery. NTIS, Springfield, Virginia.

Bulnes, A. C., and R. U. Fitting, Jr. 1945. An introductory discussion of the reservoir performance of limestone formations. Trans. AIME 160:179-201.

Chang, H. L. 1978. Polymer flooding technology-yesterday, today and tomorrow. J. Petrol. Technol. 30:1113-1128.

Chang, Y. I., and T. F. Yen. 1985. Effects of sodium pyrophosphate additive on the huff-and-puff/nutrient flooding MEOR process, p. 247-256. In J. E. Zajic and E. C. Donaldson (ed.), Microbes and Oil Recovery, vol. 1. Bioresources Publications, El Paso.

Chilingar, G. V., R. W. Mannon, and H. H. Rieke, III. 1972. Oil and Gas Production from Carbonate Rocks. American Elsevier Publishing Co., New York.

Chilingarian, G. V., and T. F. Yen. 1983. Some notes on wettability and relative permeability of carbonate reservoir rocks, II. Energy Sources 7:67-75.

Choquette, P. W., and L. C. Pray. 1970. Geologic nomenclature and classification of porosity in sedimentary carbonates. Amer. Assoc. Petrol. Geolog. Bullet. 54:207-250.

Clark, J. B., D. M. Munnecke, and G. E. Jenneman. 1981. In situ microbial enhancement of oil production. Dev. Industr. Microbiol. 22:695-701.

Craze, R. C. 1950. Performance of limestone reservoirs. Trans. AIME 189:287-294.

Davis, J. B. 1967. Petroleum Microbiology. Elsevier Publishing Co., New York.

Davis, J. B., and H. F. Yarbrough. 1966. Anaerobic oxidation of hydrocarbons by Desulfovibrio desulfuricans. Chem. Geol. 1:137-144.

Dockins, W. S., G. J. Olson, G. A. McFeters, and S. C. Turbak. 1980. Dissimilatory bacterial sulfate reduction in Montana groundwaters. Geomicrobiol. J. 2:83-98.

Donaldson, E. C., G. V. Chilingarian, and T. F. Yen. 1989. Microbial Enhanced Oil Recovery. Elsevier Science Publishing Co. Inc., New York.

Dostalek, M., and M. Spurny. 1957a. Release of oil through the action of microorganisms. I. Preliminary study on a petroleum deposit. Cesk. Mikrobiol. 2:300-306.

Dostalek, M., and M. Spurny. 1957b. Release of oil through the action of microorganisms. II. The influence of physical and physical-chemical conditions on oil-bearing rock. Cesk. Mikrobiol. 2:307-317.

Dunham, R. J. 1962. Classification of carbonate rocks according to depositional texture, p. 108-121. In W. E. Ham (ed.), Classification of Carbonate Rocks-a Symposium. Memoir 1, Amer. Assoc. Petrol. Geologists, Tulsa.

Ekzertsev, V. A., and S. I. Kuznetsov. 1954. Investigations of the microflora of the oil fields of second Baku. Mikrobiologiya 23:3-14.

Elkins, L. F. 1969. Internal anatomy of a tight fractured Hunton lime reservoir revealed by performance-west Edmond field. J. Petrol. Technol. 21:221-232.

Etris, E. L., D. S. Brumfield, R. Ehrlich, and S. J. Crabtree, Jr. 1988. Relations between pores, throats and permeability: a petrographic/physical analysis of some carbonate grainstones and packstones. Carbonates & Evaporites 3:17-32.

Felsenthal, M., and H. H. Ferrell. 1972. Fluid flow in carbonate reservoirs, p. 83-142. In G. V. Chilingar, R. W. Mannon, and H. H. Rieke, III (ed.), Oil and Gas Production from Carbonate Rocks. American Elsevier Publishing Co., New York.

Fiala, G., and K. O. Stetter. 1986. Pyrococcus furiosus sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100° C. Arch. Microbiol. 145:56-60.

Finnerty, W. R., and M. E. Singer. 1983. Microbial enhancement of oil recovery. Bio/Technology 1:47-54.

Folk, R. L. 1959. Practical petrographic classification of limestones. Bullet. Amer. Assoc. Petrol. Geolog. 43:1-38.

Folk, R. L. 1962. Spectral subdivision of limestone types, p. 62-84. In W. E. Ham (ed.), Classification of Carbonate Rocks-a Symposium. Memoir 1, Amer. Assoc. Petrol. Geolog., Tulsa.

Forbes, A.D. 1980. Micro-organisms in oil recovery, p. 169-180. In D. E. F. Harrison, I. J. Higgins, and R. Watkinson (ed.), Hydrocarbons in Biotechnology. Institute of Petroleum, London.

Friedman, G. M., K. Ruzyla, and A. Reckman. 1980. Effects of Porosity Type, Pore Geometry, and Diagenetic History on Tertiary Recovery of Petroleum from Carbonate Reservoirs. NTIS, Springfield, Virginia.

Ghiorse, W. C., and J. T. Wilson. 1988. Microbial ecology of the terrestrial subsurface. Adv. Appl. Microbiol. 33:107-173.

Ginsburg-Karagicheva, T. L. 1933. Microflora of oil waters and oil-bearing formations and biochemical processes caused by it. Bullet. Amer. Assoc. Petrol. Geolog. 17:52-65.

Gogarty, 1978. Micellar/polymer flooding-an overview. J. Petrol. Technol. 30:1715-1716.

Goldburg, A., and H. Price. 1980. Selection of Reservoirs Amenable to Micellar Flooding. NTIS, Springfield, Virginia.

Gottschalk, G. 1986. Bacterial Metabolism, 2nd ed. Springer-Verlag, New York.

Harbaugh, J. W. 1967. Carbonate oil reservoirs, p. 349-398. In G. V. Chilingar, H. J. Bissell, and R. W. Fairbridge (ed.), Carbonate Rocks. Elsevier Publishing Co., New York.

Henry, R. L., G. L. Feather, L. R. Smith, and D. D. Fussell. 1981. Utilization of composition observation wells in a west Texas CO_2 pilot flood. Proceedings of the 1981 SPE/DOE Second Joint Symposium on Enhanced Oil Recovery. Society of Petrol. Engin., Dallas.

Hitzman, D. O. 1983. Petroleum microbiology and the history of its role in enhanced oil recovery, p. 162-218. In E. C. Donaldson and J. B. Clark (ed.), Proceedings, 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS, Springfield, Virginia.

Hitzman, D. O. 1988. Review of microbial enhanced oil recovery field tests, p. VI-1 - VI-41. In T. E. Burchfield and R. S. Bryant (ed.), Proceedings of the Symposium on Applications of Microorganisms to Petroleum Technology. NTIS, Springfield, Virginia.

Huber, R., J. K. Kristjansson, and K. O. Stetter. 1987. Pyrobaculum gen. nov., a new genus of neutrophilic, rod-shaped archaebacteria from continental solfataras growing optimally at 100° C. Arch. Microbiol. 149:95-101.

Huber, R., M. Kurr, H. W. Jannasch, and K. O. Stetter. 1989. A novel group of abyssal methanogenic archaebacteria (Methanopyrus) growing at 110° C. Nature 342:833-834.

Javaheri, M., G. E. Jenneman, M. J. McInerney, and R. M. Knapp. 1985. Anaerobic production of biosurfactant by Bacillus licheniformis JF-2. Appl. Environ. Microbiol. 50:698-700.

Javor, B. 1989. Hypersaline Environments. Springer-Verlag, New York.

Jenneman, G. E. 1989. The potential for in-situ microbial applications. Devel. Petrol. Sci. 22:37-74.

Jenneman, G. E., R. M. Knapp, M. J. McInerney, D. E. Menzie, and D. E. Revus. 1984. Experimental studies of in situ microbial enhanced oil recovery. Soc. Petrol. Engin. J. 24:33-37.

Jenneman, G. E., M. J. McInerney, and R. M. Knapp. 1985. Microbial penetration through nutrient saturated Berea sandstone. Appl. Environ. Microbiol. 50:383-391.

Jenneman, G. E., M. J. McInerney, R. M. Knapp, J. B. Clark, J. M. Feero, D. E. Revus, and D. E. Menzie. 1983. A halotolerant, biosurfactant-producing Bacillus species potentially useful for enhanced oil recovery. Dev. Indust. Microbiol. 24:485-492.

Jodry, R. L. 1972. Pore geometry of carbonate rocks (basic geologic concepts), p. 35-82. In G. V. Chilingar, R. W. Mannon, and H. H. Rieke, III (ed.), Oil and Gas Production from Carbonate Rocks. American Elsevier Publishing Co., New York.

Johnson, A. C. 1979. Microbial oil release technique for enhanced recovery, p. 30-34. In Conference on Microbial Processes Useful in Enhanced Oil Recovery. NTIS, Springfield, Virginia.

Jones, R. E., R. E. Beeman, and J. M. Suflita. 1989. Anaerobic metabolic processes in the deep terrestrial subsurface. Geomicrobiol. J. 7:117-130.

Jones, D. T., and D. R. Woods. 1986. Acetone-butanol fermentation revisited. Microbiol. Rev. 50:484-524.

Kane, A. V. 1979. Performance review of a large scale CO_2 WAG project, SACROC Unit, Kelly-Snyder Field. J. Petrol. Technol. 31:217-231.

Klein, C., and C. S. Hurlbut, Jr. 1977. Manual of Minerology, 20th ed. John Wiley & Sons, New York.

Krumbein, W. C., and L. L. Sloss. 1963. Stratigraphy and Sedimentation. W. H. Freeman & Co., San Francisco.

Kuznetsov, S. I., M. V. Ivanov, and N. N. Lyalikova. 1963. Introduction to Geological Microbiology, Engl. trans. McGraw-Hill Book Co., Inc., New York.

Lake, L. W. 1989. Enhanced Oil Recovery. Prentice Hall, Englewood Cliffs, New Jersey.

La Moreaux, P. E., B. M. Wilson, and B. A. Memon (ed.). 1984. Guide to the Hydrology of Carbonate Rocks. UNESCO, Paris.

Landes, K. K. 1970. Petroleum Geology of the United States. Wiley-Interscience, New York.

LaRiviere, J. W. M. 1955. The production of surface active compounds by microorganisms and its possible significance in oil recovery. II. On the release of oil from oil-sand mixtures with the aid of sulphate-reducing bacteria. Anton. van Leeuwenhoek J. Microbiol. Serol. 21:9-27.

Lazar, I. 1978. Microbiological methods in secondary oil recovery, p. 279-287. European Symposium on Enhanced Oil Recovery. Heriot-Watt University, Edinburgh.

Lazar, I. 1987. Research on the microbiology of MEOR in Romania, p. 124-151. In J. W. King and D. A. Stevens (ed.), Proceedings of the First International MEOR Workshop. NTIS, Springfield, Virginia.

Lazar, I., S. Dobrota, and M. Stefanescu. 1988. Some considerations concerning nutrient support injected into reservoirs subjected to microbiological treatment, p. XIV-1 - XIV-6. In T. E. Burchfield and R. S. Bryant (ed.), Proceedings of the Symposium on Applications of Microorganisms to Petroleum Technology. NTIS, Sringfield, Virginia.

Marquis, R. E. 1983. Barobiology of deep oil formations, p. 124-128. In E. C. Donaldson and J. B. Clark (ed.), Proceedings, 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS, Springfield, Virginia.

Marquis, R. E., and P. Matsumura. 1978. Microbial life under pressure, p. 105-158. In D. J. Kushner (ed.), Microbial Life in Extreme Environments. Academic Press, London.

McInerney, M. J. 1983. Physiological types of microorganisms useful for enhanced oil recovery, p. 38-42. In E. C. Donaldson and J. B. Clark (ed.), Proceedings, 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS, Springfield, Virginia.

McInerney, M. J., M. Javaheri, and D. P. Nagle, Jr. 1990. Properties of the biosurfactant produced by Bacillus licheniformis strain JF-2. J. Indust. Microbiol. 5:95-102.

McInerney, M. J., and D. W. S. Westlake. 1990. Microbially enhanced oil recovery, p. 409-445. In H. L. Ehrlich and C. L. Brierly (ed.), Microbial Mineral Recovery. McGraw-Hill Publishing Co., New York.

Melrose, J. C., and C. F. Brandner. 1974. Role of capillary forces in determining microscopic displacement efficiency for oil recovery by waterflooding. J. Can. Petrol. Technol. 13:54-62.

Moses, V. 1990. Downhole microbially enhanced oil recovery systems. Dev. Indust. Microbiol. 31:143-148.

Moses, V., J. P. Robinson, D. G. Springham, M. J. Brown, M. Foster, J. Hume, C. W. May, T. S. McRoberts, and A. Weston. 1983. Microbial enhancement of oil recovery in North Sea reservoirs: a requirement for anaerobic growth on crude oil, p. 154-161. In E. C. Donaldson and J. B. Clark (ed.), Proceedings, 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS, Springfield, Virginia.

Myers, G. E., and R. G. L. McCready. 1966. Bacteria can penetrate rock. Can. J. Microbiol. 12:477-484.

Myers, G. E., and W. D. Samiroden. 1967. Bacterial penetration in petroliferous rock. Producers Monthly 31:22-25.

National Petroleum Council. 1976. EOR: An Analysis of the Potential for Enhanced Oil Recovery from Known Fields in the United States--1976-2000. National Petroleum Council, Washington, D.C.

National Petroleum Council. 1984. Enhanced Oil Recovery. National Petroleum Council, Washington, D.C.

Northrop, D. A., and L. W. Teufel. 1990. In situ stress and fracture permeability: a cooperative DOE-industry research program, p. 59-61. In J. A. Wampler, M. Singer, J. D. Batchelor, A. Hartstein, J. J. Stosur, T. C. Wesson, R. M. Ray, F. W. Burtch, and H. A. Teidemann (ed.), Contracts for Field Projects and Supporting Research on Enhanced Oil Recovery, Progress Review no. 60. NTIS, Springfield, Virginia.

Novelli, G. D., and C. E. ZoBell. 1944. Assimilation of petroleum hydrocarbons by sulfate-reducing bacteria. J. Bacteriol. 47:447-448.

Olsen, D. K., M. D. Hicks, B. G. Hurd, A. A. Sinnokrot, and C. N. Sweigart. 1990. Design of a novel flooding system for an oil-wet central Texas carbonate reservoir, p. 507-516. In Proceedings of the SPE/DOE Seventh Symposium on Enhanced Oil Recovery. Soc. Petrol. Engin., Richardson, Texas.

Olson, G. J., W. S. Dockins, G. A. McFeters, and W. P. Iverson. 1981. Sulfate-reducing and methanogenic bacteria from deep aquifers in Montana. Geomicrobiol. J. 2:327-340.

Oppenheimer, C. H., and F. K. Heibert. 1988. Microbial enhanced oil production field tests in Texas, p. XII-1 - XII-15. In T. E. Burchfield and R. S. Bryant (ed.), Proceedings of the Symposium on Applications of Microorganisms to Petroleum Technology. NTIS, Springfield, Virginia.

Orr, Jr., F. M., and J. J. Tabor. 1984. Use of carbon dioxide in enhanced oil recovery. Science 244:563-569.

Pickell, J. J., B. F. Swanson, and W. B. Hickman. 1966. Application of air-mercury and air-oil capillary pressure data in the study of pore structure and fluid distribution. Soc. Petrol. Engin. J. 6:55-61.

Postgate, J. R. 1984. The Sulfate-Reducing Bacteria, 2nd ed. Cambridge University Press, New York.

Raiders, R. A., R. M. Knapp, and M. J. McInerney. 1989. Microbial selective plugging and enhanced oil recovery. J. Indust. Microbiol. 4:215-230.

Raleigh, J. T., and D. L. Flock. 1965. A study of formation plugging with bacteria. J. Petrol. Technol. 17:201-206.

Rieke, III, H. H., R. W. Mannon, G. V. Chilingar, and G. L. Langnes. 1972. Classification and performance of carbonate reservoirs, p. 255-308. In G. V. Chilingar, R. W. Mannon, and H. H. Rieke, III (ed.), Oil and Gas Production from Carbonate Rocks. American Elsevier Publishing Co., New York.

Rodriguez-Valera, F. (ed.). 1988. Halophilic Bacteria, vol. I. CRC Press, Boca Raton.

Roehl, P. O., and P. W. Choquette (ed.). 1985. Carbonate Petroleum Reservoirs. Springer-Verlag, New York.

Rowe, H. G., S. D. York, and J. C. Ader. 1982. Slaughter estate unit tertary pilot performance. J. Petrol. Technol. 34:613-620.

Ruzyla, K., and G. M. Friedman. 1982. Geological heterogeneities important to future enhanced recovery in carbonate reservoirs of the Upper Ordovician Red River formation at Cabin Creek Field, Montana. Soc. Petrol. Engin. J. 22:429-444.

Schneider, F. N., and W. W. Owens. 1976. Relative permeability studies of gas-water flow following solvent injection in carbonate rocks. Trans. AIME 276:23-30.

Scoffin, T. P. 1987. An Introduction to Carbonate Sediments and Rocks. Chapman & Hall, New York.

Stetter, K. O., H. Konig, and E. Stackebrandt. 1983. Pyrodictium gen. nov., a new genus of submarine disc-shaped sulfur reducing archaebacteria growing optimally at 105° C. Syst. Appl. Microbiol. 4:535-551.

Stout, J. L. 1964. Pore geometry as related to carbonate stratigraphic traps. Bullet. Amer. Assoc. Petrol. Geolog. 48:329-337.

Thomeer, J. H. M. 1960. Introduction of a pore geometrical factor defined by the capillary pressure curve. J. Petrol. Technol. 12:73-77.

Townsend, W. R., S. A. Becker, and C. W. Smith. 1977. Polymer use in calcareous formations, paper no. 6382. In Permian Basin Oil and Gas Recovery Conference of the Society of Petroleum Engineers of AIME. Soc. Petrol. Engin., Dallas.

Ulmer, D. C., A. R. Moreira, and J. C. Linden. 1983. The acetone-butanol fermentation of Clostridium acetobutylicum, p. 141-149. In J. E. Zajic, D. G. Cooper, T. R. Jack, and N. Kosaric (ed.), Microbial Enhanced Oil Recovery. Penn Well Publishing Co., Tulsa.

Updegraff, D. M. 1983. Plugging and penetration of petroleum reservoir rock by microorganisms, p. 80-85. In E. C. Donaldson and J. B. Clark (ed.), Proceedings, 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS, Springfield, Virginia.

Updegraff, D. M. 1990. Early research on microbial enhanced oil recovery. Dev. Indust. Microbiol. 31:135-142.

Updegraff, D. M., and G. B. Wren. 1954. The release of oil from petroleum-bearing materials by sulfate-reducing bacteria. Appl. Microbiol. 2:309-322.

Vreeland, R. H. 1987. Mechanisms of halotolerance in microorganisms. CRC Crit. Rev. Microbiol. 14:311-356.

Wardlaw, N. C. 1976. Pore geometry of carbonate rocks as revealed by pore casts and capillary pressure. Amer. Assoc. Petrol. Geolog. Bullet. 60:245-257.

Yarbrough, H. F. 1987. Laboratory studies of mechanisms of microbial oil release, p. 214-220. In J. W. King and D. A. Stevens (ed.), Proceedings of the First International MEOR Workshop. NTIS, Springfield, Virginia.

Yarbrough, H. F., and V. F. Coty. 1983. Microbially enhanced oil recovery from the upper Cretaceous Nacatoch formation, Union County, Arkansas, p. 149-153. In E. C. Donaldson and J. B. Clark (ed.), Proceedings, 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS, Springfield, Virginia.

Yen, T. F. (ed.). 1990. Microbial Enhanced Oil Recovery: Principle and Practice. CRC Press, Boca Raton.

Yu, L., and N. C. Wardlaw. 1986. The influence of wettability and critical pore-throat size ration on snap-off. J. Colloid. Interface Sci. 109:461-472.

Zehnder, A. J. B. (ed.). 1988. Biology of Anaerobic Microorganisms. John Wiley & Sons, New York.

Zillig, W., I. Holz, D. Janekovic, H.-P. Klenk, E. Imsel, J. Trent, S. Wunderl, V. H. Forjaz, R. Coutinho, and T. Ferreira. 1990. Hyperthermus butylicus, a hyperthermophilic sulfur-reducing archaebacterium that ferments peptides. J. Bacteriol. 172:3959-3965.

ZoBell, C. E. 1946. Bacteriological processes for treatment of fluid-bearing earth formations. U.S. Patent no. 2,413,278.

ZoBell, C. E. 1947. Bacterial release of oil from oil-bearing materials. World Oil 126:36-47.

ZoBell, C. E. 1958. Ecology of sulfate reducing bacteria. Producers Monthly 22:16-29.

Potential of Microbial Enhanced Oil Recovery (MEOR) in the Petroleum Reservoirs of the Mid-Continent Region

by

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Abstract

Mid-Continent reservoir rock and oil properties were studied to evaluate the potential for applying microbial enhanced oil recovery (MEOR) processes. The study included a computer search using Dwight's Energydata TOTL database system, literature search and core data from a number of Mid-Continent sandstone and carbonate reservoir rocks. The MEOR screening parameters included reservoir permeability, temperature, brine composition, oil API gravity, oil viscosity and residual oil saturation. Comparison of the Mid-Continent reservoir rock and oil properties to screening criteria developed from those reported in the literature showed that many oil reservoirs are suitable candidates and could benefit from some form of microbial treatment. A review of a number of field tests of microbial enhanced oil recovery processes showed that oil production rates increased by as much as 200% depending on reservoir and bacterial characteristics. Properly administered bacterial treatments hold promise for fields with marginal production and for which high cost EOR methods may not be economical.

Introduction

Production of oil has been an important economic factor in the Mid-Continent region and the United States as a whole for over a century. According to American Petroleum Institute, the Mid-Continent states contain about 33.4 % of the remaining-oil-in-place (ROIP) in the Lower-48 United States and about 34.9% of U.S. production in 1988 (Table 1)[1].

However, a recent study[2] of the abandonment rates for oil producing reservoirs in the Lower 48 United States concluded that about 67% of the current proven reserves may be abandoned after production by conventional (primary and secondary recovery) methods because of oil trapped or bypassed due fluid properties and/or rock heterogeneities within the reservoir. Even producing a small part of this unrecovered oil resource will require favorable oil prices, technological advances in the characterization of oil reservoirs and novel cost-effective enhanced oil recovery processes.

Costly EOR technologies are not economical for many Mid-Continent oil-producing fields. Marginal fields account for about 32% of the total Mid-Continent oil production and cannot support expensive EOR research or investments. As shown in Table 2, some Mid Continent states have an very high percentage of stripper well oil production. Stripper well oil production in Kansas (98.1%) is the highest nationally. Other states that have substantial stripper well oil production include Oklahoma (85%), Nebraska (44.9%), Arkansas (36%), New Mexico (20.2%) and Texas (19%). In general, Oklahoma accounts for about 25% of total US stripper well oil

production, followed by KS (13%), and Texas (9%). Altogether, the Mid-Continent states accounted for 52.1 % of total US stripper well oil production in 1989.

MEOR applications do not involve high investment in surface equipment as do some other chemical flooding applications. Because of their cost-effective potential, MEOR applications may be applicable in many oil fields of the Mid-Continent.

MEOR Applications

MEOR has been identified as a collection of potential EOR technologies. Different types of microbial reservoir treatments exist (Table 3) and their application should depend on the oil production problem to be mitigated.

Bypassing of mobile oil due to reservoir heterogeneity may require selective plugging[7]. Recovery inefficiency due to unfavorable mobility will require viscosity modification of the waterflood by polymer producing microorganisms, and oil trapping due to capillary forces will require solvent/surfactant producing microorganisms in order to reverse the rock wettability and reduce the interfacial tension. Research at the University of Oklahoma is showing that improvement of reservoir pore properties may occur when carbonate rocks are treated with acid- and CO_2- producing microorganisms because of the dissolution of the carbonate matrix.

Table 3 summarises different types of microbial reservoir treatments. Field MEOR applications are relatively few but are increasing. Bryant et al.[3] reported that the number of MEOR field projects in the NIPER database has increased from 39 in 1987 to 65

in 1990. Well stimulation and cleanout treatments are the most common while results from well-controlled field trials are sparse except for a recent report on the use of in situ ultramicrobacteria to increase well productivity[18]. A few reported cases have shown production increases from 50 to 300% and a decrease of BS&W (bottom sediment and water).

Microbially enhanced waterflooding projects are fewer in number. The best documented project was performed in a calcareous sandstone unit in Union County, Arkansas[4,5]. The reservoir was 610 m (2000 feet) deep and had an average temperature of 35.5°C (96.7°F). The field trial injected the bacterial strain **Clostridium acetobutylicum** and beet molasses. Oil production was increased by 250% and was limited only by the initial low residual oil saturation. The observed microbial activity was the result of indigenous bacteria rather than the injected strain. Several successful field trials have been reported in Europe[5,6]. Some of these field trials were also in calcareous sandstone or carbonate reservoirs which relied on microbial acid, gas and possibly solvent production to enhance oil recovery. Recent data suggest that the incremental cost was around three dollars per barrel of additional oil recovered suggesting that the process is economical. The University of Oklahoma is currently carrying out a field trial of a microbial selective plugging process in the South-East Vassar Vertz Sand Unit, Payne County, Oklahoma.

Screening Parameters and Criteria for MEOR Treatment

Activity of microorganisms in petroleum reservoirs will be governed by the reservoir's environmental conditions. These factors were recently reviewed by McInerney et al[8] and Jenneman[9]. The most important factors that might limit microbial activity in reservoirs are permeability of the rock, reservoir physico-chemical conditions of temperature, pressure, brine salinity and pH, and oil properties such as oil viscosity and API gravity.

The limiting criteria as proposed by Clark et al[10] were:

Salinity < 10%
pH between 4 and 9
Permeability,md > 75 md
API gravity > 17°
Temperature < 75°C (167°F)

A recent paper by Nelson and Launt[11] on microbial well stimulation gave the characteristics of candidate stripper wells as follows:

Oil Production up to 20 b/d
Temperature ~ 20 - 60°C (85 - 140°F)
API gravity > 20°
Porosity > 3 %
Permeability > 20 md
Depth ~ 1,000 m (3200 ft)

An more recent but unpublished report[12] by the University of Oklahoma on the potential of MEOR from carbonate reservoirs showed the following criteria:

Salinity < 25 %
Permeability,md > 15 md
API > 17°
Temperature < 82° C (180°F)

Bryant[2] also reviewed screening criteria for MEOR processes and

a reported number of treated reservoirs. In the Mid-Continent region, it is reported that 44% of reservoirs in Kansas, 14% in Oklahoma and 25% in Texas are treatable. The method for reaching this conclusion was not discussed.

A survey of the factors that could limit MEOR applications in Mid-Continent reservoirs was made using Dwight's Energydata TOTL database system. In addition,the physicochemical environment and petrophysics of six Mid-Continent formations were studied and reported. These formations and (their ages) are Bartlesville (Pennsylvanian),Red Fork (Pennsylvanian), Oil Creek (Ordovician), Viola limestone (Ordovician), Springer (Pennsylvanian) and Vassar (Pennsylvanian) sands. The factors that were reviewed are reservoir temperature and pressure, brine chemistry, oil properties and rock properties.

Reservoir temperature and Pressure

Microbial growth is affected by temperature and can be predicted by the following relationship[13]:

$$\log_{10} K = -\frac{\tau}{2.303T} + C$$

where K = growth rate

τ = constant for given bacteria (an energy term)

T = temperature in degree Kelvin

C = constant

Usually there is an optimal temperature where the maximum rate of growth occurs and beyond which microbial growth diminishes rapidly. Each bacterial species has its own characteristic optimal growth temperature. There is a growing list of thermophilic microorganisms that have optimal temperatures of 100°C or higher. Thus, it cannot be assumed that even hot reservoirs are devoid of microbial activity and cannot be potential target for MEOR.

The indigenous microbial population within a reservoir are capable of withstanding its physical environment. The growth and metabolism of these indigenous microorganisms can be controlled by altering the availability and concentration of certain nutrients such as the main carbon/energy source, electron acceptor, nitrogen, sulfur, phosphorous sources etc. This approach seems to be a more practical one than injection of a specific microorganism due to the following reasons:

(a) useful bacteria are present that are already selected for growth under the prevailing environmental conditions of the reservoir;

(b) cell injection requires the development of specialized strains for each new reservoir to be treated and will increase the development costs;

(c) porous media acts as a filter to remove microorganisms from the flowing phase making cell injection difficult and, at best, localized to regions near the wellbore;

(d) normal homeostatic mechanisms present in any established microbial community will act to prevent the establishment of a newly introduced species. In fact, the results of some field trials

clearly show that the observed microbial activity was the result of indigenous rather than the injected microorganisms.

