

Fundamental Aspects of Microbial Enhanced Oil Recovery: A Literature Survey

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March, 2008

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Contents

1. Introduction	4
2. Physical and Environmental Constraints	4
2.1 Pore Size	4
2.2 Acidity	5
2.2.1 Surface Charge.....	5
2.2.2 Enzyme Function	5
2.3 Oxidation Potential.....	5
2.4 Oligotrophy and Heterotrophy	6
2.5 Water and Electrolytes	6
2.6 Temperature	7
2.7 Pressure	8
2.8 Relation to MEOR.....	9
3. Petroleum Microbiology	9
4. Biosurfactants	10
5. Bacterial Effects on Porous Media	12
5.1 Field Studies	12
5.2 Effects on Fluid Properties	12
5.3 Effects on Reservoir Properties.....	13
5.4 Gas Generation	14
6. Experimental Studies of Microbial Behavior	15
7. Mathematical Modeling	17
7.1 Transport Properties.....	18
7.2 Conservation Law	19
7.2.1 Local Equilibrium.....	19
7.2.2 Breakdown of Filtration Theory	19
7.2.3 Physical Straining	20
7.3 Biofilm Clogging and Related Phenomena.....	21
7.4 Fully-Numerical Approaches	22
7.4.1 Logistic Growth of Bacteria	23
7.4.2 Monod Equation.....	24
8. Conclusions	26
Acknowledgements	27
References	28

1. INTRODUCTION

Increased demand for petroleum products has given strong impetus to the development of Enhanced Oil Recovery (EOR) technologies. A review of several EOR technologies that have been supported by the US Department of Energy was recently published by Felber (2004). Among these, microbially enhanced oil recovery (MEOR) is among the oldest (ZoBell, 1946), but has become the subject of extensive investigation only within the last two decades. From the chemical perspective, the most outstanding advantage to be realized by use of bacteria or some other organism is the enhancement of kinetics. Thus, although the energetics of the overall process cannot be changed, the biocatalysis performed by the enzymes often allows reactions to proceed under ambient or near-ambient conditions, often with far greater specificity than could be achieved in conventional reactors. On the other hand, living things are highly complex and delicate systems, and can operate over much narrower ranges of temperature and pressure than can be achieved in chemical plant. The purpose of the following review is to consider the literature concerning MEOR in relation to the environmental constraints to which the microbes concerned are subject, and the fundamental aspects of the underlying physical and chemical processes.

2. PHYSICAL AND ENVIRONMENTAL CONSTRAINTS

Gregory (1984) begins his exposition of the fundamentals of MEOR by identifying physical (temperature, pressure, pore size/geometry), chemical (pH, E_h , electrolyte composition) and biological factors that constrain microbial activity in hydrocarbon reservoirs. In the following subsections, these constraints, and their interactions, are discussed. More generally, it is to be observed that the same factors control the existence and behavior of bacteria in other subterranean environments, which are of relevance in other practical contexts - most notably the management and remediation of groundwater resources. The observation that numerous species of bacteria found in such environments can withstand, or even thrive, under physical conditions that are inimical to most life forms has given fresh impetus to study of this topic, in relation to research on the origins of life on earth and the possible existence of life on other planets.

2.1 Pore Size

The existence of bacteria in deep subsurface rocks has been disputed in the past, but since the advent of modern tracer techniques and improved sampling protocols (Frederickson and Phelps, 1996), is now generally accepted. Perhaps the most obvious constraint that applies to deep-subsurface microbes is the size of the pores. In some studies, the lower limit of mean pore sizes has been shown to be smaller than the size of known bacteria. For example, through phospholipid fatty acid assays and measurements of ^{14}C acetate mineralization, Frederickson et al. (1997) assessed shale and sandstone cores from a site in northwestern New Mexico for microbial activity. They found no metabolic activity was detected in core samples with pore throats narrower than $0.2\ \mu\text{m}$, although in some cases it was after extended incubation. The observation of much higher levels of metabolic activity in more permeable samples led these authors to conclude that sustained bacterial activity require interconnected pores of diameter at least $0.2\ \mu\text{m}$.

2.2 Acidity

The acidity or (alkalinity) of the surrounding aqueous medium, measured by the pH, is significant in several respects:

2.2.1 Surface Charge

On the cellular scale, pH controls the extent of ionization of the protein molecules that are embedded in the cell walls. As a result, cellular surfaces are generally charged and surrounded by diffuse double layers, the thickness of which is controlled by the overall electrolyte concentration. Interaction of these ionic space-charge regions with those that also surround small particles of mineral phases can strongly affect the motion of the cells through a natural porous medium. The effect of pH on the surface charge of a protein depends on the relative numbers of acidic and basic groups in the side chains. Protein molecules are often characterized by a pH called the isoelectric point, at which the positive and negative charges resulting from ionization of side chains are balanced.

2.2.2 Enzyme Function

Some of the embedded cell wall proteins play a crucial role in the uptake of nutrients, elimination of waste products, and maintenance of correct electrolyte concentrations; on a molecular scale, their ability to perform these functions also depends on their extent of ionization. The rates of the enzymic processes that occur in respiration is strongly dependent on the pH. There generally exists an optimal pH, lying between 2 and 9.5, for the rates of such processes. The mineral phases in a porous medium (particularly carbonates), and the proteins themselves can exert a buffering effect, which can mitigate the lowering of the pH by the acids generated by primary metabolism.

2.3 Oxidation Potential

Cellular respiration consists of enzymically mediated electron transfers from an electron donor (reducing agent, in chemical parlance) to a terminal electron acceptor (oxidizing agent). Apart from a few rare cases where only one mole of electrons is transferred from each mole of reductant, this electron transfer almost always involves a number of intermediate electron transfer steps, which can be quite numerous if the original electron sources are complex molecules such as sugars. The thermodynamic driving force for these electron transfer processes is expressed quantitatively in terms of the oxidation potential, E_h (measured in volt), which is the Gibbs energy change divided by the number of moles of electrons transferred. According to the Nernst equation (discussions of which can be found in most textbooks of physical chemistry), this quantity depends logarithmically on the concentrations (strictly speaking, the activities) of not only the oxidized and reduced forms of the electron acceptor, but also of hydrogen ions and other species that might be involved. Thus, for aerobic respiration, the terminal electron acceptor is oxygen, which is reduced to water according to the overall equation



A particularly important electron acceptor in hydrocarbon reservoirs that are not supplied by surface water is sulfate:



Some organisms can use ferric ions:



The oxidation potentials corresponding to the first two of these reactions depend on pH, while that of the third does not.

2.4 Oligotrophy and Heterotrophy

To explain the existence of active microbial communities in environments such as deep granitic and basaltic aquifers, where nutrient levels are expected to be extremely low, it was suggested by Stevens and McKinley (1995) that such organisms can be sustained by hydrogen generated by reduction of minerals by groundwater. Although many species of hydrogen-consuming lithotrophic bacteria have been described, and it is well known that appreciable hydrogen fugacities can be 'buffered' by some naturally-occurring mineral assemblages, the suggestion that microbial communities could be sustained geochemically in this way has, however, been disputed by Anderson et al. (1998). These authors argued that basalt does not produce hydrogen under slightly alkaline conditions, and that the production of hydrogen under slightly acidic conditions cannot be sustained over geological time scales. But a more recent discussion presented by Nealson et al. (2005) points out that the most important and difficult issue to be established is the long-term independence of such communities from the products of photosynthesis; at present this is best regarded as an open question.

Considerable attention has been devoted to the study of heterotrophic microbes in sandstones and shales, and the possibility that these organisms are sustained by organic material codeposited with the sediments. In a recent review, Krumholz (2000) considers formations containing alternating layers of sandstone and shale, and discusses experimental evidence that organic matter and fermentation products present in the shales can diffuse across sandstone-shale boundaries and support microbial communities in the sandstone, adjacent to the sandstone-shale interfaces. Similar phenomena have been identified by McMahon and Chapelle (1991) and McMahon et al. (1992) in clay-sand sequences, and by Ulrich et al. (1998) in lignite/clay deposits.