Even though it is known that microorganisms can grow at temperatures above 100°C, little is known about the factors that control their metabolism. Because of this, Jenneman[9] determined that a practical temperature limit for in-situ MEOR was 75°C (167°F). However, a successful field trial in Hungary has been reported in a reservoir with a temperature of 97° C[5]. With increased understanding of the physiology of thermophilic microorganisms, the temperature limit is being raised to at least 82°C(180°F) as one limit for identifying potential Mid-Continent reservoirs suitable for MEOR. This value is not an absolutely limiting temperature in itself, but it includes organisms that have well-studied physiologies[12].

Bubela[14] reported that a pressure change from atmospheric to 20,000 KPa (2,870 psi) increased the optimal growth temperature of a microorganism from 55°C to 65°C and decreased its doubling time from 17 to 12 hours. In general, pressure increases up to 20,000 KPa have little or no effect on bacterial activity; however, pressures exceeding 50,000 KPa (7,175 psi) can be inhibitory to microbial activity[14]. It follows therefore that in most reservoirs, particularly in the Mid-Continent region, pressures will not be great enough to inhibit microbial growth.

Table 4 shows the number of current oil-producing reservoirs with temperature records, statewide average reservoir temperatures, and the percentage of current oil-producing reservoirs having temperatures less than 82°C (180°F) in the Mid-Continent region and

Colorado. Judging from the available temperature records, this result shows that a majority of Mid-Continent reservoirs have temperatures below the stated temperature limit (180°F).

Brine Chemistry

Oilfields produce brines with sodium chloride content ranging from less than 0.01% to more than 20%. Present emphasis in microbial EOR is either injection of microbial cultures with their nutrients into reservoirs already undergoing active waterflooding or stimulation of microbial population native to the reservoirs by the injection of necessary nutrients. In the second case, indigenous populations are already selected for growth in the brine present in the reservoir. In the first case, the brine composition must be studied to assure that it is suitable for growth and metabolism of the chosen microbial species and will require the isolation of specific microorganisms for each reservoir condition.

McInerney et al[8], Jenneman[9] and Clark et al.[10] discussed the biochemical parameters which affect microbial growth and metabolism. Clark et al.[10] proposed that the NaCl content should be less than 10% and the brine pH between 4 and 9. In a recent study, Tanner et al.[12] showed that there are several halophilic or halotolerant microbial systems that are active in environments with brine salinity as high as 25% sodium chloride.

According to Jenneman[9],the pH values in most petroleum reservoirs are within the range of growth of most known micro-organisms. In addition, carbonate reservoirs provide a natural buffering environment which prevents reduction of pH by microbial

acids to inhibitory levels.

A survey of the percentage of Mid-Continent reservoirs having total dissolved solids content less than 20% is shown on Table 5. It can been seen that Kansas has the highest percentage of such reservoirs (99%) while Texas Regulatory District 9 has the smallest number (35.3%).

Oil Properties

The presence of oil itself could be another factor that limits microbial activity in oil reservoirs. Limitations imposed upon MEOR by the petroleum phase include toxicity of the light volatile fractions and difficulty in recovering heavy oils because of unfavorable mobility ratios between the brine and the petroleum. Reservoirs whose crude oil has an API gravity of less than 17° and viscosity greater than 40 cp are probably not good candidates for MEOR processes.

Table 6 shows the statewide average API gravity and oil viscosity values for the Mid-Continent region and Colorado. Reservoirs in the Kansas and Oklahoma showed the highest percentage of current oil-producing reservoirs with oil API gravity greater than 17° while Nebraska has the lowest.

Reservoir Rock Properties

Permeability, porosity and oil saturation limit the applicability of bacterial treatments in reservoirs. Prior investigations show that permeability and porosity not only limit

injectivity and transport of microbes through the porous medium but also affect microbial growth and metabolism[15].

Porosities found in commercial reservoirs do not normally impose any restrictions on microbial enhanced oil recovery. Average porosity values for commercial reservoirs vary from about 8% to over 20%.

In microbially enhanced waterflooding, the one of most critical tests to determine whether a given reservoir is suitable for MEOR is the injectivity of the applicable bacterial strains and/or nutrients. Injectivity is controlled by permeability which is characterized by the size of the largest pore openings. Bacteria have many morphologies (e.g. rods, cocci, curved rods, tetrads, chains, et c.) with dimensions of length from 0.5 to 10 microns and diameters from 0.5 to 2 microns. Many bacteria can produce ultramicrocellular forms with cell sizes around 0.1 micron or less which may facilitate their injection into tight formations. However, Jenneman[9] reasoned that pore openings of less than 0.5 microns will place restrictions on the ability of microbes to enter and move within the reservoirs.

Figure 1 shows the relationships between entry pore throat sizes and absolute permeability for sandstones and carbonates with different degrees of cementation. Absolute permeability was measured on rock core plugs with a gas permeameter while the pore throat sizes were determined from capillary pressure data measured with Beckman L5-50B ultracentrifuge apparatus. The rocks used to establish these relationships were Red Fork, Oil Creek, Vertz and Berea sandstones, and Viola, Bethany Falls and Springer limestones.

This analysis shows that carbonates with low absolute permeability values may have entry pore throat sizes one or more orders of magnitude larger than sandstones of comparable permeability. The implication is that carbonates with low permeability values may still be candidates for MEOR which means that the permeability limit for carbonates should be lower than that for sandstones.

Jenneman[9] suggested a limit of 75 md for sandstone reservoirs. However, a more recent work based on field applications of MEOR suggested a permeability limit of 20 md for wells undergoing microbial stimulation[11]. On-going work on carbonate reservoirs showed a limit of 15 md but microbial penetration into rocks of permeability less than 0.1 md has been reported[12,16]. The distribution of average absolute permeability values and the number of reservoirs having permeabilities greater than 75 md for sandstones and 15 md for carbonates are shown in Table 7 for Mid-Continent reservoirs and Colorado. Kansas showed the highest percentage of reservoirs in the Mid-Continent region with absolute permeabilities exceeding the stated limits (88.5%) while Nebraska showed the smallest percentage (0.5%).

Table 8 shows the average values of porosity and permeability in the selected oil-producing formations. The Springer limestone has the lowest porosity (2%) and absolute permeability (0.1 md). However, the Springer is known to be fractured and oil storage and production is believed to occur in and through the fractures. The Oil Creek and Vertz sandstones exhibit very good reservoir properties. An MEOR field trial has been carried out in the Bartlesville sand by NIPER[17].

Oil Reservoirs Treatable by MEOR in the Mid-Continent Region

From the discussions above, the following criteria were chosen to determine the number of reservoirs suitable for MEOR:

Permeability, md	> 75 md (for sandstones)[9]
	> 15 md (for carbonates)[12]
NaCl content	< 200,000 ppm
pH bewteen	4 - 9
Temperature	< 82°C (180°F)[12]
Oil gravity	> 17°API

Table 9 shows the percentage of Mid-Continent oil-producing reservoirs treatable by MEOR processes. This result is obtained by treating each screening parameter as an independent variable and is subject to any limitations in the statistical data used its computation. The basic probability principle applied states that "the probability of occurrence of an event at a certain extent is proportional to the product of the probabilities of the factors causing the event". Hence, the percentage of treatable reservoirs within a given state or Regulatory District can be written as:

$$T,\% = \Pi^{n}_{1} f_i$$

where, Π is a multiplication convention,

f_i is individual factor affecting MEOR application,

n is number of factors.

According to this result, Kansas has the highest percent of currently oil-producing reservoirs in the Mid-Continent region that would be treatable by MEOR. By concidence, Kansas has the highest stripper well oil production to total oil production ratio nationally. The state with the fewest treatable reservoirs is Nebraska(<0.1%).

The surest indicator of the possibility of microbial

applications in reservoirs is the presence of a water phase and if waterflooding as a secondary recovery technique could be implemented. This parameter should rather serve as a "rule of thumb" especially in fields were rock and fluid data are sparse and use of the criteria stated in this paper is not possible.

Conclusions

A significant percentage (31.6%) of oil production from the Mid-Continent region is from marginal fields. Marginal fields may not support high-cost EOR methods, but are well-suited for microbially enhanced oil recovery.

A comparison of the MEOR screening criteria developed for selecting target reservoirs to oil-producing reservoir rock and fluid properties from data in Table 9 shows that many oil reservoirs in the Mid-Continent region are candidates for MEOR and could benefit from some form of microbial treatment.

Table 1: **Mid-Continent (Mid-C.) Proven Crude Oil Reserve and Production in 1988**

State	Proven Crude Oil Reserves (millions barrels)	Crude Oil Production (thousand barrels)
AR	77	13,578
KS	327	58,930
NE	42	5,992
NM	661	71,175
OK	796	128,350
TX	7043	758,865
Total,Mid-C.	8,946	1,036,890
Other States	17,879	1,936,238
Total US	26,825	2,973,128

Table 2 - **Stripper Well Oil Production from Mid-Continent(Mid C.) Reservoirs - 1988 and 1989**[1]

State	Stripper Well Oil Production 1988 (thousand barrels)	1989	% Stripper Oil Prod./Total State(1988)
AR	4,888	6,687	36
KS	57,803	57,051	98
NE	2,688	2,446	45
NM	14,378	14,653	20
OK	109,082	109,440	85
TX	141,704	40,504	19
Total,Mid-C.	330,542	230,782	
Other States	116,294	212,019	
US Total	446,8837	442,800	

[1] Source: Ref. 1.

Table 3 - **MEOR APPLICATIONS**

Type	Stage of Development
Wellbore cleanout	
Paraffin removal	Several commercial ventures[11]
Corrosion/scale prevention	Several commercial ventures[11]
Well stimulation	
Clostridial fermentation (acid/solvent/gases)	Many commercial ventures
Ultramicrobacterial repressurization/drainage improvement	Commercial process[18]
Coning prevention or mitigation	Field demonstration stage
Microbial control of sulfide production	Laboratory investigation
Microbially enhanced waterfloods	
Clostridial fermentation (acid/solvent/gas)	Several succesful field trials[4,5,6]
Biosurfactant production	Several field trials[5,6]
Microbial selective plugging	Field trial stage[19]

Table 4 - **Average Temperatures of Mid-Continent Reservoirs Reported by State**

State/Regulatory District	No. of Oil-producing reservoirs with temperature records	Average temperature °C (°F)	% of reservoirs with T<82°C
CO[a]	82	69 (157)	78
KS	29	35 (95)	100
NE	117	50 (123)	100
NM	134	53 (127)	91
OK	41	80 (177)	48.9
TX-5	27	88 (190)	55.6
TX-6	97	71 (166)	44.3
TX-7B	107	47 (117)	100
TX-8A	140	49 (120)	97.9
TX-9	127	54 (129)	93.7
TX-10	95	60 (140)	86.3

[a] Not in Mid-Continent region. A proximal state added for contrast.

Table 5 - Properties of Brines from Mid-Continent Reservoirs

State/Regulatory District	Average TDS[1] (percent)	% with TDS < 20%	% with pH between 4 and 9
AR	26	36	88.5
CO	8.0	98.8	98.8
KS	8.1	99	98.5
NE	8.5	79	93
NM	10.8	91.6	91.6
OK	16.7	50.1	97
TX-5	11.4[a]	61.1	95.6
TX-6	11.4[a]	56	95.6
TX-7B	11.4[a]	62.5	95.6
TX-8A	11.4[a]	35.3	95.6
TX-9	11.4[a]	35.3	95.6
TX-10	11.4[a]	35.3	95.6

[1] TDS: total dissolved solids
[a] average for all Texas regulatory districts

Table 6 - Distribution of Oil Properties by State

State/Regulatory District (no. of reservoirs)	Average API	% of reservoirs with >17 API	Average Oil viscosity (cp)	Number of reservoirs with μ_o<40 cp
CO(525)	38.5	99.6	12.3	51
KS(9365)	-[a]	99.7	23	142
NE(192)	23	12	6.2	5
NM(835)	41.1	24	5.9	56
OK(8071)	-[a]	99.7	-[a]	-[a]
TX-5(147)	23.2	55.7	5.7	17
TX-6(643)	26.5	64.2	8.7	65
TX-7B(2079)	30.3	73	3.2	119
TX-8A(875)	26.7	89.2	4.4	124
TX-9(1926)	20.8	58.9	3.8	145
TX-10(310)	32.5	77.1	2.4	20

[a] data not available

Table 7 - **Distribution of Reservoir Rock Properties by State**

State/Regulatory District		Rock Type (no. of records)	Permeability (K) Min. (md)	Max.	Avg.	No. of reservoirs with K > 15md (c)[1]	K > 75md (s)[2]
CO	s	(364)	.01	605	244	364	340
	c	(1)	.01	23	10.4	1	0
KS	s	(284)	.06	209	67	274	82
	c	(1738)	.06	1000	109	1708	1629
NE	s	(367)	.03	2237	57	4	2
NM	s	(55)	.01	123	66	55	21
	c	(161)	.01	177	68	144	38
TX-5	s	(56)			841	56	47
	c	(44)			509	43	15
TX-6	s	(250)	.01	1185	538	250	145
	c	(144)	.06	113	52	143	18
TX-7B	s	(146)	.01	496	41	140	7
	c	(192)	.01	168	52	124	4
TX-8A	s	(111)	.01	419	27	49	3
	c	(377)	.01	419	80	222	84
TX-9	s	(473)	.02	241	70	465	152
	c	(43)	.03	391	86	13	16

[1]c: Carbonates
[2]s: Sandstones

Table 8 - **Average Porosity and Permeability Values in Selected Oil-Producing Formations** in the Mid-Continent Region

Formation	Average Porosity,%	Average Permeability,md
Oil Creek	16.6	153.9
Viola[a]	8.5	49
Springer[a]	2.0	0.1
Vertz sand	16.5	210
Red Fork[a]	4.0	1.0
Bartlesville	15.6	31.3

[a] Data from computer search

Table 9 - **Percent of Mid-Continent oil-producing reservoirs potentially treatable by MEOR processes**

State/Regulatory District	Salt <20%	pH 4-9	Permeability ss:<75md;C<15md	°API >17	% Treatable Reservoirs[a]
AR[c]	36	88.5	–[b]	–[b]	–[b]
CO	98.8	98.8	93.4	99	90.3
KS	99	98.5	88.5	99.7	86
MO[c]	100	–[b]	–[b]	50	–[b]
NE	79	93	0.5	12	0.04
NM	91.6	91.6	76.3	24	15.4
OK	50.1	97	31	99.7[c]	15.1
TX-5	61.1	95.6[d]	90	55.7	24
TX-6	56	95.6[d]	64	64.2	24
TX-7B	62.5	95.6[d]	41.2	73	16
TX-8A	35.3	95.6[d]	46.1	89.2	24
TX-9	35.3	95.6[d]	37.6	58.9	7.5
TX-10	36.3	95.6[d]	38	77.1	10.2

[a] % treatable reservoirs calculated by assuming each parameter as an independent variable.
[b] insufficient data for calculation
[c] Ref. 9
[d] average of all Texas regulatory districts is used.

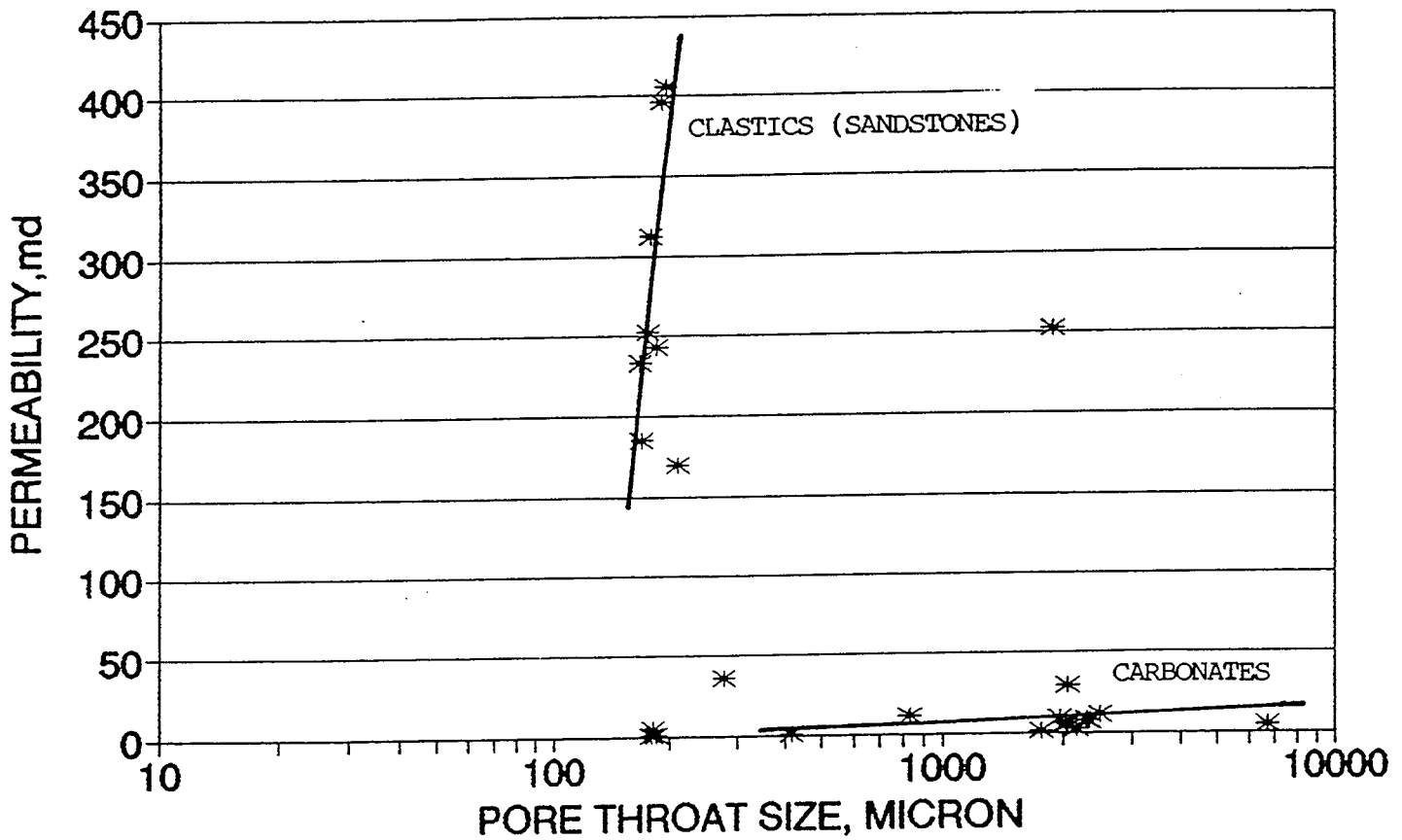


Figure 1 - Variation of permeabilities with pore throat sizes in reservoir rocks

References

1. ---,1990. Basic Petroleum Data Book,Petroleum Industry Statistics: American Petroleum Institute, Volume X,No. 2, Washington D.C.
2. ---,1990. Abandonment Rates of the Known Domestic Oil Resource: U.S.Department of Energy Report No. DOE/BC--89/6/SP,39p.
3. Bryant,Rebecca S.,1990. Screening Criteria for Microbial EOR Processes: NIPER Report for U.S. DOE No. NIPER-478,17p.
4. Yarborough H.F.; and Coty V.F.,1982,Microbially Enhanced Oil Recovery from the Upper Cretaceous Nacatoch Formation, Union County, Arkansas: Proc. International Conf. on Microbial Enhanced Oil Recovery, Afton, OK. (May 16-21), DOE Report No. Conf-8205140, pp.149-153.
5. Hitzman D.O., 1988. Review of Microbial Enhanced Oil Recovery Field Tests: Proc. Symposium on Applications of microorganisms to Petroleum Technology, US DOE Report No. NIPER-351, (September).
6. Lazar,I.,1990. Preliminary results of some recent MEOR field trials in Romania, In: Proceedings of the International Conf. on MEOR, May 27- June 1,1990, Norman, Oklahoma.
7. Jenneman,G.E.;Knapp R.M.;Menzie,D.E.; McInerney,M.J.; Revus,D.E.;Clark,J.B.; and Munnecke,D.M., 1983. Transport and plugging in Berea sandstone using microorganisms: In E.C. Donaldson & J.B. Clark (eds): Proceedings of the 1982 International Symposium on MEOR, Dept. of Energy, Bartlesville, Oklahoma,71 -75.

8. McInerney,M.J. and Westlake D.W.S., 1990. Microbially enhanced oil recovery,In: H.L. Ehrlich and C.L. Brierly (eds), Microbial Mineral Recovery, McGraw-Hill Publishing Co., N.Y., pp. 409 - 495.

9. Jenneman G.E. 1989. "The potential for in-situ Microbial applications" , Chapter 3 in Microbial Enhanced Oil Recovery, E.C. Donaldson, G.V. Chiligarian and T.F. Yen, eds. Elservier Books, NY, pp. 38-74.

10. Clark,J.B., D.M. Munnecke, and G.E. Jenneman. 1981. In situ microbial enhancement of oil production. Devel. Indust. Microbiol. 22: 695-701.

11. Nelson S.J.; and Launt, P.D., 1991. Stripper well production increased with MEOR treatment: Oil and Gas J.,(March),pp. 114- 118.

12. Tanner,R.S,;Knapp,R.M.;McInerney,M.J.;Udegbunam,E.O. and Adkins J.P., 1991. Review on MEOR from Carbonate Reservoirs: Unpublished Report being prepared for U.S. DOE.

13. Stainer,R.Y.; Ingraham,J.L.; Wheelis,M.L. and Painter, P.R.,1986. Microbial World:Prentice-Hall, Englewood Cliffs, NJ,689pp.

14. Bubela, B.; and McKay B.A., 1985. Assessment of Oil Reservoirs for Microbiological Enhanced Oil Recovery-Microbes and Oil Recovery: International Bioresources Journal,vol. 1,pp 99-107.

15. Zvyagintsev,D.G. and A.P. Pitryuk, 1973. "Growth of micro-organisms in capillaries of various sizes under continous flow and static conditions", Mikrobiologiya, Vol.42, pp.60-

64.

16. Meyers G.E.and R.J.L. McCready, 1966. Bacteria can penetrate rocks :Canadian J. of Microbiology, vol. 12, pp. 477-484.

17. Bryant,R.S.;Burchfield,T.E.;Dennis D.M. and Hitzman,D.O., 1988. Microbial-Enhanced Waterflooding: Mink Unit Project: Paper SPE/DOE 17341 presented at the SPE/DOE EOR Symposium, Tulsa, Oklahoma, April 17-20.

18. Sheehy,A.J., 1990. Field studies of microbial EOR: Paper SPE/DOE 20254 presented at the SPE/DOE seventh symposium on Enhanced Oil Recovery, Tulsa, Oklahoma,April 22-25.

19. Knapp,R.M., M.J. McInerney, D.E. Menzie and J.L. Chisolm, 1989. Microbial Field Pilot Study: Annual Report performed under DOE contract no. DE-FG22-89BC14246 at University of Oklahoma, Norman, DOE/BC/14246-5,96pp.

C. MICROBIAL ECOLOGY OF CARBONATE RESERVOIRS

A. Introduction

The microbial ecology of oil reservoirs, as that of all deep subterranean environments, is poorly understood. Early research in this area, summarized by Davis (1963) and Kuznetsov et al. (1963), does not address the basic question regarding MEOR in carbonate reservoirs: is there a microbial population extant in the reservoir with enough metabolic diversity which can be successfully stimulated for in situ MEOR by the injection of appropriate nutrients? Conversely, do microorganisms have to be introduced into the reservoir for successful MEOR? Beyond a general perception that sulfate-reducing bacteria (SRB) are present in most reservoir environments (Postgate, 1984), little of the earlier work in reservoir ecology is useful in answering this question. Only recently has research been done on the microbiology of deep aquifers (Jones et al., 1989) that incorporates techniques for "clean" sampling (Phelps et al., 1989) and the cultivation of strictly anaerobic bacteria (Balch et al., 1989; Balch and Wolfe, 1976). In that study, evidence for diverse microbial populations capable of in situ metabolism was consistently obtained through a sampled depth of 150 meters below the surface (Jones et al., 1989). The purpose of our research was to determine if carbonate reservoirs have microbial populations capable of sustaining in situ MEOR.

B. Materials and Methods

1. Media and Culture Techniques. The basal medium used for these studies is given in Table 1. Composition of the solutions used to prepare this medium, minerals, trace metals, and vitamins, are given in Table 2, 3, and 4, respectively. Specific groups of microorganisms were enumerated by amending this medium as described below.

Aerobes were cultured in 16 x 150 mm screw cap tubes containing 5 ml of medium. Most anaerobes were cultured in Hungate-type anaerobic culture tubes (Bellco, #2047-16125). The more fastidious anaerobes (some SRB, iron reducers, acetogens, and methanogens) were cultured in aluminum seal anaerobic culture tubes (Bellco, #2048-18150). Anaerobic cultures were prepared and manipulated using strict anaerobic technique (Balch et al., 1979; Balch and Wolfe, 1976). Media for acetogens and methanogens were pre-reduced by adding cysteine and sulfide to a final concentration of 1.1 and 0.8 mM, respectively.

General heterotrophs were cultured using plate count broth (Difco) at half (8 g/L) the recommended concentration. Glucose-using heterotrophs were enumerated using the basal medium with 1 or 2 g/L glucose for aerobes or anaerobes, respectively. Molasses has been frequently used as a carbon/energy source in MEOR. Cattle-feed molasses was used at 2 g/L (solids) in these studies. Sucrose, the major carbohydrate in molasses, was added at 5 g/L; a higher concentration of this substrate was used to permit the detection of extracellular polysaccharide. Nitrate was added to the basal medium at a final concentration of 9.9 mM to select

Table 1. Basal Microbiological Medium.[a]

Component	Per Liter
Minerals solution[b]	20 ml
Trace Metals solution[c]	5 ml
Vitamins solution[d]	10 ml
Buffer; adjust pH with NaOH[e]	

[a]A general purpose microbiological medium adaptable to pure culture or ecological studies with appropriate selection of carbon sources, energy sources, and electron acceptors. For most ecological studies, yeast extract (0.2 g/L) was added to enhance recovery of cells. For study of the microbial ecology of carbonate reservoirs, $CaCO_3$ (precipitated; 0.2 g/L) was added.

[b]Minerals solution described in Table 2.

[c]Trace Metals solution described in Table 3.

[d]Vitamins solution described in Table 4.

[e]The buffer varied depending on the type of medium used: TES or PIPES for neutral pH's; $NaHCO_3$ for CO_2-containing gas phases; MES for acidic culture; phosphate for lactic acid bacteria or thiobacilli. Final medium pH was generally 7.0-7.5; the pH was adjusted accordingly for each medium.

Table 2. Minerals Solution.[a]

Component	Grams per Liter
$NaCl$	40
NH_4Cl	50
KCl	5
KH_2PO_4	5
$MgSO_4 \cdot 7H_2O$	10
$CaCl_2 \cdot 2H_2O$	1

[a]A solution containing the major minerals required for microbial growth. Additional phosphate will be required by some species, including lactic acid bacteria and thiobacilli.

Table 3. Trace Metals Solution.[a]

Component	Grams per Liter
Nitrilotriacetic acid adjust pH to 6 with KOH	2.0
$MnSO_4 \cdot H_2O$	1.0
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.8
$CoCl_2 \cdot 6H_2O$	0.2
$ZnSO_4 \cdot 7H_2O$	0.2
$CuCl_2 \cdot 2H_2O$	0.02
$NiCl_2 \cdot 6H_2O$	0.02
$Na_2MoO_4 \cdot 2H_2O$	0.02
Na_2SeO_4	0.02
Na_2WO_4	0.02

[a]A trace metals solution formulated to meet the requirements of anaerobes. Store at 5° C. Adapted from Wolin et al. (1963) by Tanner (1989).

Table 4. Vitamins Solution.[a]

Component	Milligrams per Liter
Pyridoxine·HCl	10
Thiamine·HCl	5
Riboflavin	5
Calcium pantothenate	5
Lipoic acid	5
p-Aminobenzoic acid	5
Nicotinic acid	5
Vitamin B_{12}	5
Biotin	2
Folic acid	2
MESA[b]	10

[a]A vitamins solution formulated to fulfill the water-soluble vitamin requirements of anaerobic microorganisms. Store at 5° C. Adapted from Wolin et al. (1963) by Tanner (1989).

[b]Mercaptoethanesulfonic acid.

for nitrate-reducing bacteria. SRB were enumerated as previously described (Tanner, 1989), except that levels of sodium lactate and yeast extract were reduced to 2.5 and 0.5 g/L, respectively. Iron-reducing bacteria were cultured in an acetate-ferric citrate medium (Lovely and Phillips, 1988). For acidogens/acetogens, fructose replaced glucose, and the initial pH of the basal medium was lowered from 7 to 6. For CO oxidizers (acetogens), cultures were incubated under a gas phase of N_2:CO:CO_2 (44:44:12) at a gauge pressure of 200 kPa, and the initial pH was 6. All media for methanogens contained 1 g/L sodium acetate as a carbon source; in medium where acetate was the methanogenic substrate, its concentration was increased to 20 g/L. Hydrogen-oxidizing methanogens were cultured under a gas phase of H_2:CO_2 (80:20) at a gauge pressure of 200 kPa. Formate-oxidizing methanogens were cultured using a basal medium containing 20 g/L sodium formate. Methanogens capable of utilizing methanol were cultured with 2 g/L methanol.