2.5 Water and Electrolytes

The concentrations of electrolytes and other dissolved species required for proper cellular function is maintained by enzymically mediated exchange of solutes or solvent with the surrounding medium. Dissolution of electrolytes reduces the thermodynamic activity of water, a_w . Dissolution of electrolytes reduces the thermodynamic activity of water, a_w . This effect is measured by the ratio of the fugacity of water above the solution to that of pure water. At temperatures far below the critical point of water, the fugacity of water is approximately equal to the vapor pressure. For example, a_w in sea water is about 0.98, while in inland salt lakes it can be as low as 0.75. Since the water activity corresponding to appreciable electrolyte concentrations differs only slightly from 1, an alternative measure is provided by the osmotic pressure of the solution, which is defined as the hydrostatic pressure that must be applied to a

solution to raise its vapor pressure to that of pure water. Thus, for sea water (of approximately 3.3% salinity), the osmotic pressure estimated from van't Hoff's equation is about 2.8 MPa.

Differences in ionic strength across membranes provides a powerful driving force for diffusion of water into cells (when the surrounding medium is a more dilute electrolyte) or out of cells (when it is more concentrated.) While most bacteria are incapable of surviving in media with a_w below about 0.95, minimum water activities for *Pseudomonas* species (which are of interest as candidates for MEOR) are considerably lower (0.91). Extreme halophiles, such as *Halococcus*, can survive when $a_w=0.75$ (Todar, 2008). Aerobic degradation of benzene, toluene and xylene by halotolerant *Marinobacter* species in soil contaminated with oilfield brines was demonstrated by Nicholson and Fathepure (2004), suggesting potential usefulness in environmental remediation. Anaerobic bacteria from hypersaline environments are of particular interest to MEOR, considering the high salinity of connate water often found in oil-bearing formations. A review of such organisms by Ollivier et al. (1994) devoted considerable attention to sulfate-reducing organisms that feed on polymeric substrates such as starch, cellulose, and chitin, and the work of McMeekin et al. (1993) on anaerobic microorganisms isolated from concentrated salt lakes in Antarctica suggests applications to hydrocarbon degradation. In addition to MEOR and environmental remediation, the study of halotolerant bacteria is also relevant to food preservation (Vilhelmsson et al., 1997).

In addition to the specific chemical and biochemical effects that are often associated with high electrolyte concentrations, non-specific effects can be expected. Solubility of the vast majority of nonelectrolytes decreases with increasing ionic strength. This phenomenon, which is known as the 'salting out' effect, is particularly pronounced for nonpolar solutes (which tend to have low solubilities in pure water). Important examples are oxygen (the concentration of which controls the thermodynamic driving force for aerobic metabolism), and carbon dioxide, ionization of which controls the pH of many natural waters. In this way, high electrolyte concentration could affect both pH and E_h . In this connection it is also worth mentioning that the position of the autoprotolysis equilibrium



also depends weakly on ionic strength, primarily through the activity of water (see, for example, Kron et al., 1995.)

2.6 Temperature

The increase in random molecular motion resulting from an increase in temperature generally exerts negative effects on enzyme function, since the active-site configurations required for catalysis are disrupted. At still higher temperatures, the hydrogen-bonded three-dimensional arrangement of the amino-acid chains also becomes disordered, resulting in irreversible denaturation. This molecular picture of the effects of temperature on enzyme function is generally accepted, but it is also to be observed that the temperatures at which these phenomena occur vary widely between organisms. In general, microbes can be classified according to their optimum temperature range as psychrophiles (< 25 °C), mesophiles (25-45 °C), and thermophiles (45-60 °C). The relatively recent discovery of microbes that can survive in water at temperatures above 100 °C has considerably extended the range of conditions under which life can be expected to exist. Microbes that thrive under such extreme conditions are generally referred to as 'extremophiles'. Since the mean geothermal gradient beneath the continents is of

the order of 25 °C per km, and assuming more conservative upper limit of 110 °C for bacterial activity, as suggested by the work of Blöchl et al. (1995), the biosphere could extend up to 5 km beneath the surface of the earth (compare Gold, 1992).