The sodium chloride level of the medium used for enumerations was adjusted according to the source of the inoculum: 10% NaCl for the rock pile; 15% NaCl for the Great Salt Plains; adjusted according to the water analysis for the carbonate reservoirs sampled. To estimate population from the rock pile (incubated at room temperature), 30° C was used. Cultures from the Great Salt Plains were incubated at 37° C. Since all of the carbonate reservoirs had relatively elevated temperatures (44-62° C), cultures from these environments were incubated at both 37° and 60° C. Samples from one reservoir were incubated at 50° C to enumerate halophilic thermophiles.

The most-probable-number (MPN) method was used to enumerate microorganisms; either a three-point MPN in duplicate or a five-point MPN was used (Banwart, 1981; Gerhardt et al., 1981). Values differing by 0.9 log were significantly different ($P < 0.05$).

2. Chemical Analyses. Water analysis was done by Oilabs, Inc., Oklahoma City, Oklahoma. The analysis of calcium, total hardness, iron, sulfate, nitrate, and phosphate was confirmed using test kits (Hach Chemical Co., 1975). Nitrite and ammonium were also measured using Hach test kits. Total dissolved solids analysis was confirmed gravimetrically. Salinities were confirmed by conductivity measurements. pH was measured both by meter and colorpHast narrow range pH strips. Sulfide was measured in the field using a methylene blue assay (Cline, 1969; Fogo and Popowsky, 1949).

3. Sources of Inocula. An upward-flow packed-rock anaerobic filter (rock pile) was established in February, 1988, initially to provide a stable SRB-based biofilm for examination of the anaerobic biotransformation of crude oil marker compounds. It was inoculated with cells concentrated from a produced brine, showing obvious signs of microbial activity, from a well in Blaine County, Oklahoma. The rock pile was maintained with the following anaerobic medium (g/L): NaCl, 100; $(NH_4)_2SO_4$, 0.5; KCl, 0.125; $MgSO_4 \cdot 7H_2O$, 4; $CaCl_2 \cdot 2H_2O$, 0.5; sodium lactate, 0.3; yeast extract, 0.03. Fluid retention time in the rock pile was nine hours. The rock pile was periodically injected with one ml aliquots of crude oil.

Sediment samples from the Great Salt Plains (Alfalfa County, Oklahoma) were collected near sites of inflowing surface waters (Clay Creek and Cottonwood Creek) during March, 1990. Cultures were enriched for and isolated on the glucose heterotroph medium containing 15% NaCl and incubated at 37° C under aerobic conditions. Acid-producing colonies were selected.

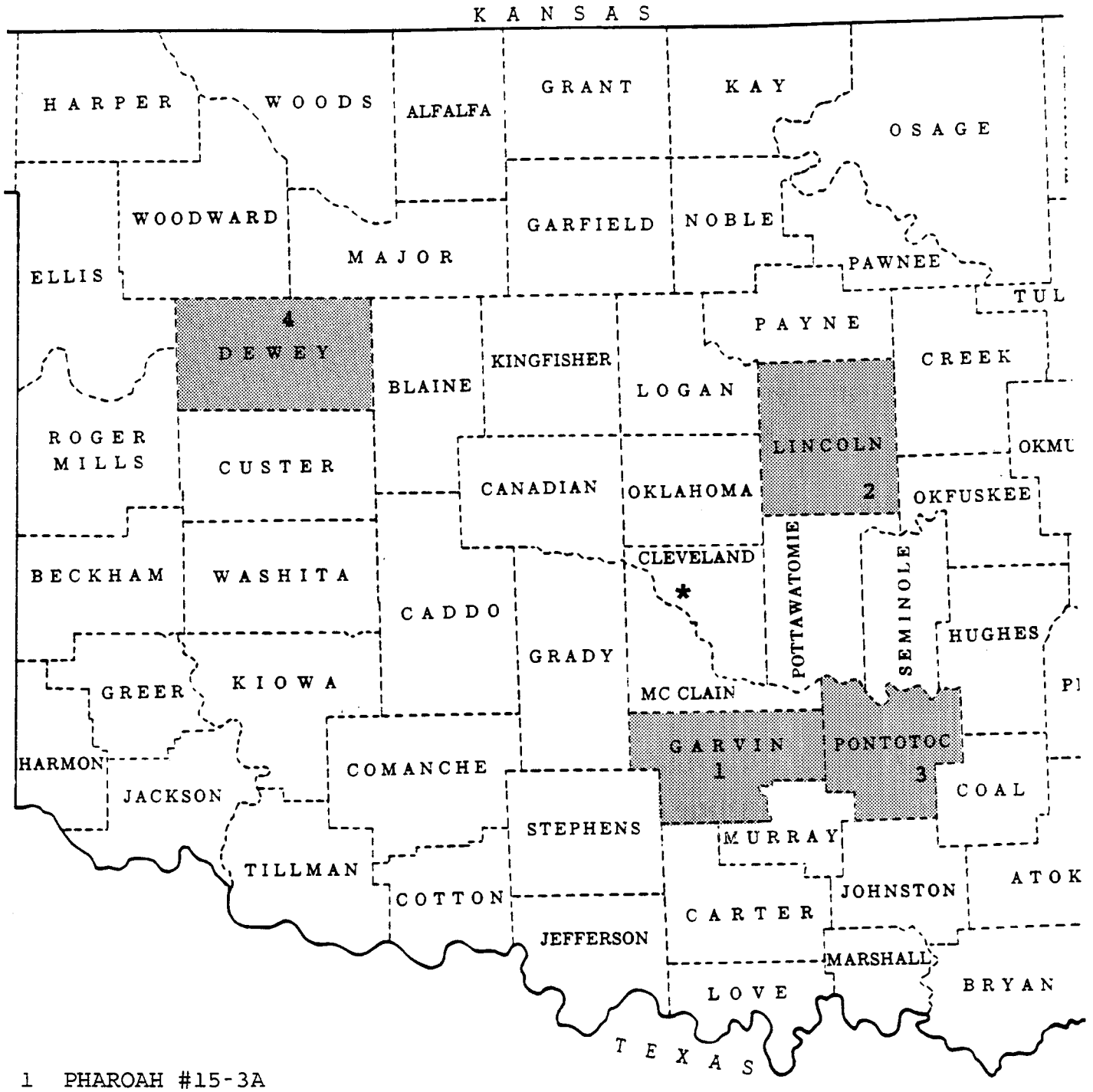
Oil wells producing only from a single zone in a carbonate formation were identified (Table 5; Figure 1). Samples were collected from as near the wellhead as possible. After at least six L of produced fluid was flushed from the collecting line, three two-L samples were collected in sterile bottles by filling them completely with liquid and sealing them. The temperature, pH, and sulfide of the brine were assayed during sampling. The final two L collected was used for the microbiological study, and the other samples were used for the water analysis. Samples were kept at room temperature overnight. The aqueous phase was centrifuged anaerobically at 15,000 x g for 30 minutes at 20° C, the centrifuge bottles decanted, and the pelleted material re-suspended to a tenth volume in the original brine. This concentrated suspension was used to inoculate media for enumeration of the various microbial populations. The produced water from the Ward well was collected from the oil-water separator. produced fluid from this well had a small water cut, making it impractical to collect a water sample directly at the wellhead. The water retention time in the separator was about eight hours. The second and third samples from the Pharoah well were processed the same day they were collected.

Table 5. Carbonate Reservoirs Sampled.

Well	County	Formation	Date	Depth (meters)	Temperature (° C)
Pharoah #15-3A	Garvin	Viola	6/12/90 7/20/90 2/23/91	1749-1847	55
Froug #1	Lincoln	Hunton, gas[a]	6/21/90	1239-1244	44
Mid Fitts #7-15	Pontotoc	Hunton	6/28/90	1033-1038	45
Ward #2-4	Dewey	Chester J	7/12/90	2530	62

[a]This was a gas-producing reservoir; the other reservoirs sampled produced a mixture of oil, water, and natural gas.

FIGURE 1. Oklahoma County Map Showing Sampled Well Locations.



1 PHAROAH #15-3A

2 FROUG #1

3 MID FITTS #7-15

4 WARD #2-4

* University of Oklahoma

C. Results and Discussion

1. Rock Pile. Results of the enumeration of microbial populations from the rock pile are given in Table 6. This work was done to test media, procedures, process logistics, and for training. In spite of the low level of nutrient input through the rock pile, more than 10^8 SRB/ml were routinely recovered from rock pile effluent. The cells exiting the rock pile were probably ultramicrocells since the effluent had no visible turbidity. Several of the cultures recovered under aerobic conditions were examined for the ability to grow anaerobically; all of these did grow anaerobically, consistent with their isolation from an anaerobic environment. As expected, hydrogen and formate-oxidizing methanogens were not recovered from this sulfidogenic environment. Interestingly, methanol-utilizing methanogens were recovered from the rock pile. Generally, methanol is a noncompetitive substrate for methanogenesis. However, it was surprising that these methanogens had maintained themselves in this environment for pver two years, given that the only potential source of methanol or other methylated compounds would be trace amounts in the yeast extract or produced by other microorganisms in the rock pile.

2. Great Salt Plains. Seventeen pure cultures of acid-producing, halophilic microorganisms were isolated from sediments from the Great Salt Plains (Table 7). In general, the isolates were gram-negative, halophilic, facultatively anaerobic rods. These cultures were preserved for potential use in carbonate core studies.

Table 6. Microbial Populations Enumerated from the Effluent of an Upward-Flow Packed-Rock Aanerobic Filter (Rock Pile).[a]

Aerobes:	
General heterotrophs[b]	3×10^5 [c]
Glucose heterotrophs	3×10^0
Anaerobes:	
General heterotrophs	3×10^5
Glucose heterotrophs	2×10^3
Molasses	8×10^7
Molasses-nitrate	1×10^7
Sucrose	3×10^3
Sucrose-nitrate	4×10^3
SRB	2×10^8
Iron reducers	2×10^0
Acidogens/fructose	nd[d]
Methanogens/H_2:CO_2	nd
Methanogens/formate	nd
Methanogens/acetate	nd
Methanogens/methanol	3×10^0

[a]The rock pile was constructed using quartz pebbles in February, 1988, for the production of a SRB biofilm. It was inoculated with cells from a well in Blaine County, Oklahoma. The rock pile was periodically treated with crude oil.

[b]Denotes the type of microorganism being enumerated and/or the substrate used to recover various populations.

[c]Average of two three-point MPNs after incubation at 30° C for 28-30 days (60 days for iron reducers and methanogens).

[d]None detected, indicative of a MPN $<2 \times 10^{-1}$/ml.

Table 7. Characteristics of Acid-Producing, Halophilic Eubacteria Isolated from the Great Salt Plains.

Gram reaction	Cell morphology	Relation to oxygen[a]	Catalase reaction	Relation to salt[b]	Number of strains
-	rod	fac. anaer.	-	obligate	6
-	rod	fac. anaer.	+	obligate	3
-	rod	obl. aer.	+	obligate	3
-	rod	fac. anaer.	-	tolerant	1
+	rod	fac. anaer.	-	obligate	1
+	rod	fac. anaer.	-	tolerant	1
+	rod	fac. aer.	-	obligate	1
+	rod	fac. aer.	+	obligate	1
+	rod	obl. aer.	-	obligate	1
-	coccus	fac. anaer.	-	obligate	1
-	coccus	fac. anaer.	+	tolerant	1
-	coccus	obl. aer.	+	obligate	1
+	coccus	fac. anaer.	+	tolerant	1

[a]Fac. anaer., facultatively anaerobic; obl. aer., obligately aerobic; fac. aer., facultatively aerobic.

[b]Obligate, obligately halophilic; tolerant, halotolerant.

3. Carbonate Reservoirs.

a. Water analysis. Analysis of the produced water from the four carbonate reservoirs sampled is given in Table 8. Produced waters from the Pharoah, Froug, and Mid Fitts wells were, as expected, highly saline brines (17.7%, 15.0%, and 11.2% NaCl, respectively). Water produced from the Ward well was essentially fresh water (o.4% NaCl). All water samples exhibited active outgassing during, and for at least one day after, collection. This was probably due to CO_2 released once the samples were no longer under pressure. All of the produced waters were apparently highly buffered, since the pH of the samples did not change significantly from time of collection to one week later, after which all outgassing had ceased. Analysis of the three brine samples from the Pharoah well were in general agreement.

Sulfide was detected in the brine from the Mid Fitts reservoir, which was under waterflood. Sulfide was also detected in the water from the Ward well; this may have been produced by SRB in the separator rather than in the reservoir.

The water analysis indicated that microbial activity/numbers would be limited in these reservoirs by lack of two key nutrients: nitrogen and phosphorous. Phosphate, nitrate, nitrite, and ammonia were not detected in the produced water samples either by the testing laboratory or our laboratory.

b. Microbial populations. All samples of produced water were concentrated 10X and examined microscopically, but microorganisms were not observed. This could be the result of the original sample having $<10^4$ cells/ml or the cells being present as

Table 8. Water Analysis of Brine from Sampled Carbonate Reservoirs.

	Well[a]				Other Waters	
Component (ppm)[b]	Pharoah [c]	Froug	Mid Fitts	Ward	1A-9 [d]	Sea Water[e]
Chloride	137,550	113,970	90,000	2,750	89,690	18,980
Sodium	69,342	58,912	44,000	1,630	45,315	10,560
Calcium	13,070	10,800	8,560	100	8,300	400
Magnesium	2,465	1,390	2,400	55	1,930	1,272
Potassium	40	490	70	12	110	380
T. D. Solids	222,821	186,414	145,228	4,740	146,330	
Total hardness	42,425	32,800	31,400	480	28,800	
Silica (SiO_2)	9	16	14	20	12	
Sulfate	30	800	130	16	830	
Sulfide	<1	<1	21	7	<5	
Bicarbonate	59	46	67	167	152	
"M" alkalinity	53	38	55	137	125	
Resistivity @ 77 F	0.043	0.049	0.060	1.450	0.059	
Sp. gravity @ 72 F	1.152	1.126	1.095	1.006	1.099	
pH	6.45	6.51	6.76	6.62	7.00	

[a] Reservoirs sampled given in Table 7.

[b] Results from water analysis performed by Oilab, Inc., Oklahoma City, OK. For all samples: carbonate (CO_3), hydroxide (OH), and "P" alkalinity (as $CaCO_3$) = 0 ppm: barium <0.2 ppm: nitrate and phosphate not detected. Sulfide assay performed in the field. pH measured in the field slightly higher.

[c] Average values for three samples from the Pharoah well sampled on 6-12-90, 7-20-90, and 2-23-91.

[d] Analysis of brine from a well in a non-carbonate reservoir, the Vassar Vertz sand in Payne County, given for comparison.

[e] From Handbook of Chemistry and Physics, 63rd edition.

ultramicrobacteria. The enumeration studies (below) show that the population levels were low.

The results of the enumeration studies from each well are given in Tables 9 through 14. These results are summarized in Table 15.

In general, the results are consistent with an anaerobic environment. Several of the aerobic enrichments were subcultured anaerobically; these all grew, indicating that they contained facultative anaerobes. All the wells contained microorganisms that could grow with molasses, which is often the substrate used in MEOR field trials. In general, fewer cells were detected when sucrose, the major carbohydrate present in molasses, was used as the substrate. Nitrate was added to the molasses and sucrose media in this study, since it may also be used in MEOR field trials both as as electron acceptor and/or a nitrogen source (all media in this study contained ammonium as a nitrogen source). Similar MPNs were obtained from wells with molasses and sucrose media in either the presence or absence of added nitrate. The exception was water from the Ward well, where significantly more cells were detected using the molasses medium compared to the molasses-nitrate medium.

SRB were recovered from the water produced from the Pharoah well using the molasses and the molasses-nitrate media. Recovery in the molasses-nitrate medium was unexpected, since nitrate (86 ppm) was still present when sulfide (6 ppm) was produced. Previous work indicated that sulfidogenesis does not occur in the presence of nitrate. If nitrate reducers were in the brine sample, reducing equivalents should have shifted from sulfate reduction to nitrate reduction. This process itself can inhibit SRB if nitrous oxide is produced as an intermediate (Jenneman et al., 1986). It

Table 9. Microbial Populations Enumerated from the Pharoah #15-3A Well Sampled 6/12/90.[a]

Aerobes:	
General heterotrophs[b]	24[c]
Glucose heterotrophs	2.3
Anaerobes:	
General heterotrophs	2.3
Glucose heterotrophs	nd[d]
Molasses	2.3
Molasses-nitrate	4.3
Sucrose	0.36
Sucrose-nitrate	4.3
SRB	0.43
Iron reducers	nd
Acidogens/fructose	nd
Acetogens/CO	nd
Methanogens/H_2:CO_2	nd
Methanogens/formate	nd
Methanogens/acetate	nd
Methanogens/methanol	nd

[a]Reservoir properties are given in Table 7; water analysis is given in Table 8. 10% NaCl was used in the enumeration media.

[b]Denotes type of microorganism being enumerated and/or substrate used to recover various populations.

[c]Average of two three-point MPN's per ml after incubation at 37° C for 28-30 days (60 days for iron reducers, CO acetogens, and methanogens).

[d]None detected, indicative of a MPN $<2 \times 10^{-1}$/ml.

Table 10. Microbial Populations Enumerated from the Pharoah #15-3A Well Sampled 7/20/90.[a]

Aerobes:	
General heterotrophs[b]	0.92[c]
Glucose heterotrophs	0.92
Anaerobes:	
General heterotrophs	43
Glucose heterotrophs	2.3
Molasses	2.3
Molasses-nitrate	2.3
Sucrose	nd[d]
Sucrose-nitrate	2.3
SRB	2.3
Iron reducers	nd
Acidogens/fructose	0.43
Acetogens/CO	nd
Methanogens/H_2:CO_2	nd
Methanogens/formate	nd
Methanogens/acetate	0.36
Methanogens/methanol	1.5

[a]Reservoir properties are given in Table 7; water analysis is given in Table 8. 15% NaCl was used in the enumeration media.

[b]Denotes type of microorganism being enumerated and/or substrate used to recover various populations.

[c]Average of two three-point MPN's per ml after incubation at 37° C for 28-30 days (60 days for iron reducers, CO acetogens, and methanogens).

[d]None detected, indicative of a MPN $<2 \times 10^{-1}$/ml.

Table 11. Microbial Populations Enumerated from the Froug #1 Well.[a]

Aerobes:	
General heterotrophs[b]	nd[c,d]
Glucose heterotrophs	nd
Anaerobes:	
General heterotrophs	0.7
Glucose heterotrophs	0.044
Molasses	1.7
Molasses-nitrate	1.1
Sucrose	0.019
Sucrose-nitrate	nd
SRB	nd
Iron reducers	0.019
Acidogens/fructose	nd
Acetogens/CO	nd
Methanogens/H_2:CO_2	nd
Methanogens/formate	nd
Methanogens/acetate	nd
Methanogens/methanol	nd

[a]Reservoir properties are given in Table 7; water analysis is given in Table 8. 20% NaCl was used in the enumeration media.

[b]Denotes type of microorganism being enumerated and/or substrate used to recover various populations.

[c]A five-point MPN per ml after incubation at 37° C for 28-30 days (60 days for iron reducers, CO acetogens, and methanogens).

[d]None detected, indicative of a MPN $<2 \times 10^{-1}$/ml.

Table 12. Microbial Populations Enumerated from the Mid Fitts #7-15 Well.[a]

Aerobes:	
General heterotrophs[b]	0.13[c]
Glucose heterotrophs	nd[d]
Anaerobes:	
General heterotrophs	7.9
Glucose heterotrophs	2.3
Molasses	3.3
Molasses-nitrate	2.3
Sucrose	2.3
Sucrose-nitrate	2.3
SRB	3.3
Iron reducers	0.23
Acidogens/fructose	nd
Acetogens/CO	nd
Methanogens/H_2:CO_2	nd
Methanogens/formate	nd
Methanogens/acetate	nd
Methanogens/methanol	0.23

[a]Reservoir properties are given in Table 7; water analysis is given in Table 8. 12% NaCl was used in the enumeration media.

[b]Denotes type of microorganism being enumerated and/or substrate used to recover various populations.

[c]A five-point MPN per ml after incubation at 37° C for 28-30 days (60 days for iron reducers, CO acetogens, and methanogens).

[d]None detected, indicative of a MPN $<2 \times 10^{-1}$/ml.

Table 13. Microbial Populations Enumerated at 37° C from the Ward #2-4 Well.[a]

Aerobes:	
General heterotrophs[b]	9.3[c]
Glucose heterotrophs	0.36
Anaerobes:	
General heterotrophs	930
Glucose heterotrophs	0.92
Molasses	93
Molasses-nitrate	1.5
Sucrose	0.36
Sucrose-nitrate	0.36
SRB	4.6
Iron reducers	0.93
Acidogens/fructose	93
Acetogens/CO	nd[d]
Methanogens/H_2:CO_2	0.011
Methanogens/formate	2.8
Methanogens/acetate	2.3
Methanogens/methanol	92

[a]Reservoir properties are given in Table 7; water analysis is given in Table 8. 0.5% NaCl was used in the enumeration media.

[b]Denotes type of microorganism being enumerated and/or substrate used to recover various populations.

[c]Average of two three-point MPN's per ml after incubation for 28-30 days (60 days for iron reducers, CO acetogens, and methanogens).

[d]None detected, indicative of a MPN $<2 \times 10^{-1}$/ml.

Table 14. Microbial Populations Enumerated at 60° C from the Ward #2-4 Well.[a]

Aerobes:	
General heterotrophs[b]	nd[c,d]
Glucose heterotrophs	nd
Anaerobes:	
General heterotrophs	150
Glucose heterotrophs	0.36
Molasses	nd
Molasses-nitrate	nd
Sucrose	nd
Sucrose-nitrate	nd
SRB	0.024
Iron reducers	nd
Acidogens/fructose	nd
Acetogens/CO	nd
Methanogens/H_2:CO_2	0.22
Methanogens/formate	nd
Methanogens/acetate	nd
Methanogens/methanol	nd

[a]Reservoir properties are given in Table 7; water analysis is given in Table 8. 0.5% NaCl was used in the enumeration media.

[b]Denotes type of microorganism being enumerated and/or substrate used to recover various populations.

[c]Average of two three-point MPN's per ml after incubation for 28-30 days (60 days for iron reducers, CO acetogens, and methanogens).

[d]None detected, indicative of a MPN $<2 \times 10^{-1}$/ml.

TABLE 15. Microbial Populations Enumerated from Carbonate Reservoir Produced Water.[a]

	Sampled Well					
	PHAROAH		FROUG	MID FITTS	WARD	
Sampled	6-12-90	7-20-90	6-21-90	6-28-90	7-12-90	
Incubation T, °C	37	37	37	37	37	60
AEROBIC:						
Heterotrophs,[b] general	20	0.9		0.1	9	
Heterotrophs, glucose	2	0.9			0.4	
ANAEROBIC:						
Heterotrophs, general	2	40	0.7	8	900	200
Heterotrophs, glucose		2	0.04	2	0.9	0.4
Molasses	2	2	2	3	90	
Molasses-nitrate	4	2	1	2	2	
Sucrose	0.4		0.02	2	0.4	
Sucrose-nitrate	0.4	2		2	0.4	
SRB	0.4	2		3	5	0.02
Iron reducers			0.02	0.2	0.9	
Fructose[c]		0.4		9		
H_2:CO_2[d]					0.01	0.2
Formate[d]					3	
Methanol[d]		2		0.2	90	
Acetate[d]		0.4			2	

[a] Population values are in MPN per ml. Incubation period for all cultures was 4 weeks except for iron reducers and methanogens, which was 8 weeks.

[b] Denotes type of microorganism being enumerated and / or substrate used to recover viable microorganisms.

[c] Lowered pH for enrichment of acetogens / acidogens.

[d] Substrates for enrichment of methanogens; methanogenesis confirmed by GC.

has also been reported that some SRB will reduce nitrate, if available, and use this as a growth-supporting metabolism (Seitz and Cypionka, 1986). However, the ratio of concentration of electron donor to electron acceptor is important in regulating the flow of electrons to terminal electron acceptors. The reason why both reductions occurred simultaneously will require further study.

Acetate- and H_2-oxidizing SRB were also enumerated from the second brine sample collected from the Pharoah well, using the respective methanogenic media with the addition of 7 mM Na_2SO_4. Acetate-utilizing SRB were not detected. However, H_2-oxidizing SRB were detected at a level of 23/ml. This suggests that H_2 oxidation can occur in a highly saline environment, and will be discussed further in the context of methanogenesis.

Sulfate was found in all of the carbonate reservoir produced waters. Consistent with a model of a low-activity microbial ecosystem, none or a low level of sulfide was detected in these waters, and none or a low level of SRB were enumerated from these samples. Sulfide was detected in the two most "impacted" environments: brine from the Mid Fitts well, which was under waterflood, and water from the Ward well, which was recovered from the oil-water separator. Still, the above results, coupled with the low numbers of iron reducers recovered from the samples, suggest that the potential for delecterious effects from SRB, such as corrosion, in the reservoirs is low. All of the results presented in Tables 9 through 15 are consistent with a hypothesis that SRB are not the dominant microorganisms in these environments.

Few acidogens/acetogens were recovered using the fructose media and no microorganisms were recovered with CO as the energy source.

Only low numbers of cells were detected using any of the single-sugar media (glucose, sucrose, or fructose); these substrates are not present at appreciable concentrations in a carbonate reservoir environment. Given the likely highly-buffered nature of these reservoirs, use of an initial pH to recover acidogens may have been too restrictive. This acidic pH was used because, in general, environmental isolates show a degree of tolerance to the effects of their end-products of metabolism, and it was thought that this would be an effective means to obtain strong acid producers. In the case of CO, the amount present in culture may have been high enough to be toxic to CO-oxidizing anaerobes; for future work, the CO concentration will be reduced, with a gas phase of N_2:CO:CO_2 (53:33:14) at a gauge pressure of 100 kPa.

Formate- and H_2-oxidizing methanogens were recovered only from the water produced from the low-salinity reservoir. This result was expected, since sulfate reduction is generally the terminal electron-accepting reaction in these environments, and since there have been no confirmed reports of H_2-oxidizing methanogens isolated from highly saline environments. It has been suggested that H_2-oxidizing methanogens would not be found in highly saline environments because of the low solubility of the substrate. This point may be mute, given that H_2-oxidizing SRB could be enumerated from the Pharoah well. Very few methanogens were detected using acetate, another competitive substrate. A non-competitive substrate, methanol, was the most successful for recovering methanogens from these environments.

Thermophiles at 60° C were recovered only from the low-salinity produced water (Ward well). This result was unexpected, since all of the reservoirs were somewhat thermophilic (44-62° C). However, there have been no confirmed isolations of thermophilic microorganisms from highly saline environments. A possible explanation for the inability to detect thermophiles is that only microorganisms from the producing wellbore were sampled. However, there is no a priori reason to assume that we were not recovering cells from the reservoir. One might also propose some undocumented interaction between pressure and temperature, perhaps influenced by high levels of dissolved CO_2, was a reason. The inability to detect thermophiles could suggest that in situ MEOR may not be feasible. The recovery of thermophiles from these highly saline brines merited re-examination.

The third sample from the Pharoah well (55° C) was examined for thermophiles able to grow at 50° C in media containing 15% NaCl and 0.02% $CaCO_3$ at pH 7. In addition to the media used previously, acetate- and H_2-oxidizing SRB were enumerated, and brines were directly amended for the detection of aerobic general heterotrophs, anaerobic general heterotrophs, sucrose heterotrophs, sucrose-nitrate heterotrophs, molasses heterotrophs, molasses-nitrate heterotrophs, CO-oxidizers, and H_2-oxidizers. Microorganisms were enumerated on the molasses-nitrate, iron-reducing, and H_2-oxidizing SRB media at 0.02, 0.13, and 0.79 cells/ml, respectively. The only direct amendment that yielded growth was with molasses-nitrate.

The problem of recovering thermophilic halophiles remains. Two approaches which would be useful to address this issue include

incubating samples under pressure in media saturated with $CaCO_3$ and CO_2 to better mimic the subterranean environment, and the study of more readily accessible environments, such as the Great Salt Plains, for thermophilic halophiles.

In many natural environments, microorganisms tend to adhere to surfaces. Thus, examining produced waters tends to underestimate the actual level of microbial populations by several orders of magnitude. However, from these data we can conclude that a mesophilic microbial community exists in these carbonate reservoirs.

REFERENCES

Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe. 1979. Methanogens: reevaluation of a unique biological group. Microbiol. Rev. 43:260-296.

Balch, W. E., and R. S. Wolfe. 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of Methanobacterium ruminantium in a pressurized atmosphere. Appl. Environ. Microbiol. 32:781-791.

Banwart, G. J. 1981. Basic Food Microbiology. AVI Publishing Co., Inc., Westport.

Cline, J. D. 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14:454-458.

Davis, J. B. 1967. Petroleum Microbiology. Elsevier Publishing Co., New York.

Fogo, J. K., and M. Popowsky. 1949. Spectrophotometric determination of hydrogen sulfide. Anal. Chem. 21:732-734.

Gerhardt, P., R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.). 1981. Manual of Methods for General Bacteriology. Amer. Soc. Microbiol., Washington, D.C.

Hach Chemical Co. 1975. Hach Water and Wastewater Analysis Procedure Manual, 3rd ed. Hach Chemical Co., Ames, Iowa.

Jenneman, G. E., M. J. McInerney, and R. M. Knapp. 1986. Effect of nitrate on biogenic sulfide production. Appl. Environ. Microbiol. 51:1205-1211.

Jones, R. E., R. E. Beeman, and J. M. Suflita. 1989. Anaerobic metabolic processes in the deep terrestrial subsurface. Geomicrobiol. J. 7:117-130.

Kuznetsov, S. I., M. V. Ivanov, and N. N. Lyalikova. 1963. Introduction to Geological Microbiology, Engl. trans. McGraw-Hill Book Co., Inc., New York.

Lovely, D. R., and E. J. P. Phillips. 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl. Environ. Microbiol. 54:1472-1480.

Phelps, T. J., C. B. Flierman, T. R. Garland, S. M. Pfiffner, and D. C. White. 1989. Methods for recovery of deep terrestrial subsurface sediemnts for microbiological studies. J. Microbiol. Methods 9:267-280.

Postgate, J. R. 1984. The Sulfate-Reducing Bacteria, 2nd ed. Cambridge University Press, New York.

Seitz, H.-J., and H. Cypionka. 1986. Chemolithotrophic growth of *Desulfovibrio desulfuricans* with hydrogen coupled to ammonification of nitrate or nitrate. Arch. Microbiol. 146:63-67.

Tanner, R. S. 1989. Monitoring sulfate-reducing bacteria: comparison of enumeration media. J. Microbiol. Methods 10:83-90.

Wolin, E. A., M. J. Wolin, and R. S. Wolfe. 1963. Formation of methane by bacterial extracts. J. Biol. Chem. 238:2882-2886.

EFFECTS OF ADSORPTION ON SUBSTRATE AVAILABILITY IN CARBONATE RESERVOIRS

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Keywords: adsorption, substrates, carbonates,reservoirs,isotherms

Abstract

The investigation of microbial substrate adsorption onto reservoir rocks is described. Substantial adsorption of substrates onto rock surfaces could affect substrate availability for in situ microbially enhanced oil recovery or bioremediation of subterranean wastes. Adsorption of ammonium, nitrate, phosphate and glucose onto reservoir rocks was examined experimentally using Viola limestone chips and Ottawa quartz sand grains. Substantial amounts of ammonium, phosphate and glucose adsorbed onto water-wetted limestone surfaces. Only ammonium ions adsorbed onto oil-wetted limestone surface. There was no nitrate adsorption. Only ammonium ions adsorbed significantly onto the water-wetted sand grains. The level of adsorption of these compound from a multicomponent substrate media was less than adsorption of the individual compounds. Desorption occurred when the limestone rock was re-suspended in brine. The following percentages of adsorbed species were desorbed under the experimental conditions: 93.5% of carbohydrates, 25% of ammonium ions and 2.7% of phosphate ions. The desorption of these species shows that adsorbed nutrients may be available for microbial utilization as the concentration of the substrate media in the reservoir is depleted. Adsorption isotherms were used to describe the adsorption data. These provide site-specific relationships that have not yet been described in modeling of MEOR.

Introduction

Microbially enhanced oil recovery (MEOR) and bioremediation of subterranean wastes may involve the injection of nutrients in an aqueous phase into a lithic environment: sand, sandstone or limestone. The native microbial flora may be employed or microorganisms may be injected along with the nutrients. However, in either situation, pre-determined amounts of carbohydrates and other nutrients such as ammonium nitrate and phosphate are often required for desired microbial activity.

Depletion of substrates in reservoirs is not only due to microbial utilization; a considerable percentage of the substrate molecules and ions is adsorbed onto the reservoir rock surfaces. As Bubela (1989) pointed out, substrate adsorption onto the pore walls of the reservoir rock is one of the parameters that should be considered in the assessment of a given reservoir for MEOR. Adsorption and subsequent limitation of nutrient availability could interfere with the goals of a MEOR project. Furthermore, concentration of nutrients on pore surfaces could result in localised formation of biomass and may cause a decrease in rock permeability to the detriment of oil production.

Sorptive Phenomena in Rocks

Sorptive phenomena in reservoir rocks involve absorption (especially of liquids) into, and adsorption/desorption of molecules and ions onto, rock matrices. Adsorption is caused by imbalance of molecular and ionic forces at the adsorbate/rock interfaces. If the forces are intermolecular in nature, such as Van

der Waals' and London's forces, then the adsorption phenomena is physical. Physical adsorption or physiosorption can be reversible and may involve more than one layer of the adsorbate molecules. On the other hand, existence of valency forces will result in sharing of electrons between the adsorbent and adsorbate. The adsorbent and the primary layer of the adsorbate form a chemical compound. Chemical adsorption or chemisorption is practically irreversible and involves only one layer.

Theories and relationships for describing simple adsorption phenomena and data are well-documented (Oscik, 1982 & Parfit et al,1983). Corapcioglu and Haridas (1985) assumed the Freundlich adsorption isotherm for nutrient adsorption in the modeling of bacterial metabolism and transport through porous media. Islam (1990,1990) assumed a Langmuir model. However, none of the above workers had site specific parameters for their models.
Corapcioglu and Haridas (1985) cited estimates for the adsorption constants and exponents from Sykes et al. (1982) and Selim et al (1977) who investigated adsorption of landfill leachate and herbicide onto soil and clay particles respectively. In general, there is a lack of information in literature on the adsorption of microbial nutrients on the different types of reservoir rock surfaces under commonly encountered conditions. The adsorption isotherm to be chosen for a given adsorbate/rock interaction depends on a number of physiochemical conditions. Assumption of an isotherm for a given process without the benefit of prior experimentation may be in serious error. Although several studies have been undertaken on adsorption of surfactants on reservoir rock

surfaces (Refs.1,5,11-13), these results may not be transfered to substrate adsorption on rock surfaces because the fluid/rock interactions are different.

This paper describes the results of the adsorption of glucose, ammonium, nitrate and phosphate on carbonate surfaces. Adsorption on clean quartz sand was also studied for comparative purposes.

Materials and Methods

Minerals

Chips of the Viola limestone (20 - 50 mesh) were obtained from a quarry near Davis, Oklahoma. Viola limestone is of the Ordovician age and is oil-bearing in some parts of the state. Ottawa III sand (50 - 70 mesh) was also used. Fines were removed by repeated washing and agitation with pure water. The minerals were then dried and stored dry. Water-wetted and oil-wetted rock samples were prepared by soaking the grains in 2% brine or hexadecane, respectively, for 24 hours.

Adsorbate Solutions and Assay Methods

Solutions of glucose,ammonium chloride, sodium nitrate, and potassium phosphate were prepared. Ammonium chloride, nitrate, and phosphate were assayed with test kits: CHEMetrics K2505A, 0-250 mg/l free and total NH3; CHEMetrics K45678D,0-200 mg/l free and total nitrogen; CHEMetrics F6789D, 0 -10 mg/l phosphate). Glucose was assayed using the phenol/sulphric acid method described by duBois et al(1956).

Adsorption Procedure

Adsorption tests were conducted by mixing and agitating 10 ml of the adsorbate solution in a 25 ml test tube with a known weight of the rock chips or grains at room temperature. After several minutes of agitation, the sample was centrifuged for 10 minutes or more and the supernatant solution was assayed for the residual concentration of the adsorbate being tested. The same test was

repeated for other weights of rock materials but the same initial adsorbate concentration. This procedure was repeated using other concentrations of the adsorbate solution.

To test whether adsorbed species can be recovered from the rock surfaces, the test sample was drained of all supernatant liquid, re-suspended in 2% brine and agitated. The resulting supernatant solution was then assayed for the adsorbate. A non-absorbed compound, nitrate, was used to estimate apparent adsorption due to fluid retention.

Results and Data Interpretation

Adsorption was measured as the difference between the initial and supernatant concentrations according to the following equation:

$$n^{\sigma} = \frac{(C^{o} - C)\,V}{m}$$

where V = volume of test solution, l

m = weight of the rock chips or grains,g

C_o= initial concentration of test solution, mg/l

C = supernatant concentration, mg/l

and n^{σ}= adsorption in mg of adsorbate/g of rock.

For a given test solution, the average adsorption was calculated by taking an average of adsorption values determined from Eq. 1 with different rock weights. This average value may be obtained from the slope of Figure 1 which is the plot of the adsorbed weight of species (NH_4^+ ions in this case) at different weights of rock chips for a given test solution.

The adsorption data were regressed by the BET equation:

$$n^{\sigma} = \frac{C}{(1-C)\left(\frac{1}{a_m K} + \frac{K-1}{a_m K} C\right)}$$

which reduces to the Langmuir isotherm for very large K:

$$n^{\sigma} = \frac{a_m KC}{1+C}$$

where K is related to the pure heat of adsorption and a_m is an adsorption constant. The following results were obtained.

Carbohydrate Adsorption

Initial concentrations of glucose in 2% brine ranged from 10g/l (1%) to 0.05 g/l (0.005%). Tests were conducted on water-wetted and oil-wetted Viola limestone chips as well as water-wetted Ottawa sand. Glucose adsorption was substantial on the water-wetted limestone but was not appreciable on the oil-wetted limestone nor water-wetted sands. This data is shown in figure 2.

Carbohydrate adsorption on water-wetted Viola limestone follows adsorption isotherms described by

$$BET: n^{\sigma} = \frac{C}{(1-C)\,(.000102+.2045C)}$$

and

$$Langmuir: n^{\sigma} = \frac{9804C}{(1+2006C)}$$

where C = equilibrium concentration in g/ml.

and n^{σ} = adsorption in mg of glucose per g of rock.

Up to 17% of 10g/l glucose was adsorbed onto water-wetted Viola limestone from the glucose/brine solution.

These results imply that carbohydrates may not adsorb on oil-wetted porous media nor on water-wetted clean sands. However, the adsorption behavior of carbohydrates on carbonate rocks of mixed

wettability or sandstone formations with clays and appreciable amounts of multivalent cations is not yet known.

Ammonium Ion Adsorption

Ammonium ions were strongly adsorbed onto water-wetted Viola limestone chips and, to a lesser extent on the oil-wetted chips. Experimental data for ammonium adsorption on water-wetted and oil-wetted Viola limestone are shown in Figures 3 and 4 and are described by the following isotherms:

<u>water-wetted limestone</u>

$$BET: n^{\sigma} = \frac{C}{(1-C)\,(.00063+3.8C)}$$

or

$$Langmuir: n^{\sigma} = \frac{1594.90C}{(1+6062.4C)}$$

and

<u>oil-wetted limestone</u>

$$BET: n^{\sigma} = \frac{C}{(1-C)\,(.000408+5.9223C)}$$

or

$$Langmuir: n^{\sigma} = \frac{2451C}{(1+14516.35C)}$$

where C = equilibrium ammonium concentration in g/ml

and n^{σ} = adsorption in mg of NH4+ /g of rock

The coefficients of correlation for this regression are 0.98 and

0.89 respectively. Figures 3 and 4 show that ammonium ions are adsorbed at the same order of magnitude on the Viola limestone irrespective of the rock's wettability.

Ammonium ions also adsorbed water-wetted Ottawa sand but not appreciably on the oil-wetted sand. The experimental data for ammonium adsorption on water-wetted sand is shown on Figure 5 and is described by:

$$n^{\sigma}=\frac{628.93C}{(1-C)}$$

Phosphate Ion Adsorption

Phosphate was strongly adsorbed onto water-wetted Viola limestone surfaces. The adsorption data, shown in Figure 6, is described by the BET equation:

$$n^{\sigma}=\frac{C}{(1-C)\,(.003971+19.692C)}$$

and Langmuir equation:

$$n^{\sigma}=\frac{251.83C}{1+4960C}$$

Phosphate ion adsorption on oil-wetted Viola limestone and water-wetted Ottawa sand is not significant.

Nitrate Ion adsorption

Nitrate ions did not adsorb onto carbonate or sandstone surfaces. This suggests that all of the nitrate injected into an aqueous subterranean environment will be readily available to

microorganisms. Nitrate is important because it can serve as a terminal electron acceptor. The behavior of nitrate in the clayey environment of most reservoirs is not yet known.

Multicomponent Nutrient Media

The multicomponent adsorption phenomena was1 studied at various glucose concentrations while the ammonium and phosphate ions in all substrate media were fixed at initial concentrations of 200 mg/l and 10 mg/l, respectively. Table I shows the amount of glucose adsorbed as a function of the initial glucose concentration in the multicomponent media. From Figure 2 and Table I, it can be seen that glucose adsorption onto water-wetted Viola limestone from the multicomponent media and from the 2% brine media are on the same order of magnitude (10-20% of 10 g/l of glucose).

Adsorption from multicomponent solutions is considered a very complex process and presents many difficulties (Oscik, 1982). The adsorption behaviour of each component in the media is likely to differ depending on the concentrations of each component species. Glucose adsorption data from the test solutions fitted the following isotherms with a correlation coefficient of 0.993:

BET:

$$n^{\sigma}=\frac{C}{(1-C)\,(.00392+1.0565C)}$$

and

Langmuir:

$$n^{\sigma}=\frac{255.1C}{1+270.5C}$$

Desorption Phenomena

Physiosorptive processes are characterized by reversibility. The possibility of substrate media desorbing from the rock surfaces in response to the depletion of the substrate in the aqueous phase can increase substrate availability for bacteria. Results of the desorption experiment showed that glucose, phosphate and ammonium species were recovered when the adsorbent (Viola limestone) were re-suspended in brine (Table 2). Initial concentrations were 0.01% of glucose, 200 mg/l of NH_4^+ ions, 10 mg/l of PO_4= ions and 22.5 ppm of NO_3^- ions. Nitrate ions were neither adsorbed nor desorbed and served as a control for estimating amounts of other ions and glucose possibly entrained in the water wetting the rock surface. Incidentally, all free water was drained off before re-suspension and the entrained nitrate ion concentration was not appreciable in the supernatant solution. Table 2 shows that glucose had the highest degree of desorption with about 93.5% of the total adsorbed molecules desorbing. Phosphate showed the least desorption with only 2.7% of the total adsorbed amount desorbing. One quarter of the adsorbed ammonium ions desorbed on re-suspension.

Discussion of results

These results and methods are useful in modeling, design and execution of both laboratory experiments and field trials of microbial enhanced oil recovery or bioremediation of subterranean

wastes. Nutrient adsorption onto porous media may limit its availability for microbial utilization. The implication for field applications of MEOR and bioremediation of subterranean wastes is that nutrient concentrations in excess of the stoichiometric requirements for the desired microbial activity should be injected in order to accomodate the adsorption losses. The excess amounts to be used are likely rock specific because of the varying quantities of minerals that exist in different porous media.

Observations

(1) Isotherms for adsorption of solutions of glucose, ammonium and phosphate on Viola limestone at different wetting conditions and on water-wetted Ottawa sand were determined experimentally.

(2) Rock wettability was found to alter substrate adsorption behavior in reservoir rocks.

(3) The adsorption behavior of the substrates on Viola limestone and Ottawa sand differed showing that a common generalized adsorption isotherm for different rock types is not realistic.

(4) Adsorption from multicomponent nutrient solutions onto rock surfaces is more complex. Amount of each component adsorbed depended on the initial concentrations of each participating species.

(5) Nutrient adsorption onto the pore walls of the reservoir rock can be considerable.

(6) Desorption occurred when the rock adsorbent was re-suspended in brine. Glucose desorbed the most while $PO_4^{=}$ ions desorbed the least.

Acknowledgement

This work was supported by DOE Contract No DE-AC22-90BC14202. Assistance with the experimental work from Jon Adkins, Hong Wong and J. Troy Turcott is gratefully acknowledged. Technical support from the School of Petroleum & Geological Engineering is also acknowledged.

References

1. Bae, J.H. and C.B. Petrick(1977): "Adsorption/Retention of Petroleum Sulfonates in Berea Cores", **Soc. of Pet. Eng. J.**, (October),353-357.

2. Bubela, B.(1989): "Geobiology and Microbiologically Enhanced Oil Recovery",Chapter 4 in **Microbial Enhanced Oil Recovery**, E.C. Donaldson, G.V. Chiligarian and T.F. Yen, eds.,Elservier Science Publishers, Amsterdam.

3. Corapcioglu, M.Y. and Haridas,A.(1985): "Microbial transport in soils and groundwater: numerical model", **Advances in Water Resources**,8, **4**,(Dec).

4. Du Bois,N., K. A. Giles, K. Hamilton,A. Rebes and F. Smith: **Analytical Chem.** 28, 350-356.

5. Dubey S.T.and M.H. Waxman(1989): "Asphaltene Adsorption and Desorption from Mineral Surfaces", **SPE Paper 18462** presented at the Int'l Symposium on Oilfield Chemsitry in Houston, TX, Feb. 8-10.

6. Islam,M.R.(1990): "Mathematical Modelling of Microbial Enhanced Oil recovery", **SPE Paper 20480** presented at 65th Annual Technical Conference and Exhibition in New Orleans, LA, Sept. 23-26.

7. Islam, M.R.(1990):"Mathematical modelling and scaling up of microbial enhanced oil recovery", **Paper No. CIM/SPE 90-131 Presented at Int'l Tech mtg of CIM and SPE, Calgary, June 10-13.**

8. Oscik,J.(1982): **Adsorption**, John Wiley & Sons, N.Y.,206pp.

9. Parfit, G.D. and C.H. Rochester (eds)(1983): **Adsorption from Solution at the Solid/Liquid Interface**, Academic Press, N.Y., 416pp.

10. Reynolds,P.J., P. Sharma, G.E. Jenneman and M.J. McInerney (1989): "Mechanisms of Microbial Movement and Subsurface Materials", **Appl. & Environ. Microbio.**,55, 2280-2286.

11. Selim, H.M., J.M. Davidson and P.S. Rao (1977): "Transport of reactive solutes through multilayered soils", **Soil Sci. Soc. Amer. J.**, **41**, 3-10.

12. Somasundaran, P. and H.S. Hanna (1979): "Adsorption of Sulfonates on Reservoir Rocks", **J.Pet. Eng.**, August,221-232.

13. Sykes,J.F., S.Soyupak and G.J. Faruhar (1982): "Modelling of leachate organic migration and attenuation in ground water below sanitary landfills", **Water Resources Res.**,**18**,135-145.

14. Yang,C.,W. Jao and Huang,Y.(1989):"The mechanism of adsorption and a new method to reduce surfactant loss in chemical flooding",**J. of Petroleum Sci. and Eng.**,3,97-109.

Table 1. Adsorption of Glucose in a Multicomponent Medium onto Viola Limestone.

Initial Glucose Concentration (mg/liter)	Glucose Adsorbed (%)
100	43
200	29
1,000	11
5,000	11
10,000	12

Ten grams of sized (20-50 mesh) Viola limestone chips was the adsorbent.

Table 2. Adsorption and Desorption of Compounds in 2% Brine on Viola Limestone.

Compound	Initial Concentration	Adsorbed mg	Desorbed mg	Desorbed %
Glucose	100 mg/liter	0.63	0.59	93.5
Ammonium	200 mg/liter	0.67	0.17	25.0
Nitrate	22 mg/liter	0	-	-
Phosphate	10 mg/liter	0.062	0.0017	2.7

Adsorption and subsequent desorption of individual compounds in 2% NaCl was determined using water-wet, sized (20-50 mesh) Viola limestone chips (10 grams). Ten ml of brine, containing the test compound as required, were used for the adsorption and desorption.

Figure 1 - Weight of Phosphate Adsorbed versus Weight of Adsorbent Rock

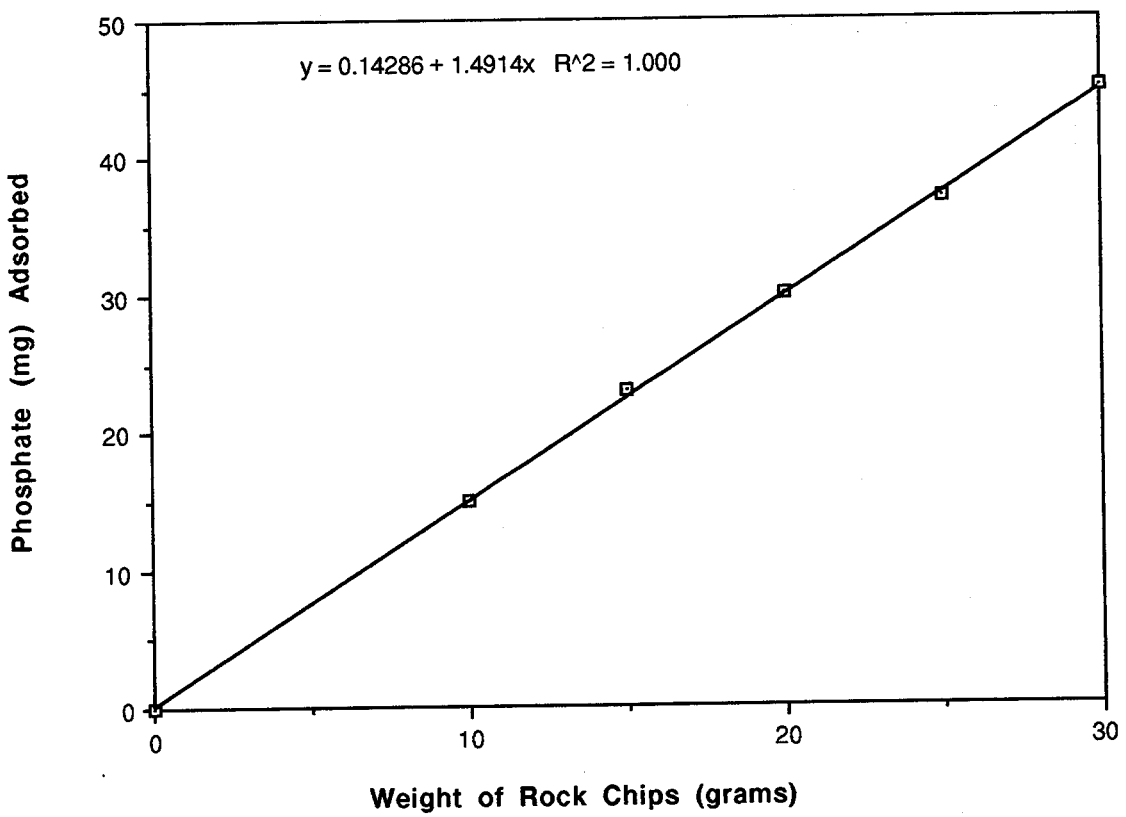


Figure 2: Glucose Adsorption on Water-wetted Viola Limestone

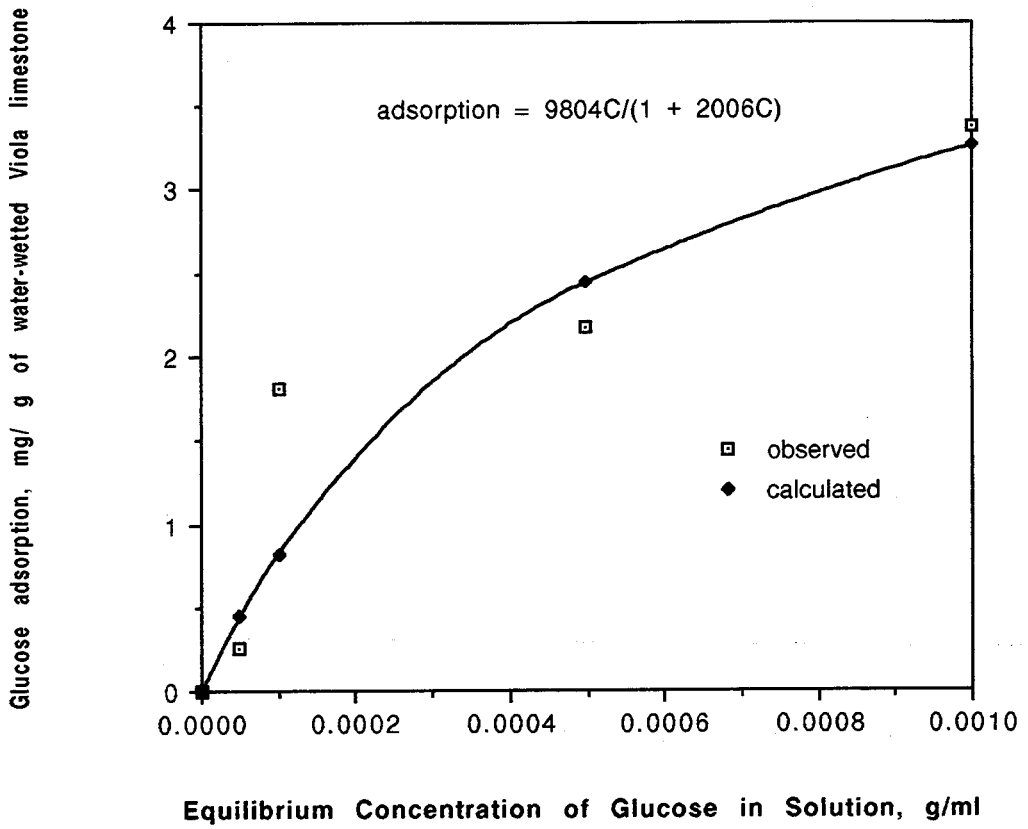


Figure 3: Ammonium ion adsorption on Water-wetted Viola limestone

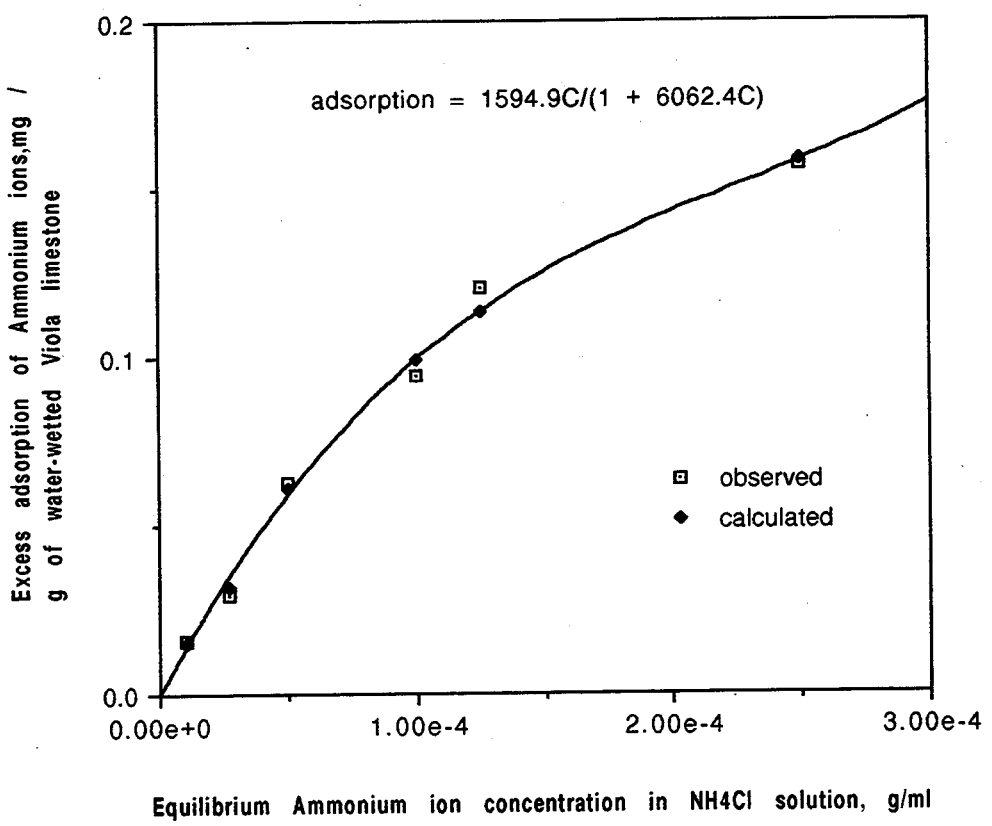


Figure 4 : Ammonium Adsorption on Oil-wetted Viola limestone

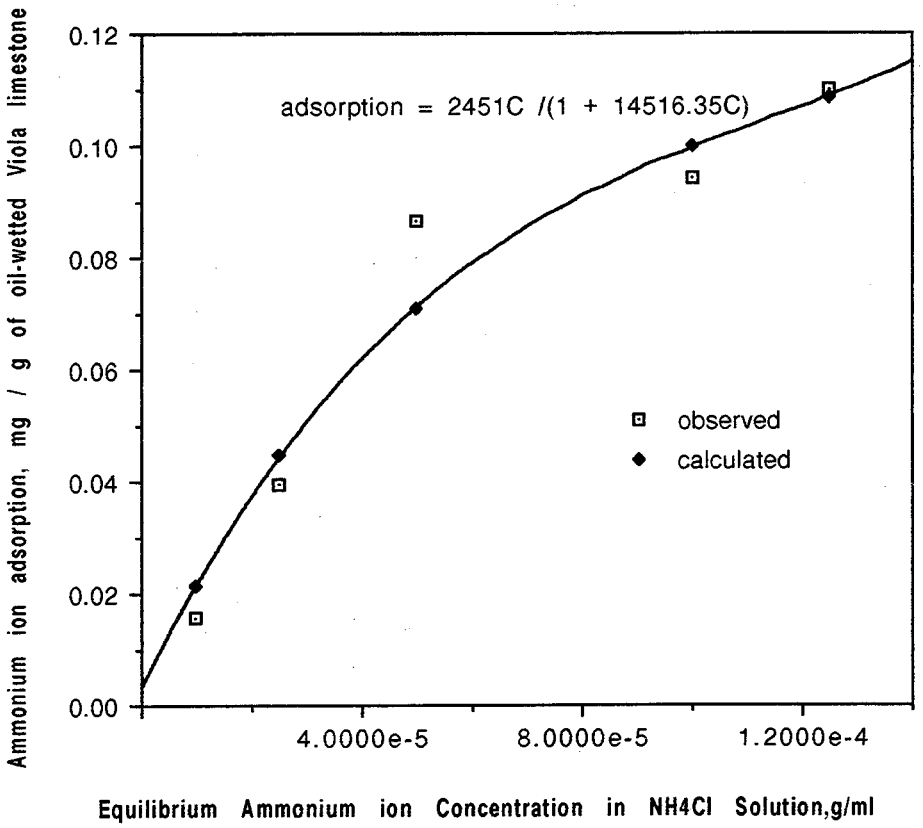
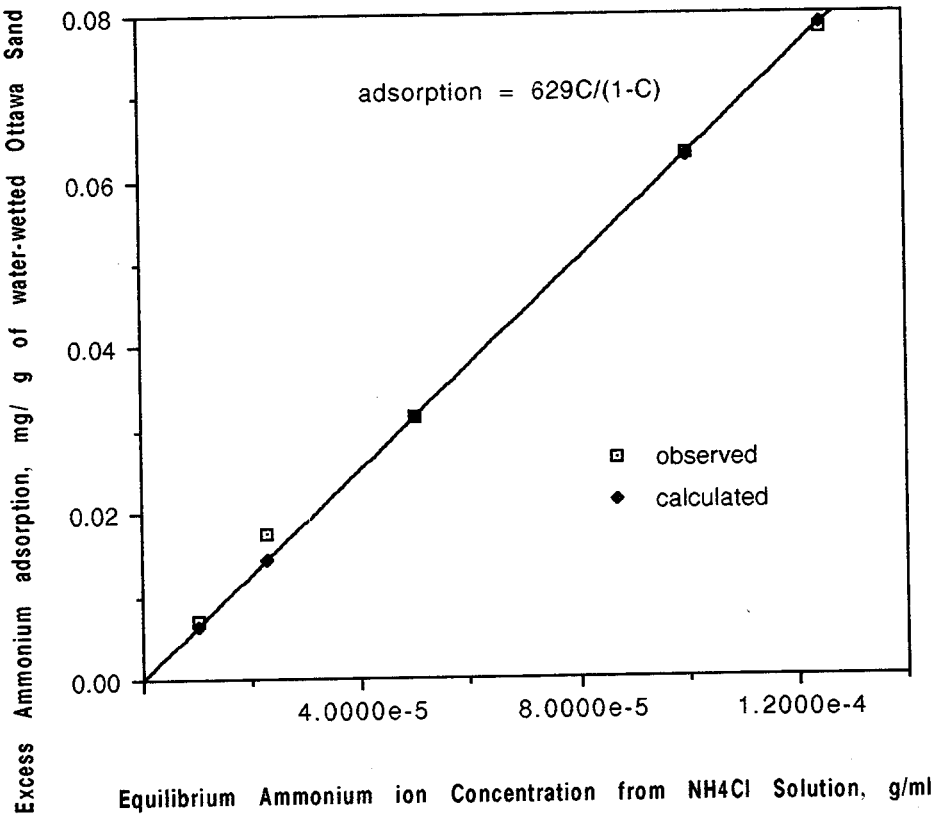