2.7 Pressure

The effects of pressure on microorganisms are closely associated with those of temperature, since elevated pressures in natural environments are always associated with temperature variations. Specifically, the pressure in the ocean increases by about 10 MPa for every km of depth, while the temperature of the ocean is about 3 °C below about 100 m. On land, the pressure increases by about 3 MPa per km depth, but the temperature increases by about 25 °C per km. Thus, in terms of the earlier terminology introduced to describe the temperature-tolerance of bacteria, a marine bacterium that thrives on the seafloor at a depth of 3 km would be a psychrophile, while its terrestrial counterpart at the same depth underground would be a thermophile. An obvious exception to this generalization would be the bacteria in the vicinity of hydrothermal vents on the seafloor (the so-called 'black smokers'), some of which can withstand temperatures as hot as 121 °C (Miroshnichenko and Osmolovskaya, 2006).

Indirect and direct effects of pressure on cellular function can be identified. An example of an indirect effect is the augmentation of gas solubility with increasing pressure; this could affect the oxidation potentials if the gases concerned are electron donors or acceptors (such as hydrogen or oxygen, respectively.) The study of direct pressure effects originated in the pioneering work of ZoBell and Johnson (1949) on bacterial growth under deep ocean conditions, and on bacteria isolated from marine sediments (ZoBell and Morita, 1957). Growth rates of normal bacteria decrease to zero as hydrostatic pressure approaches about 40 MPa. ZoBell and Johnson used the term 'barophilic' to describe bacteria whose growth rate is enhanced at elevated pressure. (The prefix 'baro-' is sometimes replaced by 'piezo-'.) It is also customary to refer to bacteria for which the diminution of growth rate commences at pressures above 40 MPa as 'piezotolerant'. A third class of bacteria, which cannot be grown under ambient conditions, are referred to as 'obligatory piezophiles'.

The microbiology of bacteria isolated from the deepest oceans has been reviewed by Jannasch and Taylor (1984) and Yayanos (1995). Kato and Bartlett (1997) describe the identification of pressure-regulated genes from deep-sea bacteria of the genus *Shewanella*. Imposition of high pressure affects the fluidity and water-permeability of the cell walls, causing the phospholipid bilayers to pack more tightly and assume a more ordered configuration. Piezotolerant organisms apparently compensate for this by increasing the proportion of unsaturated fatty acids, which have a much lower tendency towards such packing (DeLong and Yayanos, 1985, 1986; Kamimura et al., 1993). A more recent review by Daniel et al. (2006) describes other impressive advances that have been made in the molecular-level understanding of pressure effects on aspects of bacterial physiology. Under high pressures, the DNA double helix becomes more dense, which can interfere with gene expression and the associated protein synthesis. Another important factor is the sterol content; membrane lipids that have a high cholesterol content are more pressure resistant than those that contain ergosterol instead.

DNA analysis reveals the extremophiles to be among the most ancient life forms known. This fact has given rise to intriguing speculations that life on earth could have originated in these extreme environments. The idea that life originated in the depths of the oceans about 3.8 billion years ago is also explored in some detail by Daniel et al. (2006). In a more practical context, pressure-induced deactivation of bacteria has been investigated as a possible way of sterilizing food (Spilimbergo et al., 2002; Aoyama et al., 2004).

2.8 Relation to MEOR

The purpose of the preceding discussion was to identify the factors that constrain the growth of bacteria in subsurface environments, thereby providing a set of criteria by which the suitability of organisms for use in EOR can be assessed and compared. For oil-bearing formations, it is to be observed that some of these constraints are somewhat less rigorous. For example, the salinities of connate brines are typically greater than that of seawater, but much less than those occurring in salt lakes, and pressures of up to 20 MPa and temperatures to 80° are well within the limits observed for survival of bacteria. But the combination of these constraints can be expected to limit the number of suitable organisms. Among the best 'all-round performers' are the *Bacillus* bacteria. Yakimov et al. (1997) described a detailed study of several strains of *Bacillus licheniformis*, and concluded that this organism is potentially useful for enhanced oil recovery. It is capable of functioning at moderately elevated temperatures (55° C) and salinities to 12% NaCl, produces significant quantities of biomass, and a surfactant similar to surfactin (produced by *B. subtilis*) which is known to possess antimicrobial properties. A more recent report by McInerney et al. (2004) describes a particularly thorough examination of over 200 strains of *Bacillus subtilis*, *B. licheniformis*, *B. mojavensis*, and *B. sonorensis*, which were compared with respect to surfactant production under anaerobic conditions at 5% salinity. In the course of their work, these authors developed new and improved procedures for isolating biosurfactants produced by the organisms, established quantitative relationships between the surfactant concentration and interfacial tensions, and performed numerous experiments involving mobilization of oil from Berea sandstone cores.