Figure 5: Ammonium adsorption on water-wetted Ottawa quartz sand



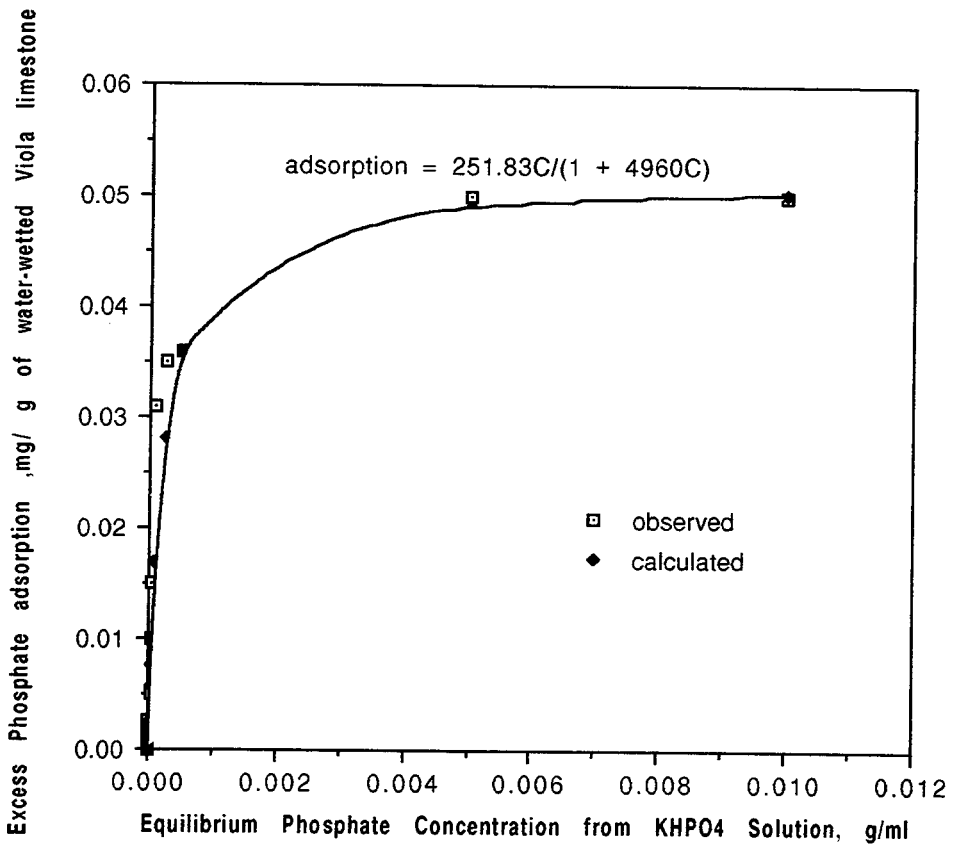


Figure 6: Phosphate Adsorption on water-wetted Viola limestone

Assessing the Effects of Microbial Metabolism and Metabolites on Reservoir Pore Structure

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ABSTRACT

The effect of microbial treatment on pore structure of sandstone and carbonate reservoirs was determined. Understanding how different bacterial strains and their metabolic bioproducts affect reservoir pore structure will permit the prudent application of microorganisms for enhanced oil recovery. The microbial strains tested included Clostridium acetobutylicum, a polymer-producing Bacillus strain, and an unidentified halophilic anaerobe that mainly produced acids and gases. Electrical conductivity, absolute permeability, porosity and centrifuge capillary pressure were used to examine rock pore structures. Modifications of the pore structure observed in the laboratory cores included pore enlargement due to acid dissolution of carbonates and pore throat reduction due to biomass plugging. This paper shows that careful selection of microbes based on proper understanding of the reservoir petrophysical characteristics is necessary for applications of microbially enhanced oil recovery. These methods and results can be useful to field operators and laboratory researchers involved in design and screening of reservoirs for MEOR. The methods are also applicable in evaluation of formation damage caused by drilling, injection or completion fluids or stimulation caused by acids.

INTRODUCTION

Applications of microbially enhanced oil recovery are increasing[1,2]. Microbially assisted well treatments are the most common. Well treatments involve wellbore cleaning, mitigation of paraffin deposits and coning in wells. In contrast, microbially enhanced waterflooding projects are fewer in number. Potential mechanisms for mobilization of remaining oil in the microbially enhanced waterflooding process include selective plugging by the cellular biomass and polysaccharides, and/or reduction of the oil/water interfacial tension (IFT) and oil viscosity by biogenic surfactants, solvents and gases. The results of most field trials of MEOR have been summarized by Hitzman[3] and Lazaar[4].

As in every case where extraneous fluids are introduced into petroleum bearing rocks, injection of microorganisms and/or nutrients into formations may affect subsequent injectivity and reservoir flow capability. Some applications of MEOR are associated with formation plugging[6,16]. This has obvious advantages in reservoirs requiring selective plugging for the recovery of bypassed oil[17]. Organic acids and carbon dioxide produced by microorganisms will dissolve rocks with a carbonate matrix. However, it is not known what effect the dissolution will have on pore structure and oil recovery. In carbonate systems where divalent cations are being dissolved as a consequence of dissolution of the rock matrix, there may be competing effects between pore structure modifications that result from the enlargement of pore throats and pores by biogenic acids and carbon

dioxide and from plugging caused by the biomass production and possible precipitation of divalent cations. Hence, it is critical that an evaluation of the effects of the selected microbial strains, substrates and metabolic products on the reservoir pore structure be conducted.

Here, the effects of three microbial strains and their metabolites on the pore structure of carbonate and sandstone rocks is examined.

Pore Structure Assessment Parameters

Formation damage caused by prior exposure to drilling, completion, and injection fluids or formation improvement caused by acid stimulation treatments has been historically and routinely assessed by dynamic core flow permeability tests[7,8]. As pointed out by Amaefule and Masuo[9], these tests can be time-consuming and expensive, especially when several samples of fluids and rocks are involved. Further, test fluids, if not properly selected, may interact with the test cores and introduce further pore structure alterations.

Several studies have confirmed that capillary pressure data can be useful for assessing modifications in pore structure because of their dependence on pore geometry. Permeability[10,11], pore size distribution[12], differences in lithology[13], and formation damage[9] have been evaluated from drainage capillary pressure data. Previous studies showed that sandstones with high water-sensitivity and mud-contaminated rock samples exhibited higher irreducible

water saturation[7,14]. Amaefule and Masuo[9] investigated the effects of extraneous injected fluids and acid stimulation with varying degrees of overflush on Berea and other sandstone rocks. This study confirmed the following:

1. damaged samples exhibited higher pore entry pressures (threshold pore pressure) and higher irreducible water saturation,
2. acidized core samples with adequate overflush resulted in lower pore entry pressures and lower irreducible water saturation, and
3. acidized core samples without adequate overflush exhibited the attributes of damaged cores.

The pore structure features that explain the above results are the ratio of pore size and pore throat size (the aspect ratio) and the pore structure connectivity described by its conductivity or its formation factor[15]. A large aspect ratio, a small pore throat size relative to pore size, increases the tendency of the phases to "snap off" and as a result leads to higher residual wetting phase saturations. Entrapment of fluids is reduced when there is high connectivity. In contrast, high residual saturations occur when the pore connectivity is low and is analogous to a high formation factor. Using percolation theory, Katz and Thompson[28] showed that rock permeabilities are intimately related to pore size and the pore structure conductivity. Since conductivity is the reciprocal

of formation factor (F), it can be shown that

$$K = C\frac{R_c^2}{F} \qquad (1)$$

where K = rock permeability, R_c = pore throat radius and

C = a constant.

Equation 1 implies that formation damage will lead to higher values of the formation factor (F) consequently, lower permeabilities, while formation improvement will give lower values of the formation factor and higher permeabilities. The formation factor can be conveniently deduced from resistivity logs or laboratory core measurements. In addition, using the Laplace equation that relates pore throat size, fluid/rock and fluid/fluid interactions to capillary pressure:

$$P_c = \frac{2\sigma\cos\theta}{\overline{R}} \qquad (2)$$

where R = average pore throat radius

σ = interfacial tension, and

θ = contact angle,

it can be seen that large average values of pore throat radius result in low pore entry pressure and higher permeability, and that even with large pore throat radii, pathways obstructed by fines or biomass lead to reduced connectivity (larger formation factor) and lower permeability.

EXPERIMENTAL WORK

Rock Selection

The rocks employed in these experiments were the Bethany Falls limestone, and the Okessa and Berea sandstones.

Samples of the **Bethany Falls** limestone (Mississippian) were collected from the McAdams Quarry near Mound City, Kansas. This limestone, an oolitic grainstone to packstone with vugular and intergranular porosity and heavy oil stains, has good reservoir properties which are locally preserved and is easily accessible at the McAdams quarry.

The **Okessa** sandstone (Pennsylvanian) is a buff-colored, fine-grained and slightly cemented formation which outcrops in the northeastern parts of Oklahoma (notably Osage County). It has been described by Hopkins[18], Oakes[19] and Tanner[20].

The **Berea** sandstone, available from a quarry in Ohio, is a well-known standard rock and is frequently used for laboratory studies.

Selection of microbial strains

Strain GSP-1 was isolated from the Great Salt Plains, Alfalfa County, Oklahoma. It is a gram-negative motile rod, obligately halophilic facultative anaerobe that produces acids and gas from glucose.

Bacillus **Strain SP018**[29], was isolated from the aquifer underlying the Norman, Oklahoma municipal landfill. It is a moderately halophilic, thermophilic, facultative anaerobe selected

for its ability to produce biopolymers.

Clostridium acetobutylicum is an anaerobic bacterium that produces acids, gases and solvents.

Media and Growth conditions

The basal media used in this study are described in Table 1. The trace metal and water soluble vitamin solutions are described by Tanner[5]. The cultures were grown at 37°C under static conditions.

Core Preparation

Core plugs of 2.54 cm diameter and 2.54 cm long were cut from Bethany Falls limestone and Okessa sandstone samples. The limestone plugs, which contained heavy oil stains, were cleaned with toluene in a Soxhlet extractor and oven dried at 125°C. The sandstone plugs were steam-cleaned for at least 10 days to remove organic matter and then oven dried. Re-saturation of the cores was carried out under vacuum with 2% brine (1.5% NaCl and 0.5% KCl or 0.5% NH_4Cl).

Growth Chambers

Growth chambers (Jenneman, et al.[27]) were used to study the effects of growth and metabolism on rock properties. The growth chambers consisted of two 250 ml Erlenmeyer flasks with a glass nipple fastened to the bottom of each flask. Stoppered growth chambers were autoclaved at 121°C for 20 min. Each end of a

sleeved core was then aseptically fastened to a flask with a hose clamp. A sterile, oxygen-free stream of nitrogen gas was flushed through the chamber for 15 min. Nutrient medium was then added to each flask, 100 ml in flask A and 90 ml in flask B, and vacuum was applied to draw 10 ml of medium through the core and establish liquid communication. The assembled core system was then placed in the incubator for 48 hours to check sterility. The core system was inoculated by adding 10 ml of a lag phase culture to flask A and the system was incubated for 10 to 14 days. Microbial penetration through the core was indicated by turbidity in flask B.

Core Measurements

Porosity, absolute permeability and formation resistivity measurements were measured for each core plug.

Porosity was measured gravimetrically. The absolute permeability was measured with both a gas permeameter with the dry core and using a liquid permeameter on the re-saturated or treated core plug. For comparing between the permeabilities measured before and after bacterial treatment, only the liquid permeability results are reported. In this way, any effects of core water sensitivity are eliminated from the effects of bacterial metabolism and metabolites. Core resistivity was measured using a Core Lab resistivity apparatus (Model CEF) on the brine- or media- saturated core plug. The formation resistivity factor was calculated by dividing the core resistivity by the resistivity of the brine or medium sample.

Ultracentrifuge Capillary Pressure

The ultracentrifuge method is the most rapid method for capillary pressure determinations and has several advantages over the mercury-injection and porous plate methods[9,21-26]. This method has been used to determine irreducible water and residual oil saturations in small core plugs with good precision and reproducibility[9]. Amaefule and Masuo also showed that damage or stimulation in rock samples can unmistakably be identified from variations in capillary pressure.

In the centrifuge capillary pressure measurement technique, brine or bacterial medium was drained from the core plug at various rotational speeds. The average brine or medium saturation in the core at a given rotational speed was determined from a material balance equation by using the displaced liquid volume:

$$\overline{S_w} = 1 - \frac{V_{wd}}{V_p} \qquad (3)$$

where V_{wd} = volume of brine or medium displaced from core plug

V_p = pore volume of the core plug

The capillary pressure corresponding to each average water saturation was determined using the angular velocities[9,24].

A number of techniques have been proposed to determine the point saturation values which correspond to the capillary pressures at the outer rotational radius of the core plug[24-26]. Rajan's approximate method[24,25] has been shown to be more accurate than other

approximate techniques and was used in this study to evaluate the point saturation values corresponding to the capillary pressures. To apply Rajan's approximate technique, the capillary pressure data were correlated with a modified Langmuir isotherm:

$$\overline{S_w} = \frac{a+bP_c}{1+cP_c} \qquad (4)$$

or a power-law equation:

$$\overline{S_w} = \frac{a}{P_c^b} + c \qquad (5)$$

where a,b,c = correlation parameters calculated from data with a Levenberg-Marquardt non-linear optimization method with a least-squares objective function.

RESULTS and DISCUSSION

The results of the core analyses are given in Tables 2 and 3 for the rock core samples and bacterial inocula used in this experiment. The capillary pressure index (given in Table 3) was defined by Amaefule and Masuo[9] as

$$J_n = \overline{r_h^2}(1-S_{wI}) \qquad (6)$$

where S_{wr} = residual wetting phase saturation

r_h = average pore radius corresponding to the pore entry pressure (at S_w = 100%)

Capillary pressure curves (Figs. 1 - 3) show the effect of each inoculum on the different types of rocks used.

Effects of GSP-1

Berea and Okessa sandstone

The Berea core sample showed a reduction in reservoir properties when treated with **GSP-1,** Figure 1 and Tables 2 and 3. The porosity of the sample was reduced from 18.9% to 15%, and liquid absolute permeability from 75 md to 10.6 md, while irreducible water saturation increased from 20.1% to 26.6% and the formation factor from 18.4 to 22.8. A similar trend was found in the Okessa sandstone. Absolute permeability and porosity decreased from 161 md to 103.5 md and 23.7% to 17.2% respectively and the irreducible water saturation and formation factor increased from 11.4% to 37.9% and 19.7 to 29.1 respectively. The increases in the irreducible water saturation and the formation factor, and capillary pressure index are indications that plugging occurred as expected the permeability reduction factors (K/K_o) were 0.14 and 0.64 showing that the bacterium could effectively plug thief zones, a function previously performed with more expensive polymer.

Bethany Falls limestone

The effects of **GSP-1**, shown in Figure 1 and Tables 2 and 3, included pore plugging due to the biomass and metabolites and matrix dissolution due organic acids produced by it. Expected metabolic products of **GSP-1** included organic acids and carbon dioxide. An experiment using carbonate chips in glass tubes showed that bacterial acids produced by **GSP-1** dissolved the carbonate material resulting in weight loss of the carbonate material and gas production due to the dissolution of the carbonates. The post treatment water saturation is less than the pretreatment values indicating an improvement of pore properties.

As shown in Table 2, the porosity of Bethany Falls core plugs increased or remained essentially the same. A decrease in permeability occurred in two core plugs, BF-16 and BF-23. These liquid-permeability measurements were performed on the cores without flushing them of loose divalent cations or biomass. To distinguish the pore enlargement effects of carbonate matrix dissolution by microbial acid production from pore plugging caused by biomass and fines, the gas permeability values of core No. BF-6 were used. No gas flow was obtained in core BF-6 before the bacterial treatment with the **GSP-1** strain. Post-treatment, the permeability was measured on a gas permeameter after centrifugation. It is believed that biomass and dissolved divalent cations impending flow were driven out of the core by centrifugation. A permeability increase from essentially zero to 4.7 md was obtained in core BF-6 after bacterial treatment. This shows that microbial acid production can result in the enlargement

of pores in carbonate rock and that this overcomes any reduction due to plugging.

The capillary pressure index is a useful measure of formation damage in the absence of absolute permeability values. A decrease in capillary pressure index indicates formation damage while an increase indicates an improvement. Amaefule and Masuo[9] correlated the permeability of sandstones on the basis of these indices. However, it appears that this relationship does not hold for carbonates as shown on Table 3. This lack of relationship is attributable to the complex structure of carbonates caused by multiple diagenetic processes.

Effects of Clostridium acetobutylicum

Products expected from the metabolism of glucose by *C. acetobutylicum* include carbon dioxide, hydrogen, butyrate, acetate, acetone, various alcohols and biomass. The effects of *C. acetobutylicum* and its metabolites on the capillary curves of Okessa sandstone and Bethany Falls limestone are shown in Figure 2. The post treatment irreducible water saturation is higher for the Bethany Falls limestone indicating a net decrease in pore properties. This result is also confirmed by porosity and permeability reductions (Table 2). The average permeability reduction factor (K/K_0) was 0. 075 in the Bethany Falls limestone. However, as mentioned above, it is not possible to explain any changes in pore properties on the basis of the capillary pressure index measurements for Bethany Falls limestone (Table 3).

There is a reduction in porosity and permeability in Okessa sandstone as shown in Table 2. Table 3 shows reduced capillary pressure index which is also an indication of pore plugging. However, the post-treatment irreducible water saturation (Figure 2) is less than the pre-treatment value. This result is contrary to the expected trend but may be explainable. The pre-treatment brine solution does not contain ammonium nor any potassium ions. These were added to the media for their stabilizing effect on clay. Okessa sandstone contains anthegenic clays and a high percentage of iron oxide. It is thought that the long incubation of the core plug affected the clay and reduced its ability to retain water. However, Okessa sandstone samples were plugged when inoculated with C. acetobutylicum.

Effects of SPO18 Inoculum

SPO18 strain plugged Bethany Falls limestone and Okessa sandstone samples used in the experiments as shown by permeability and porosity reductions in Table 2. The reduction in the capillary pressure indices of Okessa sandstone after treatment with SPO18 (Table 3) also indicated pore plugging. However, the post-treatment irreducible water saturation results shown in Figure 3 do not follow the expected trends. In each core, there was an increased entry pressure due to pore plugging by SPO18.

Bacillus strain **SP018** is a polysaccharide producer and is more effective in pore plugging than the C. acetobutylicum or **GSP-1** strains. On the average the permeability reduction factor (K/K_o)

was 0.02 for cores treated with SPO18.

CONCLUSIONS

1. The growth of each microorganism in Okessa sandstone reduced the permeability and shifted the capillary pressure curve to the left indicating that larger pores or pore entrances were plugged.
2. A polymer-producing bacterium was the most effective plugging agent.
3. In the Bethany Falls carbonate rock the effect of microbial growth and metabolism depended on competing processes between permeability reduction due to plugging and permeability improvement due to microbial and acid/gas production.
4. In carbonates, the capillary pressure index may not be correlated to permeability damage.

References

1. Nelson, S. J. and P. D. Launt, "Stripper well production increased with MEOR treatment", Oil and Gas J., **89**:114-118, March 18, 1991.

2. Bryant, R. S., Screening Criteria for Microbial EOR Processes. NIPER Report No. NIPER-478, 17pp, 1990.

3. Hitzman, D. O., "Review of Microbial Enhanced Oil Recovery Field Tests" In: Proceedings of the Symposium on Applications of microorganisms to Petroleum Technology, US DOE Report No. NIPER- 351, Sept. 1988.

4. Lazar, I., "Preliminary results of some recent MEOR field trials in Romania," in Proceedings of the International Conf. on MEOR, Norman, Oklahoma, May 27 - June 1, 1990.

5. Tanner, R. S., "Monitoring sulfate-reducing bacteria: comparison of enumeration media", J. Microbiol. Methods, **10**:83-90, 1989.

6. Jenneman, G. E., M. J. McInerney and R. M. Knapp, "Microbial penetration through nutrient-saturated Berea sandstone", Appl. Environ. Microbiol., **50**:383-391, 1985.

7. Hewitt, C. H., "Analytical techniques for recognizing water sensitivity reservoir rocks", **J. Pet. Tech.**, **15**:813-818, 1963.

8. Baptist, O. C. and S. A. Sweeny, "Effects of clays on the permeability of reservoir sands to various saline waters, Wyoming." Report of Investigation No. 1, 5180 U.S. Bureau of Mines, 1955.

9. Amaefule, J. O. and S. T. Masuo, "Use of capillary-pressure data for rapid evaluation of formation damage or stimulation." **SPE Production Eng. J.**, pp.131-142, March, 1988.

10. Purcell, W. R., "Capillary pressures - their measurements nusing mercury and the calculation of permeability therefrom." **Trans. AIME**, **186**:39-48, 1945.

11. Swanson, B. F., "A simple correlation between permeabilities and mercury capillary pressures", **J. Pet. Tech.**, p.2498-2505, Dec. 1981.

12. Burdine, N. T., L. S. Gournay and P. P. Reichertz, "Pore size distribution of petroleum reservoir rocks", **Trans. AIME**, **189**:195-202, 1950.

13. Udegbunam, E. O., R. M. Knapp, M. J. McInerney and R. S. Tanner, "Potential of Microbial Enhanced Oil Recovery (MEOR) in the Petroleum Reservoirs of the Mid-Continent Region", presented at the 1991 Oklahoma Geological Survey Symposium held in Norman, Oklahoma, March 26-27, 1991.

14. Glen,E. E. and M. L. Slusser, "Factors affecting well productivity II. Drilling fluid particle invasion into porous media", **Trans. AIME, 210**, 1957.

15. Hagoort, J., "Measurement of relative permeability for computer modeling/reservoir simulation", **Oil and Gas J.**, p.62-68, Feb. 20, 1984.

16. Jack, T. R., J. Shaw, N. Wardlaw and J. W. Costerton, "Microbial plugging in enhanced oil recovery", Chapter 7 in **Microbial enhanced oil recovery:** E. C. Donaldson, G. V. Chiligarian and T. F. Yen (eds.), Elservier Science Publishers, New York, 1989.

17. Knapp, R. M., M. J. McInerney, D. E. Menzie and J. L. Chisholm, Microbial Field Pilot Study, Annual Report performed under DOE Contract no. DE-FG22-89BC14246, 96pp, 1989.

18. Hopkins, O. B., **U. S. Geol. Survey Bull. 686-H**, p. 76-77, 1919.

19. Oakes, M. C., **Tulsa Geol. Soc. Digest, 19:**120, 1951.

20. Tanner, W. F., **Oklahoma Geol. Survey Circ. 40,** p.30-32, 1951.

21. Hassler, G. L. and E. Brunner, "Measurement of Capillary pressures in small core samples", **Trans. AIME, 160:**114-123, 1945.

22. Melrose, J. C., "Interpretation of Centrifuge capillary pressure data", **The Log Analyst, 29:**1, 1988.

23. Omoregie, Z. S., "Factors affecting the equivalency of different capillary pressure measurement techniques", **SPE Formation Evaluation**, p.145-155, 1988.

24. Ayappa,K. G., H. T. Davis, E. A. Davis and J. Gordon, "Capillary pressure: centrifuge method revisited", **AIChE J., 35:365-372**, March, 1989.

25. Rajan, R. R., "Theoretically correct analytical solution for calculating capillary pressure-saturation from centrifuge experiments", **SPWLA Trans.**, 27th Annual Logging Symposium, Houston, Texas, Paper O, June 9-12, 1986.

26. Ruth, D. and S. Wong, "Centrifuge capillary pressure curves", **J. of Can. Pet. Tech.**, **23**:67-72, no. 3, 1990.

27. Jenneman, G. E., R. M. Knapp, D. E. Menzie, M. J. McInerney, D.E. Revus, J. B. Clark and D. M. Munnecke, "Transport and plugging in Berea using microorganisms", In: Proceedings of the 1982 International Symposium on MEOR, E. C. Donaldson and J. B. Clark (eds.), Dept. of Energy, Bartlesville, Oklahoma, 71-75, 1983.

28. Katz, A. J., and A. H. Thompson, "Quantitative prediction of permeability in porous rocks", Physical Review B: The American Physical Society, **34**:8178-8181, 1986.

29. Pfiffner, S. M., "Isolation of halotolerant, thermophilic, facultative, biopolymer producing bacteria: characterization and purification of strain SPO18 exopolymer", M. S. Thesis, The University of Oklahoma, 1985.

Table 1. Media Used for Dual Flask Growth Experiments

Components	Grams/liter GSP-1	SP018	C.acetobutylicum
NaCl	100	100	0.8
NH_4Cl	1.0	1.0	1.0
KCl	0.1	0.1	0.1
KH_2PO_4	0.1	0.1	0.1
$MgSO_4 \cdot H_2O$	0.2	0.2	0.2
KNO_3	0.0	1.0	00
TES buffer	10	10	10
Glucose	20	20	20
Yeast Extract	0.2	0.5	5
Trace Metal Solution	5 ml	5 ml	5 ml
Vitamin Solution	10 ml	10 ml	10 ml

Table 2 - Results of Core Analyses Before and After Bacterial Treatment

Inoculum	Core Sample	Pre-treatment ϕ,%	Pre-treatment K,md	Pre-treatment F	Pre-treatment S_{wr},%	Post-treatment ϕ,%	Post-treatment K,md	Post-treatment F	Post-treatment S_{wr},%
GSP-1	Berea	18.9	75	18.4	20.1	15.0	10.6	22.8	26.6
GSP-1	OK-7#	23.7	161	19.7	11.4	17.2	103.5	29.1	37.9
GSP-1	BF-6*	11.0	<0.1	27.2	73.8	15.7	4.7	65.8	39.9
GSP-1	BF-16	6.8	6.6	56.0	22.8	13.5	0.42	47.5	59.5
GSP-1	BF-23	17.0	18.5	41.4	56.7	16.4	1.11	38.6	53.9
Clost.	OK-1	15.5	242	19.0	30.3	13.2	58.0	11.5	11.2
Clost.	OK-2	16.0	312	17.3	-	-	32.4	10.2	-
Clost.	BF-8	18.7	24.8	64.3	26.3	19.8	1.6	18.0	25.8
Clost.	BF-9	17.2	51.5	92.2	38.4	16.0	2.8	27.2	36.3
Clost.	BF-10	18.7	16.0	169	24.0	18.0	1.7	21.7	66.3
SPO18	OK-5	17.4	405	17.7	22.8	14.5	13.1	17.5	11.2
SPO18	OK-6	18.2	396	16.7	14.6	16.6	10.4	19.2	16.7
SPO18	BF-13	17.3	19.3	79.4	28.6	15.6	0.2	60.0	62.7
SPO18	BF-14	16.0	13.9	77.8	20.0	13.5	0.5	40.0	42.3
SPO18	BF-15	20.4	17.1	38.9	34.7	15.9	0.3	39.2	66.8

OK# - Okessa sandstone
BF* - Bethany Falls limestone

Table 3: Capillary Pressure Indices of Various Rocks Before and After Bacterial Treatment

Inoculum	Core Sample	Pre-treatment K,md	Pre-treatment J_n	Post-treatment K,md	Post-treatment J_n
GSP-1	Berea	75	2800	10.6	1127
	OK-1	161	6960	103.5	1445
	BF-16	6.6	3183	0.42	4.6×10^6
Clostridium	OK-1	242	7195	58	1073
	BF-8	24.8	2170	1.6	28430
SPO-18	OK-5	405	124	13.5	51
	OK-6	396	161	10.4	63
	BF-14	17.1	1515	0.5	1794
	BF-15	13.9	4376	0.3	960

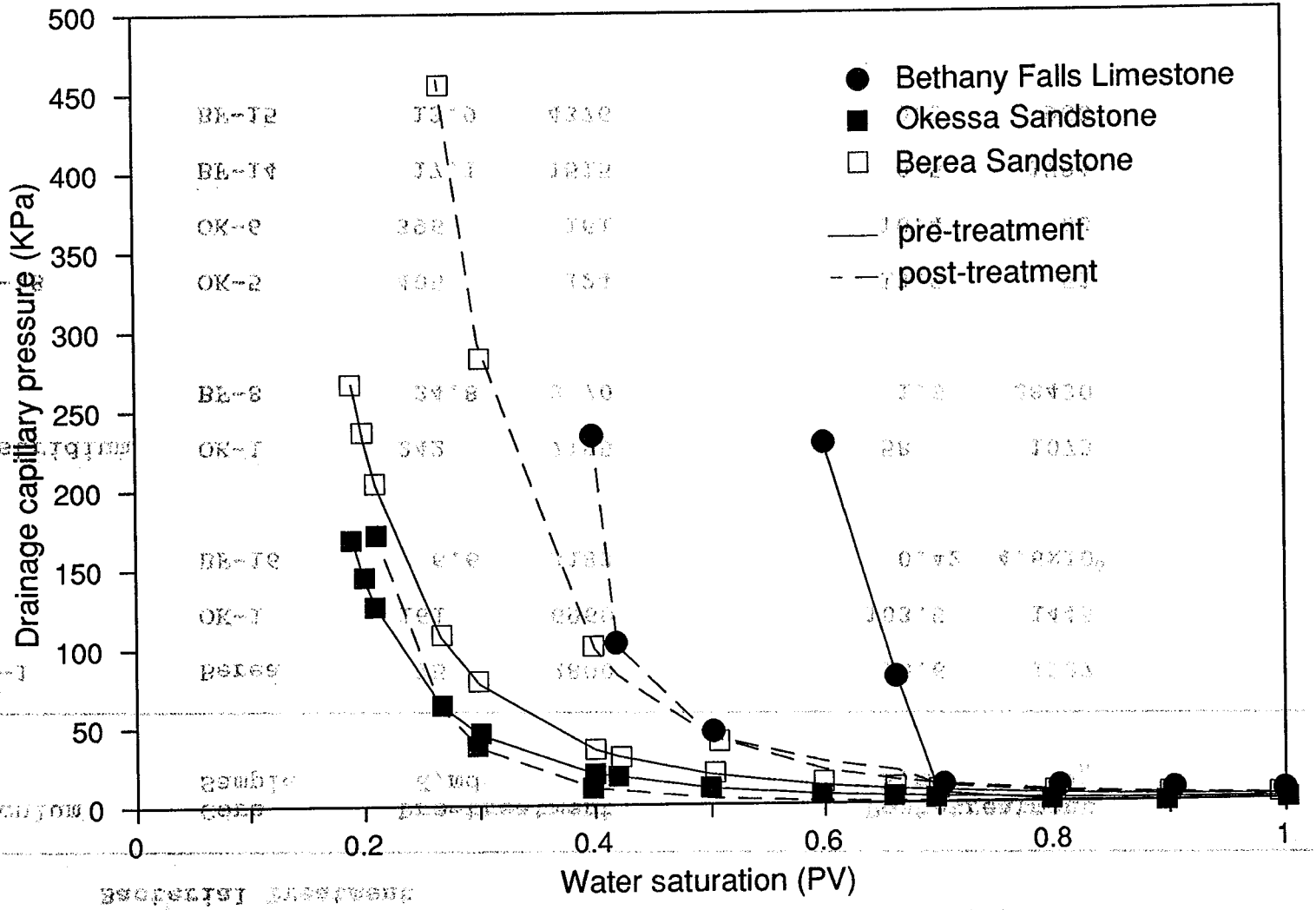

Figure 1. Capillary Pressure Curves Showing Effects of GSP-1 and Its Metabolites on Pore Structure of Rocks.

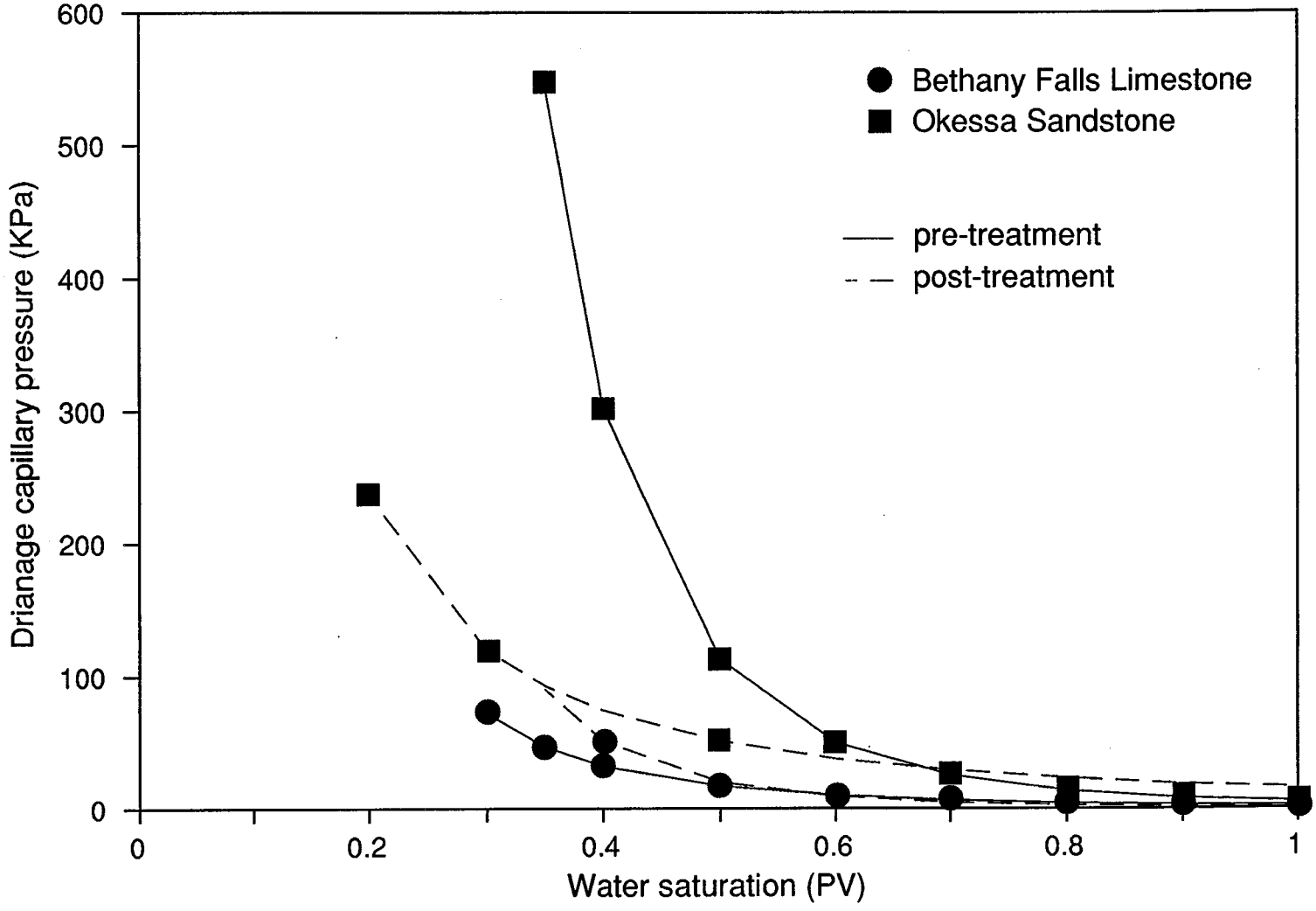

Figure 2. Capillary Pressure Curves Showing Effects of C. acetobutylicum and Its Metabolites on Pore Structure of Okessa Sandstone and Bethany Falls Limestone.

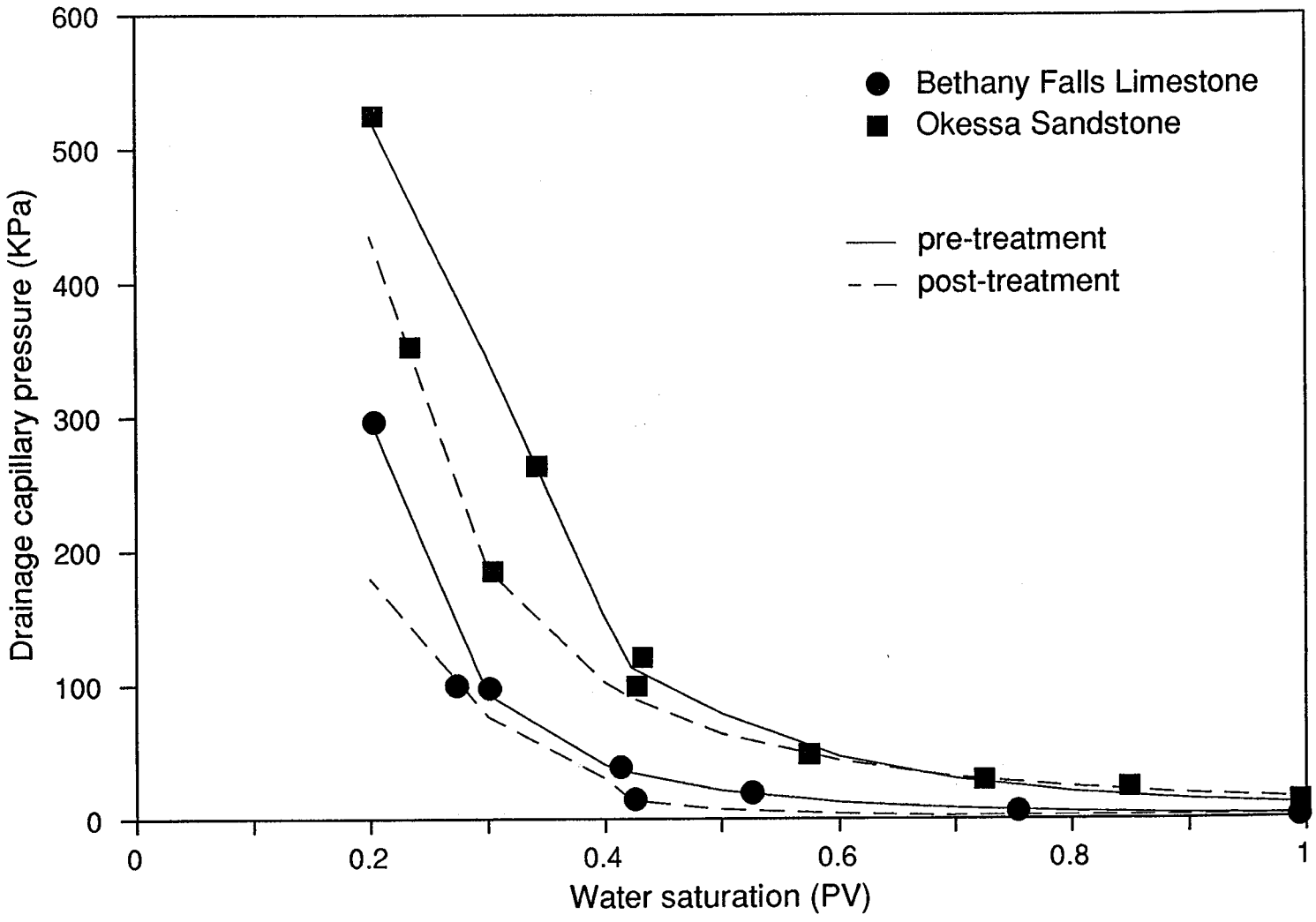

Figure 3. Capillary Pressure Curves Showing Effects of SP018 Strain and Its Metabolites on Pore Structure of Bethany Falls Limestone and Okessa Sandstone.

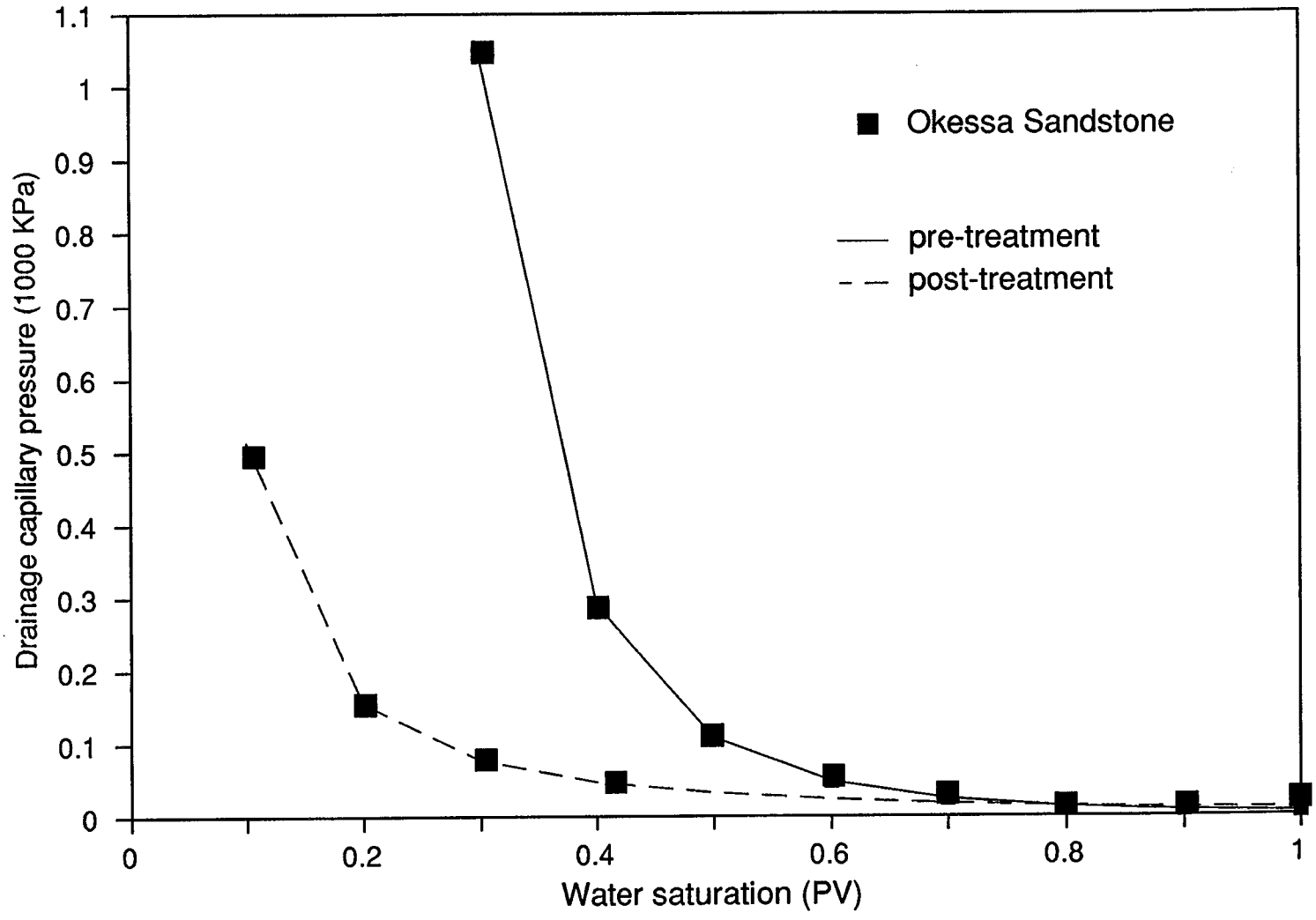

Figure 4. Capillary Pressure Curves Showing Effects of Bacillus licheniformis (JF-2) and Its Metabolites on Pore Structure of Okessa Sandstone.

MICROBIALLY ENHANCED OIL RECOVERY FROM UNCONSOLIDATED CARBONATE CORES

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ABSTRACT

A series of experiments designed to study oil recovery mechanisms likely to mobilize residual oil from unconsolidated carbonate cores was conducted. Work was done in model cores packed with chips of Viola limestone. Strains GSP-1, an acid and gas producer,and *Bacillus licheniformis* JF-2, a surfactant producer, were used as the test microorganisms. The primary hypothesis tested was that microbial treatment of a packed core system would result in the recovery of residual oil from the carbonate test system. After three treatments, 43% of the residual oil-in-place was recovered from a packed core system with strain GSP-1.Two treatments with strain JF-2 resulted in a 32% recovery of the oil-in-place. Modification of the core matrix was indicated by the presence of calcium in the core effluents and significant amounts of carbonate fines found in the dissected cores, presumably caused by metabolic acid dissolution of the carbonate matrix.

MATERIALS AND METHODS

Microorganisms. Strain GSP-1, an acid-producing obligately halophilic facultatively anaerobic microorganism isolated from sediment from the Great Salt Plains, was used as the primary test organism. GSP-1 ferments glucose producing organic acids, ethanol and gas. The strain grows in the presence of crude oil; cultures grown in the presence of crude oil exhibit some emulsification of the oil. GSP-1 is a highly motile rod often found in pairs with one cell possessing a single polar flagellum. The identified end-products of metabolism are lactic acid, formic acid, pyruvic acid, and ethanol, which may have a solvent effect in MEOR. The strain produces an unknown acidic end-product of metabolism, which is found at higher concentrations in the presence of $CaCO_3$.

Bacillus licheniformis JF-2, a surfactant-producing microorganism, was tested in one packed core. *B. licheniformis* JF-2 also produces organic acids from glucose.

Limestone. Viola limestone rock samples were obtained from a quarry near Davis, Oklahoma. These were crushed and sized to 20-50 mesh. The resulting chips were washed extensively with 10% (w/v) sodium chloride brine, pH 7, to remove fines. the chips were dried in an 80°C oven for at least three days, and then held in a 105°C oven for 30 minutes to reduce or eliminate any microorganisms.

Construction of test cores. Packed carbonate cores were constructed from two inch polyacrylate tubing (Regal Plastics, Oklahoma City, Oklahoma) mated with machined aluminum end plates. Viola limestone chips were soaked in a 10% NaCl brine, pH 7, for water-wet systems or in hexadecane or 37° API crude oil,obtained from the Pharoah #15-3A well, for oil-wet systems. Basic data for the packed limestone cores is given in Table 1. For core PV-1, which was prepared water-wet, the

pack was filled with hexadecane as a model oil, incubated 48 hours, waterflooded with a 10% NaCl brine, flushed with medium and and inoculated with strain GSP-1. The oil-wet cores were packed with chips, flushed with oxygen-free, sterile N_2 gas for 30 minutes, filled with crude oil, incubated 48 hours, waterflooded with a 10% NaCl brine, pH 7, , flushed with medium and inoculated. Test cores were incubated at 37°C, monitored continuously for pressure, and the effluents examined for glucose consumption, acid production, calcium and pH. Volumes of oil and gas produced from each treatment was also measured.

Table 1. Packed Viola Limestone Core Data.

	PV-1	PV-2	PV-3	PV-4	PV-5
Wetting Condition	WW[a]	OW	OW	OW	OW
Oil	hexadecane	crude	crude	crude	crude
S_{OR}	0.266	0.397	0.342	0.253	0.416
Permeability (mD)	22	21	18	38	22
Porosity	35%	52%	45%	44%	50%
Length (cm)	50	25	25	25	25
Inner Diameter (cm)	4.4	4.4	4.4	4.4	4.4
Pore Volume (ml)	282	196	170	166	190

a WW=water-wet, OW=oil-wet.

RESULTS AND DISCUSSION

Core PV-1. This core was packed with water-wet Viola limestone chips and used hexadecane as a model oil for the MEOR experiment. The pressure data collected over the coarse of five treatments is given in Figure 1. The core was only inoculated for the first treatment: cells remaining in the core after brine flooding and the addition of new medium served as a source of inoculum for the subsequent treatments. Monitoring of the core effluents by HPLC indicated that GSP-1 remained the dominant microorganism in this system. As indicated by the pressure measurements, metabolism in the core occurred at a faster rate from treatment one to treatment two to treatment three. This was a result of biomass building up in the system, which was also observed visually in the core effluents. Glucose was consumed in the core, organic end-products of metabolism were formed, and calcium was released from the limestone matrix as shown in Table 2. Further evidence of the dissolution of the rock matrix was the appearance of fines after breakdown of the core system.

In this, and the other core experiments, the pressure observed in the cores was primarily due to the microbial production of gas as an end-product of metabolism. Carbon dioxide was the gas detected by gas chromatography of produced gas samples. Also, in general, the dissolution of the limestone matrix, indicated by the appearance of calcium ions in the effluents, correlated to a degree with the formation of organic acid end-products of metabolism. This is consistent with the model that microbially produced acid reacts with carbonates. The pH of all the effluents were essentially neutral, ranging from 6.8-7.1. This supports the hypothesis that the pH of carbonate reservoirs will tend toward neutrality as long as carbonate remains present to react with the acids.

Figure 1. Pressure vs. Time Data for PV-1.

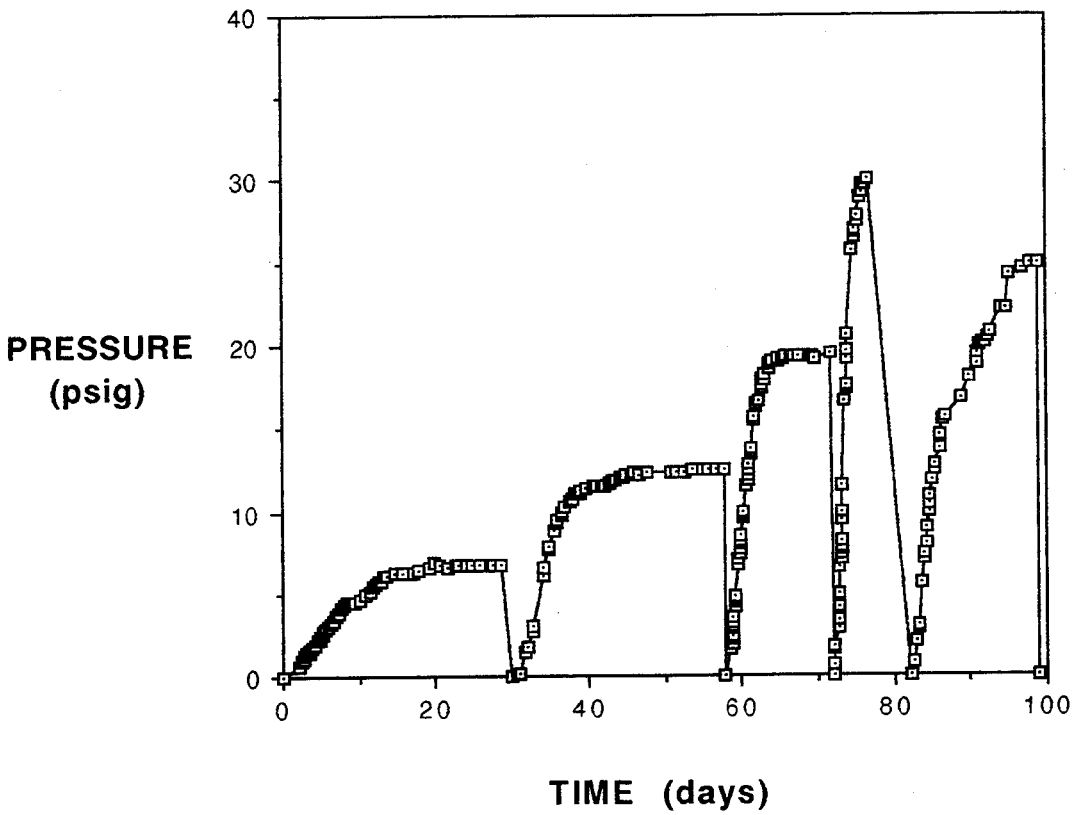

Table 2. Core Effluent Analysis Results from Packed Viola Limestone Cores.

TREATMENT	Glucose Consumed	millimoles / Liter Acid Produced	Dissolved Ca++
PV-1A	43	27	15
PV-1B	48	39	15
PV-1C	50	38	20
PV-1D	49	50	27
PV-1E	50	35	17
PV-2A	44	30	16
PV-2B	50	39	17
PV-2C	48	50	28
PV-4A	12	13	7
PV-4B	27	15	7
PV-5A	17	10	4
PV-5B	19	11	5

Additional hexadecane was not recovered after the microbial treatments of core PV-1. Upon dismantling of the packed core system after the fifth and final treatment, it was determined that essentially no hexadecane remained in the test core after the initial brine flood. For the further testing of MEOR in this laboratory model, an oil-wet limestone system was employed using a 34° API gravity crude oil rather than hexadecane.

Cores PV-2, PV-3 and PV-4. Details on the physical parameters of these packed cores are given in Table 1. PV-2 was treated with strain GSP-1. PV-3 was treated with the medium for GSP-1 alone and not inoculated; this was a control for biological activity. PV-4 was treated with spent medium from a culture of GSP-1 to evaluate the effect of organic acids in the absence of gas production.

The pressure verses time data for the initial treatments on cores PV-1, PV-2, and PV-3 are given in Figure 2. The two cores inoculated with GSP-1 responded similarly. Core PV-3 was not inoculated with any culture. However, pressure did build up in the core, especially after 260 hours of incubation. This was due to biological activity, although not to an microorganism that catabolized glucose or produced organic acid end-products of metabolism. The pressure data observed for core PV-4 is given in Figure 3. This packed core was treated with filter-sterilized spent medium from a culture of GSP-1. Core PV-4 was also not inoculated with any cells, but gas production occurred and this was due to biological activity. Glucose was consumed, organic acid end-products of metabolism were detected in the core effluent, and cells were observed in the supernatant.

As shown in Table 3, some residual oil-in-place after waterflooding was recovered after each MEOR treatment in cores PV-2, PV-3, and PV-4, even though the latter two were not inoculated. The cumulative oil-in-place recovered from the core

Figure 2. Pressure vs. Time Data for First Treatments.

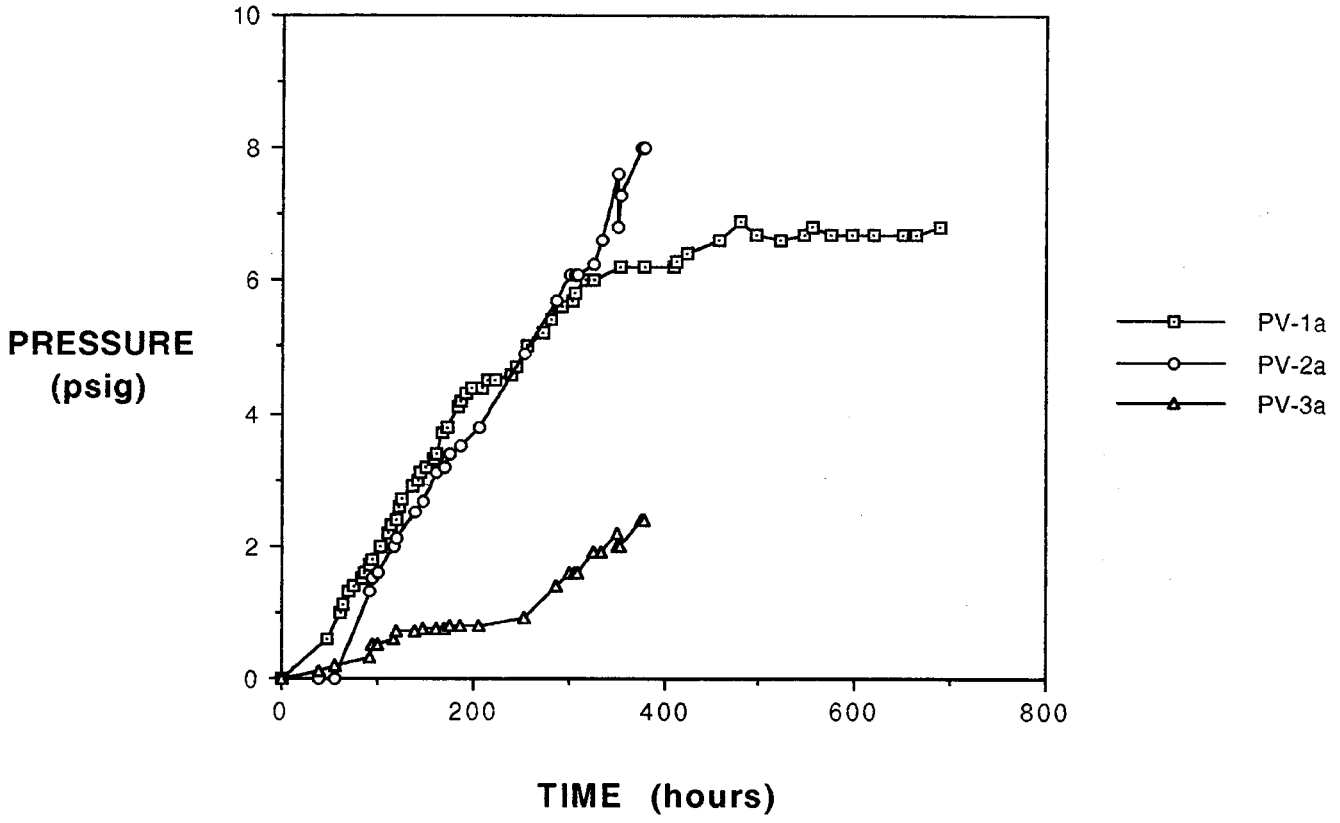

Figure 3. Pressure vs. Time Data for GSP-1.

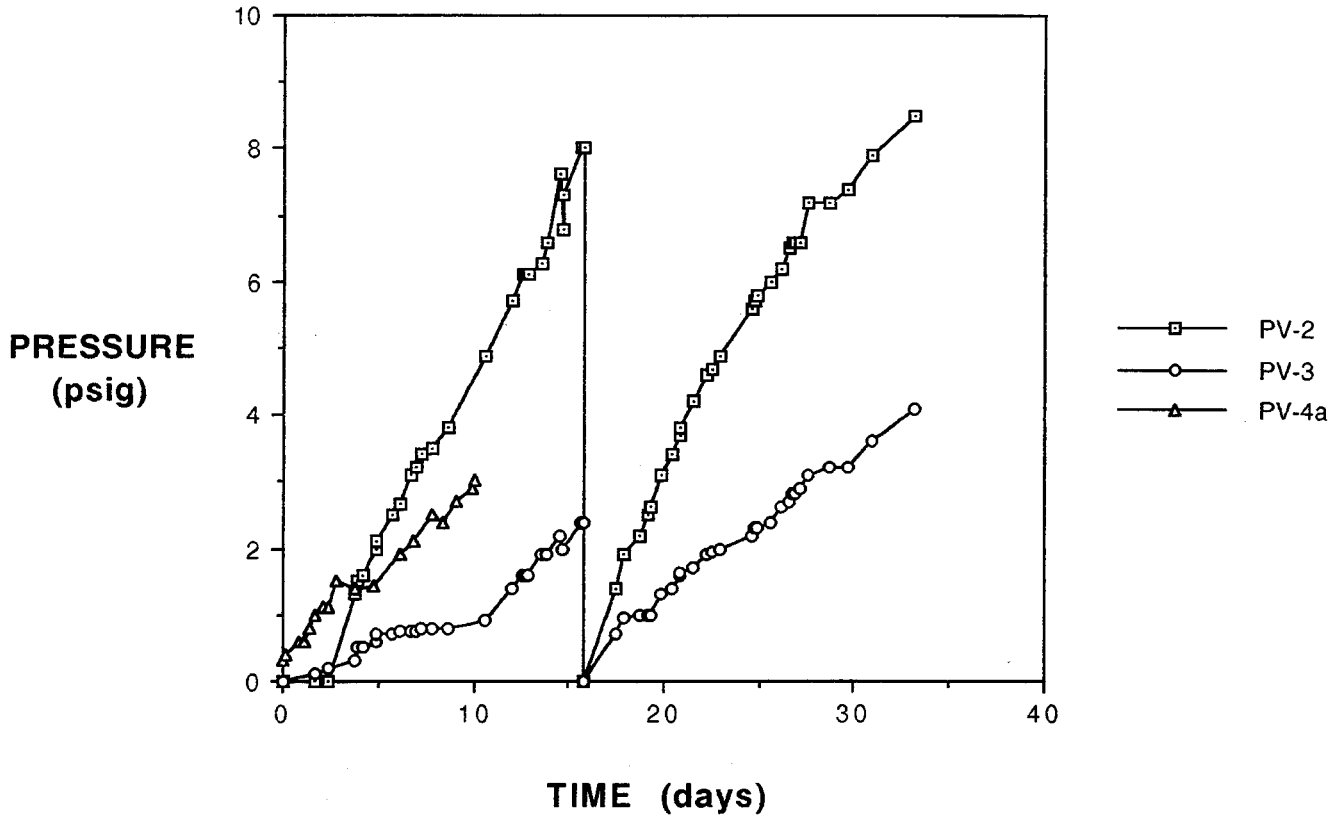

Table 3. Oil Recovery Data from Viola Limestone Chip Packs.

	CORE			
	PV-2	PV-3	PV-4	PV-5
Incubation	GSP-1	Medium	GSP-1 spt.	JF-2
Pore Volume (ml)	196	170	166	190
Residual Oil (S_{OR})	39.7%	34.2%	25.6%	41.6%
Oil In Place (OIP, ml)	77.8	58.1	42.0	79.0
TREATMENT 1				
Oil Recovered (OR, ml)	12	5	6	14
OR (% OIP)	15%	8.6%	14.3%	17.7%
OR (over control)	6.4%		5.7%	9.1%
TREATMENT 2				
OR (ml)	14	6	7	11
OR (%OIP)	18%	10.3%	16.7%	13.9%
Cumulative OR (%OIP)	33%	18.9%	31%	31.6%
OR (over control)	7.7%		6.4%	3.6%
Cum. OR (over control)	14.1%		12.1%	12.7%
TREATMENT 3				
OR (ml)	8	4		
OR (%OIP)	10.3%	6.9%		
Cumulative OR (%OIP)	43.3%	26.8%		
OR(over control)	3.4%			
Cum. OR (over control)	17.5%			

treated with GSP-1 was 15%, 33%, and 43% after the first, second, and third treatments respectively. These results are impressive. The probable mechanisms of MEOR in this test are acid production resulting in dissolution of the carbonate rock matrix, CO_2 production, which probably dissolved to some extent in the residual crude oil, and ethanol production, which could act as a solvent for oil extraction.

Core PV-3 was intended as a control, therefore, the oil recovery data from cores PV-2, PV-4, and PV-5 was analyzed against the oil recovered from PV-3. However, core PV-3 was not a true control since biological activity did occur in this core. This was not unexpected given difficulties of sterilizing limestone without altering the rock and given the inherent difficulties in maintaining these laboratory units aseptically for many hundreds of hours under basic conditions favoring microbial growth. Analysis of the core effluent from core PV-3 showed that essentially no glucose was utilized in the core and organic acid end-products were not detected. The incubation medium used to flood the cores contained 0.1% yeast extract; it is likely that the microorganisms in core PV-3 metabolized components, probably amino acids, of the yeast extract.

Packed core PV-4 was treated with filter-sterilized spent medium from a culture of GSP-1. We intended to evaluate the effects of the organic acids and ethanol produced by GSP-1 on oil recovery independent of the effects of CO_2 production and localized growth of the culture. However, flooding PV-4 with filtered supernatant from a culture of GSP-1 did not result in any immediate production of crude oil from the core. As shown in Figure 3, once shut in gas production in the core due to biological activity occurred within a few days. Some of the glucose remaining in the culture medium was consumed in core PV-4 and organic acid end-products of metabolism were produced (Table 2). The microorganism growing in this core was different from strain GSP-1, but the percent oil recovery observed in core PV-4 after two treatments was very similar to that observed for core PV-2, which was inoculated with strain GSP-1.

Packed core PV-5 was inoculated with *B. licheniformis* JF-2, a surfactant-producing microorganism. This eubacterium also produces acid and gas as end-products of glucose metabolism. Pressure data was not recovered from this core due to a faulty fitting. The cumulative oil-in-place recovered from this core after the first and second treatments was 17.7% and 31.6% respectively. The potential effect of surfactant production by strain JF-2 has not yet been determined. However, it seems clear that crude oil was released after microbiological activity occurred in these carbonate systems. Some evidence for this observation was that initial flooding of packed core PV-4 with spent culture medium of GSP-1 did not release detectable amounts of oil; this only occurred after the core was shut in and microbial activity took place. Also, the recovery of residual oil in cores PV-2, PV-3, PV-4, and PV-5 was effected by a different microorganism in each case. Organic acid and calcium ions were not detected in the effluent from packed core PV-3, suggesting that another mechanism other than acid production was responsible for MEOR. Biomass production in these packed core systems did not appear to interfere with oil recovery.

Mathematical Modeling of Bacterial Treatment of Carbonate Reservoirs

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Introduction

Many oil reservoirs are targets for improved oil recovery techniques. MEOR may be cost effective, but understanding it is still in the early stages.

Several potential mechanisms for oil recovery from reservoirs are possible with MEOR:

- selective plugging of highly permeable zones,
- pressurization by and oil mobility increase from biogenic gas,
- wettability alteration and interfacial tension (IFT) reduction, and
- possible pore enlargement.

Little work has been done on mathematical modeling of the microbial enhanced oil recovery processes[1,2,3]. Numerical prediction of MEOR processes is desirable for the purposes of design and evaluation of both laboratory and field projects. Mathematical modeling of bacterial processes is difficult because bacterial metabolic interactions within mixed populations in open systems are not well understood[4]. Microbial transport and substrate propagation may be easily modelled; however, microbial metabolism and growth, substrate consumption and generation of bioproducts depend on

parameters which are site-specific and must be empirically determined. Further, the effects of bacterial bioproducts on the porous medium are not restricted to plugging. There is evidence that some matrix dissolution occurs in carbonate rocks when acids and acidic gases are produced microbially. Models incorporating the competitive effects of both bacterial plugging and acidic dissolution of the pore walls have not been described.

The objective of this study is to quantify biomass growth, product generation and substrate consumption based on present understanding of bacterial metabolic processes[4] and to predict the net permeability modification resulting from the competitive processes of plugging and pore enlargement. This model is similar to that developed by Zhang[2] in which end-products' formation were balanced with growth and substrate utilization. The incremental work includes matrix dissolution and substrate adsorption processes. Further, the permeability modification exponent is shown here to be a function of the cementation factor of the rock.

Mathematical formulation

Mathematical equations are written for all species involved in the microbially enhanced waterflooding process. These species are broadly categorized into

- planktonic species, e.g.: bacteria, nutrients and metabolic products,
- sessile bacterial phase, and
- divalent cations dissolved from the rock matrix.

1. Planktonic species

Material transport equations are written for the planktonic bacterial phase, limiting nutrients and metabolic products. The limiting nutrients are principally glucose (carbon source), and ammonium nitrate (nitrogen source). The metabolic products modeled are biomass, carbon dioxide, nitrogen, organic acids and alcohols. The one-dimension generalized transport equation for each of the flowing phases is given by[2]:

$$\frac{\partial(\phi S_w C_i)}{\partial(t)} = -\frac{\partial(u_f C_i)}{\partial x} + \frac{\partial}{\partial x}(\phi S_w K_D \frac{\partial C_i}{\partial x}) + (\phi S_w) R_i \qquad (1)$$

where:

C_i = concentration of planktonic species in water phase (mg/cm^3),

K_D = dispersion coefficient (cm^2/hr),

S_w = water saturation (fraction),

u_f = darcy flux (cm/hr),

R_i = rate of generation or consumption of species ($mg/cm^3/hr$),

ϕ = rock porosity (fraction), and

i = species representing biomass, glucose, ammonium (NH_4^+) ions, nitrate ions, acetate, carbon dioxide, and nitrogen, respectively.

2. Biomass retention

The accumulation of the biomass attached on the pore surfaces is assumed to be the net result of biomass retention, detachment

and growth. Hence, the conservation equation for the sessile bacterial phase can be written as:

$$\frac{\partial}{\partial t}(\rho_B \sigma) = R_r - R_d + R_{Bs} \qquad (2)$$

where:

R_r = rate for biomass retention (mg/cm^3hr),

R_d = rate for biomass detachment (mg/cm^3hr),

R_{Bs} = rate for biomass growth (mg/cm^3hr),

ρ_B = biomass density (mg/cm^3), and

σ = fraction of pore space occupied by the sessile bacterial phase (fraction).

3. <u>Divalent cations in the carbonate rock matrix</u>

Calcium and magnesium ions will be dissolved from the carbonate matrix in the presence of acid-generating biomass. This is true for carbonates and calcareous sandstones. The dissolution equation is given by:

$$\frac{\partial(1-\phi_0)C_M}{\partial t} = -R_M \qquad (3)$$

where:

R_M = rate of dissolution of divalent cation species, M from the rock matrix (mg/cm^3hr),

C_M = concentration of the divalent cation in the matrix (mg/cm^3), and

M = divalent cation species (either Ca^{2+} or Mg^{2+}).

The rate (R_{Ac}) of acid consumption (due to matrix dissolution) is given by[9,10]:

$$R_{Ac} = \sum_{M} (\lambda_M R_M) \quad , \qquad R_M = K_M C_A (C_M - C^*_M) \qquad (4)$$

where:

λ_M = stoichiometric coefficients described as the moles of acid required to dissolve 1 mole of the divalent cation,

C^*_M = irreducible concentration of divalent cation species M in the rock matrix (mg/cm^3), and

K_M = specific reaction rate for the divalent cation species M,

C_A = acid concentration (mg/cm^3).

4. <u>Chemical Balance Equations</u>

An approximate chemical balance equation for anaerobic cell growth and metaobolism by consumption of both glucose and ammonium is given by[4]:

$$0.175 C_6H_{12}O_6 + 0.2 NH^+_4 \Rightarrow CH_{1.8}O_{0.5}N_{0.2} + 0.05 CO_2 + 0.45 H_2O \qquad (5)$$

where:

$C_6H_{12}O_6$ = glucose,

NH^+_4 = ammonium,

$CH_{1.8}O_{0.5}N_{0.2}$ = biomass, and

CO_2 = carbon dioxide (gas).

In addition to carbon dioxide, products of metabolic activities

include organic acids, such as formic (HCOOH), acetic (CH_3COOH), and lactic (C_3H_5OCOOH) acids, solvents such as ethanol (C_2H_6O), and nitrogen (gas), depending on the particular microbial strain and nutrients. In addition, Some microorganisms produce polysaccharides or surfactants.

For acid-producing microorganisms, such as the unidentified strain, GSP-1, which has been extensively employed in a related experimental program, there is a secondary rock matrix dissolution reaction:

$$RCOOH + MCO_3 \Rightarrow M^{2+} + 2RCOO^- + 2CO_2 + H_2O \qquad (6)$$

where:

RCOOH = organic acid,

MCO_3 = carbonate,

M^{2+} = divalent cation (either Ca^{2+} or Mg^{2+} or both),

$RCOO^-$ = organic anion, and

CO_2 = carbon dioxide (gas).

5. Porosity Modifications

There are two competitive processes occurring which may result in rock porosity modification. Pore plugging is caused by an increasing sessile biomass population while pore enlargement is caused by biogenic acid dissolution of the rock matrix. Porosity at any time is then given by:

$$\phi = \phi_0 - \Delta\phi_p + \Delta\phi_d \qquad (7)$$

where:

$\Delta\phi_p = \phi_o\sigma$,

$\Delta\phi_d = \phi_o\beta$,

ϕ_o = initial porosity of rock, and

β = volume fraction of divalent cations being dissolved (cm^3/cm^3)

It follows that the rate of change of porosity is:

$$\frac{1}{\phi_o}\frac{\partial\phi}{\partial t} = \frac{\partial\beta}{\partial t} - \frac{\partial\sigma}{\partial t} \qquad (8)$$

From a volume balance on the matrix,

$$\beta = \frac{1 - \phi}{\phi_o}\sum_M\left(\frac{C_M^o - C_M}{\rho_M}\right) \qquad (9)$$

where

ρ_M = density of the divalent cation species, M, and

C^0_M = initial concentration of divalent cation species, M, in the rock matrix.

6. <u>Rate Equations</u>

(1). *Microbial Growth*

Bacterial growth occurs with the utilization of substrate and is assumed to follow the Monod equation[2]. A double substrate limitation (glucose [S1] and ammonium [S2+]) is assumed:

$$R_{B\chi} = \mu_{Bmax}\left(\frac{C_{S1}}{K_{B/S1} + C_{S1}}\right)\left(\frac{C_{S2+}}{K_{B/S2+} + C_{S2+}}\right)C_\chi \qquad (10)$$

where:

$R_{B\chi}$ = growth rate for flowing (f) or sessile bacterial phase,

χ = represents either the flowing (f) or the sessile (s) phase, and

$C_s = \sigma\rho_B$ = sessile phase concentration, (mg/cm^3).

In addition to the above equation, rates of detachment and retention are given by

$$R_d = K_d(\sigma\rho_B)\,(u_f\frac{\mu_w}{K_f}) \qquad (11)$$

and,

$$R_r = K_r(u_f C_{Bf})\,(1 - \sigma)\,\rho_B \qquad (12)$$

where:

K_r = kinetic coefficient for biomass retention (cm^2/mg), and

K_d = kinetic coefficients for biomass detachment (cm^2 hr/mg).

(2). *Substrate Consumption*

Nutrients are consumed in two ways: by bacterial consumption and by adsorption onto the rock matrix. The major nutrient sources are glucose (carbon source), ammonium and nitrate. Glucose in the system contributes to biomass growth, production of organic acids (or acetate), carbon dioxide and the maintenance energy required to keep the microbial cells in a viable state. The ammonium ion also contributes to biomass cell buildup. A previous experiment has shown that glucose and ammonium adsorbed on carbonate chips but not on pure quartz sand[5]. Nitrate ions did not adsorb on either the

carbonate matrix or on sand grains.

The experimental data for the adsorbing substrate species can be fit with the Langmuir isotherm:

$$n^{\sigma} = \frac{aC_S}{b + dC_S} \tag{13}$$

where:

n^{σ} = amount of glucose adsorbed (mg glucose/g of rock matrix),

a,b,d = adsorption constants, and

C_S = concentration of glucose or ammonium (mg/cm^3).

The rate (R_{S1}) of consumption of glucose in the system is:

$$R_{S1} = -\frac{R_{Bf} + R_{Bs}}{Y_{B/S1}} - \frac{R_{Ap}+R_{Cp}}{Y_{(A+C)/S1}} - m_{S1}(C_B + \sigma\rho_B) - R_{a/S1} \tag{14}$$

and

$$R_{a/s1} = \frac{1 - \phi}{\phi}\rho_{rock}\frac{ab}{(b + dC_{S1})^2}\frac{\partial C_{S1}}{\partial t} \tag{15}$$

where:

R_{Bf} = rate of biomass growth in the flowing phase (mg/cm^{3}hr),

R_{Bs} = rate of biomass growth in the sessile phase (mg/cm^{3}hr),

R_{Ap} = rate of acid production (mg/cm^{3}hr),

R_{Cp} = rate of carbon dioxide production (mg/cm^{3}hr),

$Y_{B/S1}$ = yield coefficient of biomass on glucose (mg/mg),

$Y_{(A+C)/S1}$ = yield coefficient of acid and carbon dioxide on glucose (mg/mg),

m_{S1} = coefficient for biomass maintenance energy by glucose (mg/mg.hr), and

$R_{a/S1}$ = rate of glucose adsorption onto the rock matrix (mg/cm^3).

while the rate of ammonium consumption is given by:

$$R_{S2+} = -\frac{R_{Bf} + R_{Bs}}{Y_{B/S2+}} - R_{a/S2+} \qquad (16)$$

where:

$R_{a/S2+}$ = adsorption rate of ammonium ion (mg/cm^{3}hr),

$Y_{B/S2+}$ = yield coefficient for biomass on ammonium (mg/mg),

R_{S2+} = consumption rate of ammonium, (mg/cm^{3}hr).

The rate of nitrate consumption is also given by:

$$R_{S2-} = -\frac{R_N}{Y_{N/S2-}} - m_{S2-}(C_B + \sigma\rho_B) \qquad (17)$$

where:

R_N = rate of production of nitrogen (mg/cm^3.hr),

m_{S2-} = coefficient for biomass maintenance energy by nitrate,

$Y_{N/S2-}$ = yield coefficient for nitrogen on nitrate (mg/mg).

(3). *Production*

The metabolic products in this model are acetate or acetic acid, carbon dioxide and nitrogen. Organic acids are both generated and consumed in the system. The net rate of generation of an organic acid is given by:

$$R_{An} = R_{Ap} - R_{Ac} \tag{18}$$

and

$$R_{Ap} = \mu_{Am} \frac{C_{S1} - C^*_{S1}}{K_{A/S1} + C_{S1} - C^*_{S1}} (C_B + \sigma \rho_B) \tag{19}$$

where:

R_{An} = net rate of accumulation of organic acid (mg/cm^3hr),

R_{Ac} = rate of consumption of organic acid defined in Eq.(4),

μ_{Am} = maximum specific production rate of organic acid on glucose (1/hr), and

$K_{A/S1}$ = saturation constant for organic acid production on glucose (mg/cm^3), and

C^*_{S1} = critical concentration for glucose consumption (mg/cm^3).

Carbon dioxide is produced biologically and from the dissolution of the carbonate matrix. For this reason, the rate of carbon dioxide production can be evaluated from the stoichiometric ratios of carbon dioxide to acetic acid in reactions. Hence:

$$\frac{\partial C_{Cp}}{\partial t} = \omega R_{Ap} \tag{20}$$

where:

ω = sum of stoichiometric (mass) ratios of CO_2 to acid from all CO_2 production reactions.

The stoichiometric ratio from the biological reaction is obtained empirically and depends on the microbial strain. It is known for

GSP-1 that fermentation of 100g of glucose will produce about

- 7.5g of biomass,
- 23g of carbon dioxide, and
- 69.5g of organic acid[6].

It follows then that

$$\omega = \left(\frac{m_{CO_2}}{m_{ac}}\right)_{bio} + \left(\frac{m_{CO_2}}{m_{ac}}\right)_{abio} \tag{21}$$

where the first term on the right hand side describes the ratio of the mass of carbon dioxide produced biologically to mass of associated acetic acid and the second term is the ratio of the mass of carbon dioxide produced abiologically from acid dissolution of carbonate matrix to the mass of acetic acid producing it.

It is assumed in this model that the carbonate matrix dissolution due to carbonic acid is very small compared to dissolution due to the organic acids.

(4). *Carbonate Matrix Dissolution*

The generation rate of divalent cations from the matrix is given by Eq.(3). The rate of porosity development from dissolution can be obtained by differentiating Eq.(9):

$$\frac{\partial \beta}{\partial t} = \frac{\frac{\partial \sigma}{\partial t}\Sigma_M + \frac{(1-\phi)}{\phi_o}\frac{\partial \Sigma_M}{\partial t}}{(1 + \Sigma_M)} \tag{22}$$

where, $\Sigma_M = \Sigma_M(C^o_M - C_M)/\rho_M$.

7. Effect of bacterial activity on permeability

Microbial plugging and matrix dissolution have competitive effects on rock permeability (K). The net effect may be predicted from relationships among the rock petrophysical parameters. Permeability has been shown to depend on the sizes of the pore openings (R_e), and the interconnectivity of the different pores. Pore interconnectivity can be quantified by the formation resistivity factor (F). Katz and Thompson[7] have shown that

$$K = K_c \frac{R_e^2}{F} \qquad (23)$$

where, K_c = a constant. By defining the formation factor (F) in terms of porosity (ϕ):

$$F = a\,\phi^{-m} \qquad (24)$$

then the permeability modification ratio or PRF is

$$\frac{K}{K_o} = \left(\frac{\phi}{\phi_o}\right)^m \left(\frac{R_e}{R_{eo}}\right)^2 \qquad (25)$$

where:

m = cementation factor,

a = lithology factor,

K_o = original permeability,

ϕ_o = original porosity, and

R_{eo} = original pore openings.

If we define an equivalent pore throat size such that:

$$\phi = \pi R^2{}_e \frac{L}{V_B} \tag{26}$$

where:

L = core length, and

V_B = bulk volume.

then

$$\frac{\phi}{\phi_0} = \left(\frac{R_e}{R_{eo}}\right)^2 \tag{27}$$

and the permeability modification factor is given as:

$$\frac{K}{K_o} = \left(\frac{\phi}{\phi_o}\right)^{m+1} \tag{28}$$

Numerical Methods

The partial differential equations are solved by using the method of lines[8] which is a semi-discrete method. The spatial derivatives are approximated by their finite difference formulas while the temporal derivatives remain continuous.

1. <u>Finite Difference Equations</u>

(1). *Planktonic Phases*

The first-order spatial derivative corresponding to the convective transport for species i at node j is replaced by the third-order upwinding formula of Leonard[11]:

$$\left(\frac{\partial C_i}{\partial x}\right)_j = \frac{(C_i)_{j-2} - 6(C_i)_{j-1} + 3(C_i)_j + 2(C_i)_{j+1}}{6\Delta x} + \Theta(\Delta x^3) \tag{29}$$

$$j = 3, 4, \ldots, N-1$$

The third-order upstream weighting is the lowest order method that possesses the properties needed for a stable and accurate difference operator for the convection term. The first-order spatial derivative at j=2 is expressed by the two-point central weighting scheme:

$$\left(\frac{\partial C_i}{\partial x}\right)_2 = \frac{(C_i)_3 - (C_i)_1}{2\Delta x} + \Theta(\Delta x^2) \tag{30}$$

The dispersion term, a second-order spatial derivative, is approximated by the second-order central finite difference scheme:

$$\left(\frac{\partial}{\partial x}(\phi K_D \frac{\partial C_i}{\partial x})\right)_j = \frac{(\phi K_D)_{j+\frac{1}{2}}[(C_i)_{j+1} - (C_i)_j] - (\phi K_D)_{j-\frac{1}{2}}[(C_i)_j - (C_i)_{j-1}]}{\Delta x^2} \tag{31}$$

$$j = 2, 3, \ldots, N-1$$

Substituting the formulas above into the partial differential equations for species i, the resulting equations have the following forms:

$$\left(\frac{\partial C_i}{\partial t}\right)_2 = \hat{a}_2(C_i)_1 + \hat{b}_2(C_i)_2 + \hat{c}_2(C_i)_3 + (R_i)_2 \tag{32}$$

$$\left(\frac{\partial C_i}{\partial t}\right)_j = a_j(C_i)_{j-2} + b_j(C_i)_{j-1} + c_j(C_i)_j + d_j(C_i)_{j+1} + (R_i)_j \tag{33}$$

$$j = 3, 4, \ldots, N-1$$

(2). *Sessile Phase*

The sessile-phase conservation equation, when applied to the inoculation process by neglecting the growth term, can be written:

$$\left(\frac{\partial\sigma}{\partial t}\right)_j = K_r(u_f C_{Bj})(1-\sigma_j) - K_d\sigma_j u_f \frac{\mu_w}{(K_f)_j} \qquad j = 1, \ldots N \qquad (34)$$

The sessile-phase equation for the incubation process is expressed:

$$\left(\frac{\partial\sigma}{\partial t}\right)_j = (\mu_{ss})_j\sigma_j \qquad j = 1, \ldots N \qquad (35)$$

(3). *Divalent Cations in the Matrix*

The finite difference equations for divalent cations in the matrix can be written:

$$\left(\frac{\partial C_M}{\partial t}\right)_j = -\frac{K_M C_A (C_M - C^*_M)_j}{1-\phi_0} \qquad M = Ca^{++},\ Mg^{++} \qquad j = 1, \ldots N \qquad (36)$$

(4). *Dissolved Divalent Cations*

For the fractional pore space created due to the divalent cations dissolved from the rock matrix, the conservation equation can be discretized as:

$$\left(\frac{\partial\beta}{\partial t}\right)_j = \frac{\phi_0\left(\frac{\partial\sigma}{\partial t}\right)_j \sum_M \frac{(C_M^0 - C_M)_j}{\rho_M} + (1-\phi_j)\sum_M \frac{1}{\rho_M}\left(-\frac{\partial C_M}{\partial t}\right)_j}{\phi_0\left(1+\sum_M \frac{(C_M^0 - C_M)_j}{\rho_M}\right)} \qquad (37)$$

$$M = Ca^{2+},\ Mg^{2+}, \qquad j = 1, \ldots N$$

2. Computation

Through discretization in space, the model equations are translated into a set of ordinary differential equations corresponding to each node j along the one-dimensional porous medium. The resulting model was solved using the Runge-Kutta-Fehlberg method. The simulator was coded using FORTRAN 77 and implemented on the Encore Multimax computer.

Model Validation

1. Case Study

Requirements for the validation of the numerical model are:

- core data
- core treatment data, and
- microbial kinetics data.

The core data and the core treatment data were obtained from the bacterial treatment of a Viola limestone core pack (PV-1) described in the preceding Section F. The microbial kinetics data (shown in Table G-1) were obtained from various sources[2,6].

The simulation of three treatments (totalling 70 days) took about 35 minutes on the Encore Multimax computer. The simulation results describing the gas (carbon dioxide) production, the divalent cation (calcium) production, the glucose consumption and permeability modification are shown on Figures G.1 - G.4 respectively.

2. Discussion

One of the difficulties in the validation of the model is availability of measured data. The only available data from the experiment were gas volumes. In the absence of detailed experimental analysis of the produced gas, it was assumed that all gas production was carbon dioxide. Sources of carbon dioxide are two-fold: from bacterial metabolism and from acidic dissolution of the carbonate matrix. Fig. G.1 is a comparison of simulation and experimental results on gas production. A maximum relative deviation of 26% was observed between the two results.

Figures G.2 through G.4 show the calcium ion production, glucose consumption and permeability modification. The simulation result (Fig. G.3) shows that the glucose consumption was expected to increase with the number of treatments. This is because the microbial population should increase with each successive treatment. This was confirmed by the increased pressure readings for successive treatment of PV-1 (Figure 1, Section F). As seen in Figure G.4 the permeability modification model as formulated was not able to match the experimental data. This is because permeability modification was assumed to depend on the competitive processes of the sessile bacterial population and matrix acidic dissolution in the pore space. However, net accumulation of the mass in the pore space is not the only mechanism of plugging processes. Pore throat clogging and bridging by microorganisms and fine particles may be more significantly in the permeability

modification process. In addition, relative permeability effects of the biogenic gas phase may need to be considered.

Conclusions

1. A model that can quantify biomass growth, product formulation, and substrate utilization in bacterial treatment of carbonate porous media has been developed.
2. The model was tested using limited experimental data. The model gave a qualitative reasonable estimation of gas production during microbial growth and metabolism. But, the numerical prediction of permeability modification appears to be inadequate.
3. Mechanisms of microbial plugging processes on the pore surface and at the pore throat need to be further studied for improving the permeability modification model.
4. Additional experimental data for confirmation of the model shoud include primary dependent variables in the effluent fluids, such as concentrations of glucose, carbon dioxide, nitrogen, organic acid, and divalent cations, etc.

References

1. Knapp, R.M., F. Civan and M.J. McInerney, "Modeling Growth and Transport of Microorganisms in Porous Formations", **IMACS, Proceedings of 12th World Congress on Scientific Computation**, R. Vichnevetsky, P. Borne and J. Vignes (eds.), Paris, France, **3:**676-679, 1988.
2. Zhang, Xu (1990), Mathematical Modeling of Microbially Enhanced Oil Recovery, M.S. Thesis, University of Oklahoma, (Sup: R.M. Knapp),150pp.
3. Islam, M.R.and A. Gianetto, "Mathematical Modeling and Scaling Up of Microbial Enhanced Oil Recovery", Paper CIM/SPE 90-131, presented at the Int. Tech. mtg of **Pet. Soc. of CIM/Soc. of Pet. Engineers**,Calgary, Canada, June 10-13,1990.
4. Sinclair, C.G.,(1987), "Microbial Process Kinetics", Chapter 4 in Basic Biotechnology, J. Bu'lock, and B. Kristiansen,(eds.), Academic Press, New York, 561 pp.
5. Udegbunam, E.O., R.M. Knapp, M.J. McInerney, and R.S. Tanner, "Effects of Adsorption on Substrate Availability in Carbonate Reservoirs", Prepared for publication,1991.
6. Tanner, R.S., private communication,1991.
7. Katz A.J. and A.H. Thompson, "Quantitative prediction of permeability in porous rock", **Physical Review B: The American Physical Soceity**, **34:**8179-8181,1986.
8. Ames, W.F., Numerical Methods for Partial Differential Equations, 2nd ed.,Academic Press, New York, 1977.

9. Rege, S.D. and H.S. Fogler,"Competition Among Flow, Dissolution, and Precipitation in Porous Media", AIChE J., v. 35, no. 7, p. 1117 -1185.

10. Langmuir, Donald, "Physical and chemical characteristics of carbonate water", Guide to the hydrology of carbonate rocks, P. E. La Moreaux, B. M. Wilson and B. A. Memon (eds.), Unesco studies and reports in hydrology, p.69 -100.

11. Leonard, B.P., "Third-order Upwinding as a Rational Basis for Computational Fluid Dynamics," Computational Techniques and Applications: CTAC-83, Editors, J. Noye and C. F;etcher, (1984) p. 106-120.

Table G.1 - Microbial Kinetics Data for Simulation

Symbols	Definition	Values	Units	Source
μ_{Bm}	Max. specific growth rate for biomass	0.0012	1/hr	ref.(6)
μ_{Am}	Max. production rate for acetate	0.02	1/hr	"
μ_{Nm}	Max. production rate for nitrogen	0.09	1/hr	"
$K_{B/S1}$	Saturation constant for biomass on glucose	0.045	mg/cm^3	ref.(2)
$K_{B/S2+}$	Saturation constant for biomass on NH_{4+}	0.045	"	ref.(2)
$K_{B/S2-}$	Saturation constant for nitrogen on nitrate	1600	"	ref.(2)
m_{s1}	Coeff. of energy maintenace by glucose	$1.8(10^{-5})$	mg/mg.h	ref.(2)
m_{S2-}	Coeff. of energy maintenance by nitrate	$4.7(10^{-5})$	"	"
$Y_{B/S1}$	Yield coeff. for biomass on glucose	0.08	mg/mg	ref.(6)
$Y_{(A+C)/S1}$	Yield coeff. for acid and CO_2 production on glucose	6.8	mg/mg	ref.(6)
$Y_{B/S2+}$	Yield coeff. for biomass on Ammonium	1.1441	"	ref.(6)
$Y_{N/S2-}$	Yield coeff. for nitrogen production on nitrate	0.226	"	ref.(2)
K_r	Coeff. of retention of bacteria	0.001	$cm^2.h/mg$	"
K_d	Coeff. of detachment of bacteria	$1.6(10^{-23})$	"	"

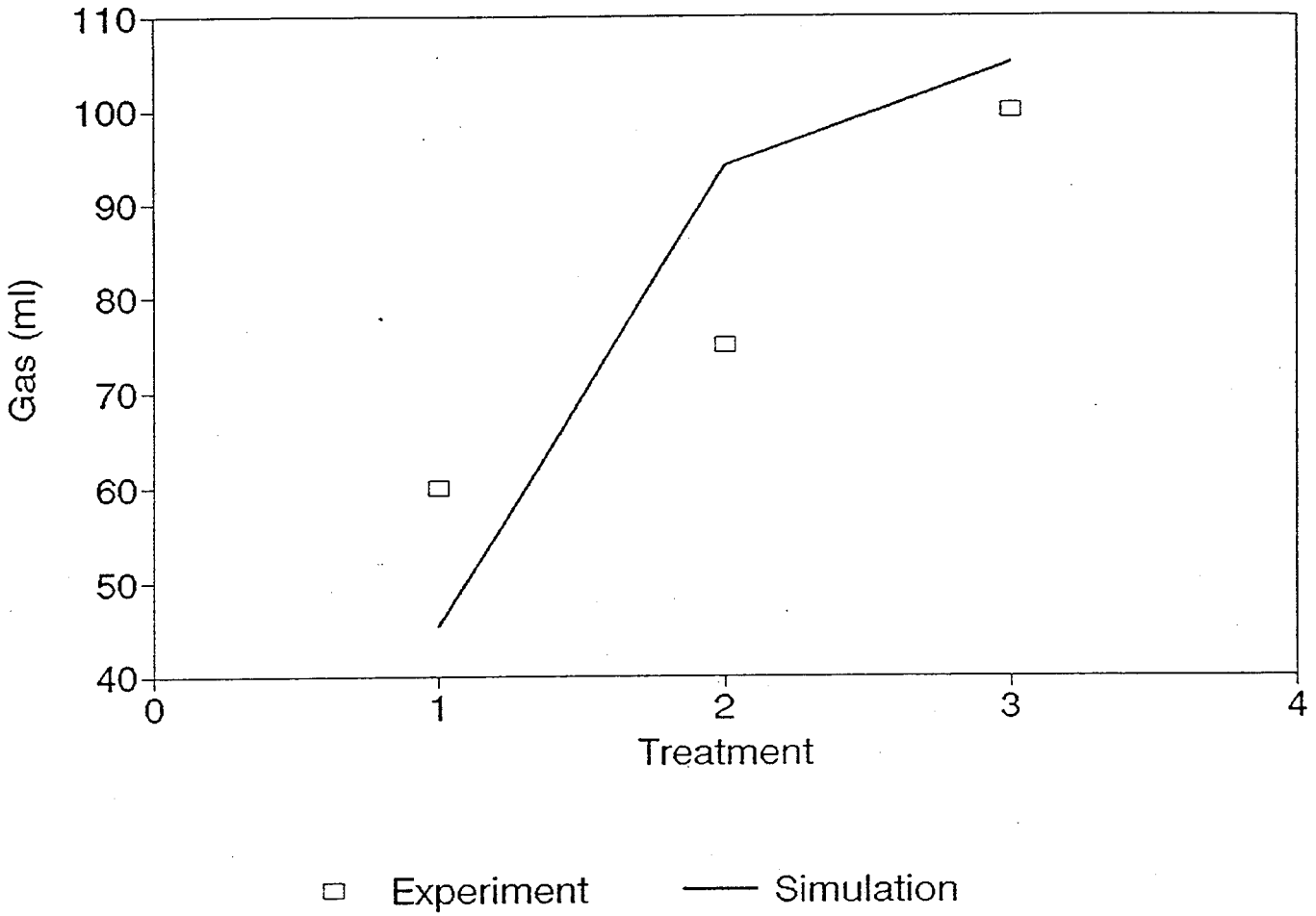

Figure G.1 - Comparison of Gas Production from Experiment and Simulation

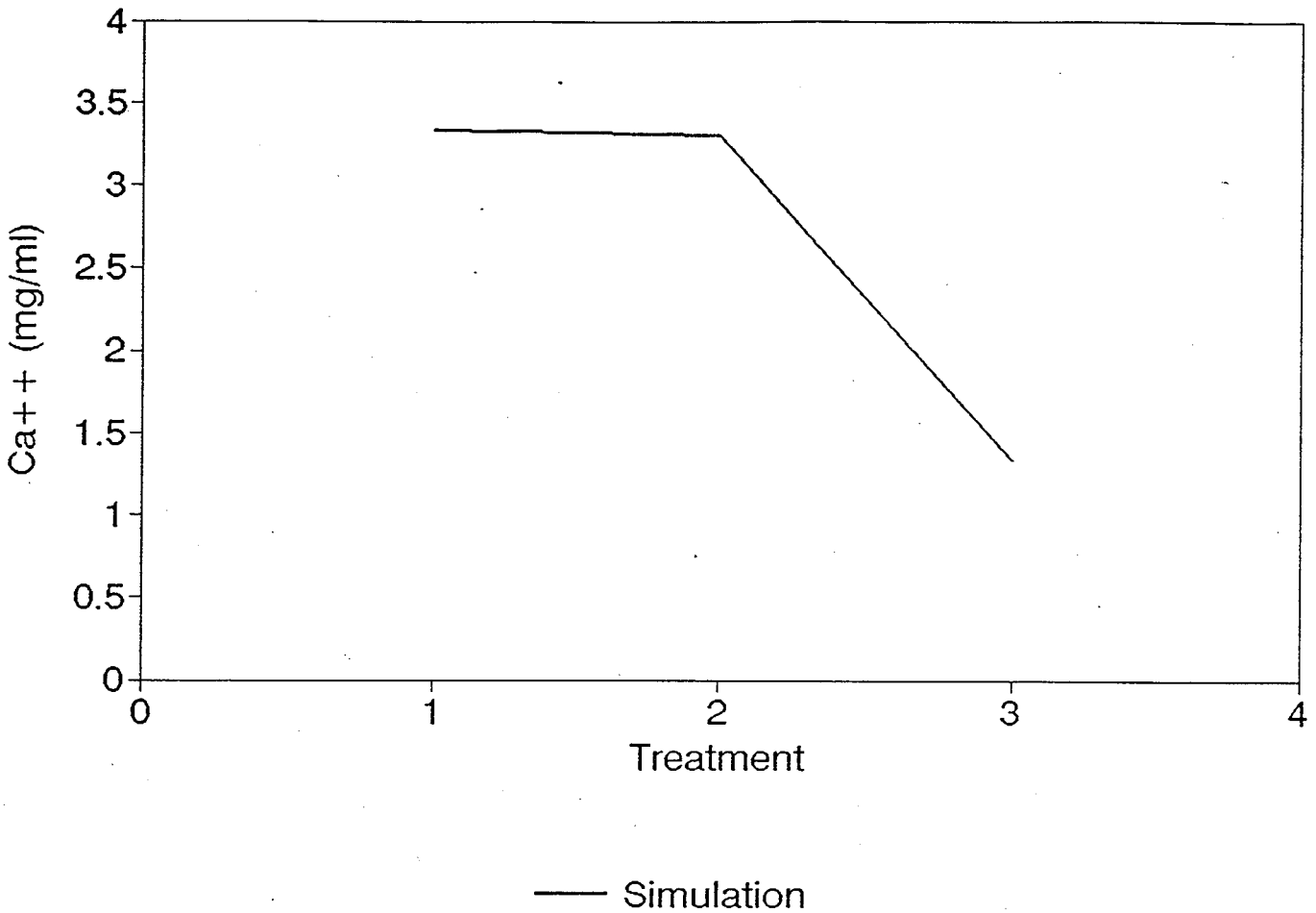

Figure G.2 - Divalent (Calcium) Cation production from Simulation

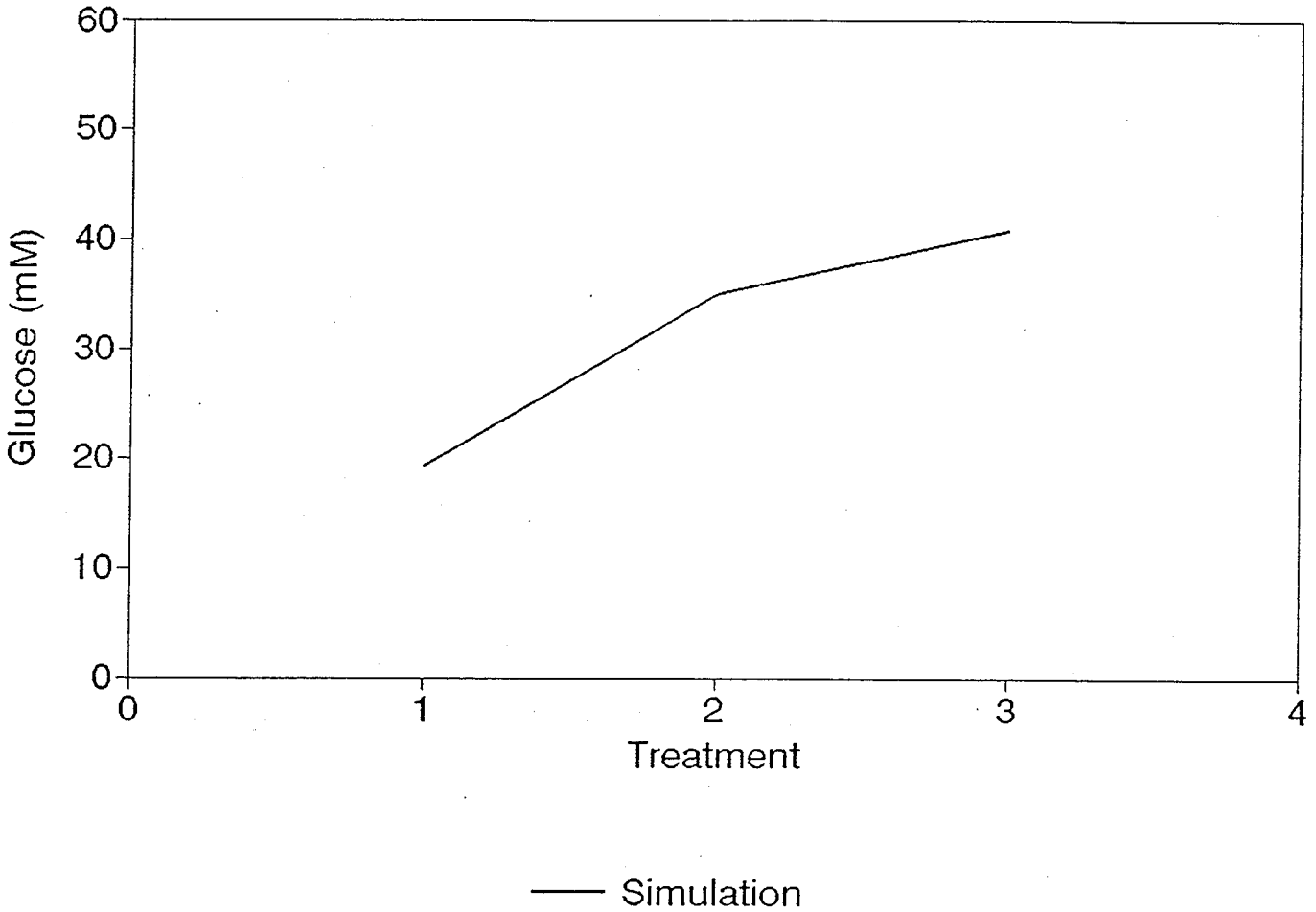

Figure G.3_Glucose comsumption result from Simulation

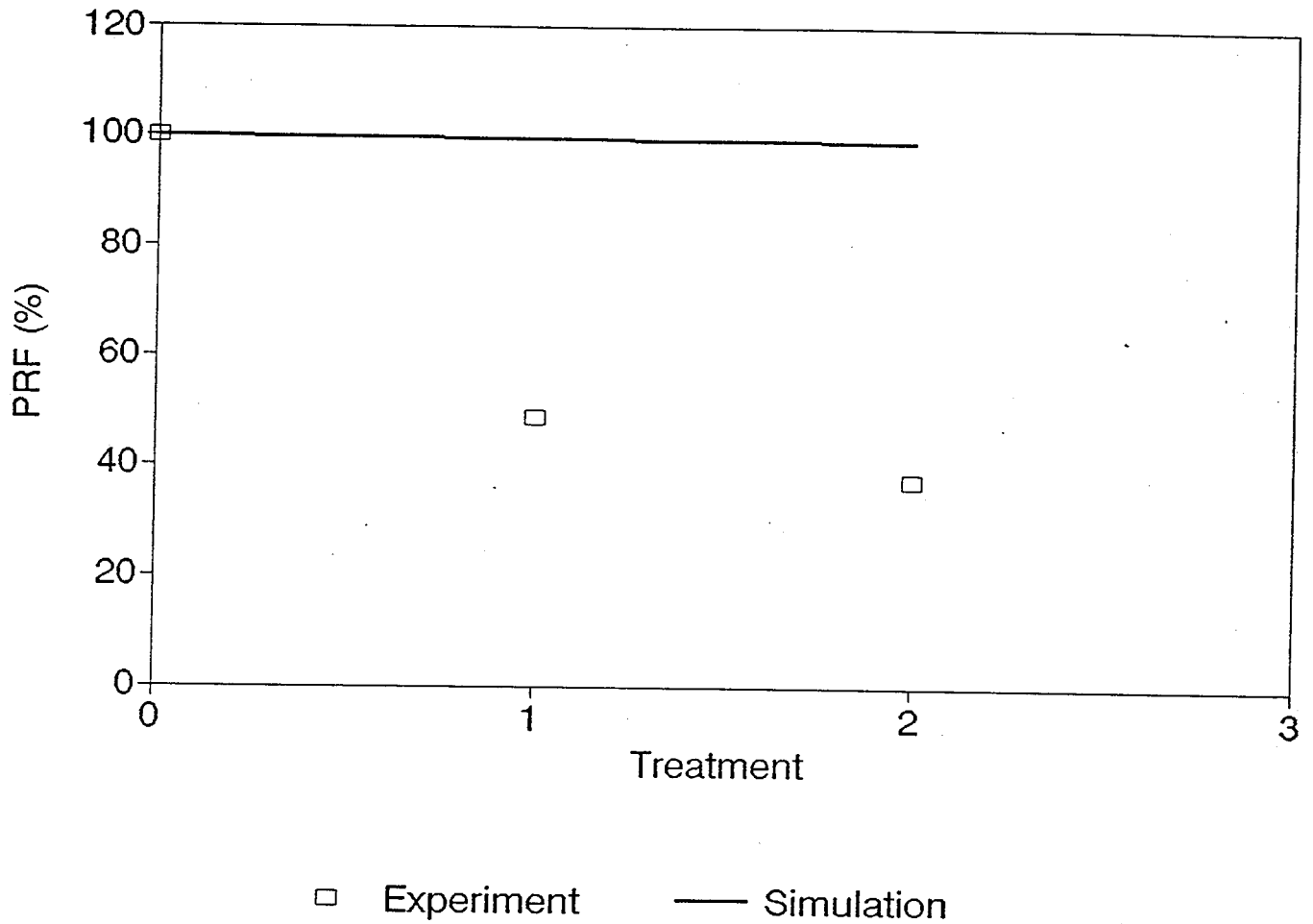

Figure G.4 - Comparison of Permeabilty reduction from the Experiment and Simulation.

CONCLUSIONS AND RECOMMENDATIONS

The problems and promise of MEOR in carbonate reservoirs were outlined in the review of the available literature. Aside from the sparsity of material, the major problem for laboratory research on MEOR in carbonate systems is the dual porosity nature of many carbonate reservoir rocks. However, review of the literature indicates that microbial acid and gas production are important mechanisms for MEOR in carbonate systems, that in situ microbial activity can affect properties of reservoirs, and that MEOR has been successfully demonstrated in carbonate systems in several field trials.

A basic microbial ecology study of produced water from carbonate reservoirs was conducted to address some questions on the feasibility of MEOR in carbonate reservoirs without the injection of microorganisms (Section C). Numerically small but metabolically diverse microbial populations of mesophiles were recovered from each of the four reservoirs sampled. The data suggest that these ecosystems were most limited by the availability of nitrogen and phosphorous in the environment. However, the populations were diverse enough that it should be possible to stimulate the activity of native microorganisms for in situ MEOR, if the physical parameters found in the reservoir permit biological activity. The microbiology of halophilic thermophiles, however, requires further investigation. Only a few of these were detected in our study, and none have been characterized in the scientific literature. The effects of dissolved carbon dioxide, divalent cations, and pressure on the recovery of and activity of halophilic, thermophilic

microorganisms would be the next logical extension of the research presented here.

Several studies were designed specifically to assist the effort to mathematically model microbial processes in carbonate systems. The adsorption and desorption of microbial nutrients by water-wet and oil-wet limestone chips was examined. The results indicated that adsorption of nutrients onto sand or limestone surfaces differed, especially in the case of glucose and water-wet surfaces. The data obtained suggest that while nutrients are adsorbed onto carbonate surfaces, this should not be a major limiting factor for in situ MEOR in carbonate reservoirs. Nutrient adsorption and desorption should be compared between sandstones and carbonates in systems normalized for rock surface area; we predict that this would emphasize the difference between the two kinds of rock. Also, the affect of adsorption and desorption of nutrient species in complex mixtures should be examined further. Given the limiting availability of nitrogen and phosphorus in the carbonate brines sampled in this work, nutrient availability could be a factor in reservoir-scale MEOR. This might best be studied by treating the rock matrix as a chromatographic medium in further research.

Microbial growth and activities had predictable effects on the permeability and porosity of sandstone and limestone rock plugs (Section E). Growth of microorganisms in sandstones, biomass production, lowered the permeability and porosity of the plugs. The same was observed when a polymer-producing microorganism, strain SP018, was grown in a limestone core plug. However, the metabolic activities of acid-producing microorganisms, especially the acid- and gas-producing strain GSP-1, increased the porosity

and permeability of limestone plugs. Thus, in carbonate systems there are competing reactions, organic acid production followed by dissolution of the rock matrix and plugging due to biomass formation, which need to be considered for mathematical modelling of MEOR in carbonate systems. This might be advantageously manipulated for in situ MEOR in carbonate reservoirs by providing substrates for microbial acid production, yet limiting biomass production by limiting availabilty of nitrogen and/or phosphate.

The effects of nutrient adsorption and matrix dissolution were incorporated into mathematical modelling of microbial processes in carbonate systems. This first generation model, based on that developed by Xu Zhang targeted at modeling MEOR by selective plugging in sandstone systems, also equalized all rates of substrate utilization and end-product formation, with the refinement that all organic acid end-products of metabolism were treated as equivalent acids rather than individual moieties (formic acid, acetic acid, butyric acid, etc.). Enhanced oil recovery in carbonate chip packed cores using strain GSP-1 was used as the experimental system to vaidate the mathematical model. An absolute deviation of 20% was obtained in comparing the model and experimental gas production in packed core results. This mathematical model will undergo refinement, but performed fairly well, especially in light of the fact that GSP-1's basic fermentation pathway for glucose has not yet been determined.

MEOR was demonstrated in four separate carbonate chip packed cores (Section F). MEOR resulted from the biological activity of four different microbial systems: strain GSP-1, which produces acid, gas, ethanol, and a substance which can emulsify crude oil;

B. licheniformis JF-2, which produces acid, gas, and a surfactant; an unknown strain(s) which produced acid and gas; and an unknown strain(s) which probably metabolized amino acids and did not produce acidic end-products of metabolism. While the exact mechanisms of oil recovery were not determined in this set of experiments, several new factors were indicated. The data indicated that the effect of trapped gas in packed cores needs to be evaluated; while cores with GSP-1 generally showed increases in permeability after microbial activity, some of the core packs were less permeable and this appeared to be due to the trapping of gas bubbles in the core. The effect of ethanol on MEOR in carbonate systems needs to be evaluated independent of gas production, independent of acid production, and independent of surfactant production. Test cores will have to prepared and handled aseptically to evaluate the effects of individual microbial end-products of metabolism on MEOR. While difficult, this is technically feasible.

MEOR needs further laboratory evaluation in consolidated carbonate rock systems. Porous channels/fractures/etc. could be produced artificially in limestone to facilitate these studies. A more attractive alternative would be to physically increase the size of the limestone rock to be studied. This should increase the likelihood of capturing the dual porosity character of carbonate reservoir rock in the laboratory. Research on the small plug cores, which had too little volume for enhanced oil recovery studies, did show that microbial activity affects the permeability and porosity of carbonate rock.

We feel that the information gleaned from the literature review coupled with the laboratory research conducted during the past year permits the planning and execution of a field trial of in situ MEOR in a carbonate reservoir in the near future. The candidate reservoir should be under waterflood and have characteristics within the physical parameters outlined in Section A. The initial MEOR treatment would probably be to stimulate microbial acid and gas production, activities which could improve the permeability and porosity characteristics of a carbonate formation and improve the flow characteristics of the petroleum trapped in the reservoir. Given that analysis of the available reservoir data indicates that 40% of the carbonate petroleum reservoirs in the United States could be candidates for MEOR, MEOR should prove a technology widely applied for tertiary petroleum recovery.