3. PETROLEUM MICROBIOLOGY

A comprehensive review (692 references) of the microbiology of petroleum was recently published by Van Hamme et al. (2003). This covers the literature up to 2002, and is mainly focused on the molecular-biological aspects of aerobic and anaerobic hydrocarbon utilization, with some reference to applications such as microbial treatment of petroleum waste, microbial recovery and improvement of petroleum, and the design of biosensors. The aspect of petroleum microbiology that is possibly of most importance for MEOR is the ability of microbes to use hydrocarbons as a carbon and energy source. The discussion of hydrocarbon catabolism presented by Van Hamme et al. (2003) can be summarized in the following paragraphs.

Aerobic alkane metabolism follows a mechanism that starts with (1) oxidation of the terminal carbon atom to a carboxylic acid, followed by a further two steps: (2) abstraction of two hydrogens to form an α, β unsaturated acid, and (3) cleavage of the double bond by reaction with a further oxygen molecule. The products of step (3) are acetate and a saturated carboxylic acid with two fewer carbon atoms. This process has been most extensively characterized for *Pseudomonas putida*, but even for this species, not all of the alkane degradation genes have been assigned a function. The gene systems and enzymes involved in alkane degradation by other species are poorly characterized.

Aerobic degradation of polycyclic aromatic hydrocarbons containing up to four rings has been observed in a large number of species. The fundamental step in ring opening is the addition of molecular oxygen across one of the 'double bonds,' to form a vicinal diol, further oxidation of which results in α, β -dehydro ε -keto carboxylic acids that can lose carbon and cyclize to lactones or ketones, among many possible transformations. The genetic regulation of these pathways and how they compete for substrates remains to be determined for most species.

Anaerobic metabolism of polycyclic aromatic hydrocarbons (PAHs) has been observed to occur with sulfate, fumarate, quinones, and manganese dioxide as terminal electron acceptors. As a result of this range of possibilities, the metabolic pathways are much less well characterized than for the aerobic degradations. Under conditions relevant to MEOR, sulfate seems to be the most common electron acceptor, being reduced in the process to hydrogen sulfide. For example, Galushko et al. (1999) reported the degradation of naphthalene to carbonate by a new species of marine sulfate-reducing organism, and verified by chemical analysis that it proceeds to completion according to



The microbial degradation of aliphatic hydrocarbons under anaerobic conditions is not considered by Van Hamme et al. (2003). This is an inherently difficult process. According to an article by Britton (1984) in a collection of papers edited by Gibson (1984), the main kinetic obstacle to microbial degradation is the initial hydroxylation. Reference is made to an alternative pathway that involves dehydrogenation to form an alkene (first demonstrated in a study of the conversion of heptane to heptene by Senez and Azoulay (1961) and Chouteau et al. (1962)), to which water can be much more easily added. Britton (1984) concluded his article by noting that evidence for the occurrence of anaerobic degradation of aliphatic hydrocarbons was at best equivocal. A citation search of some of the papers he refers to reveals that, in the following twenty years, a large body of evidence that aliphatic hydrocarbons can be degraded by sulfate- and nitrate-reducing bacteria has accumulated (Aeckersberg et al., 1991; Rueter et al., 1994; Aeckersberg et al., 1998; So and Young, 1999a,b; Spormann and Widdel, 2000; So et al., 2003), reviewed most recently by Wentzel et al. (2007). This process does not, however, involve intermediate formation of alkenes, as originally envisaged by Senez and coworkers. The first of the two mechanisms that have been proposed involves activation of the alkane by addition of a one-carbon species (presumably derived from carbonate) to form a carboxylic acid, followed by elimination of two carbon atoms. According to this scheme, even- and odd-carbon alkanes result in formation of even- and odd-carbon carboxylic acids, respectively. In the alternative scheme, the hydrocarbon is added across the double bond of fumarate, and after rearrangement of the carbon skeleton of the resulting succinate, action of Acyl-CoA synthetase forms an acyl coenzyme A, which undergoes β oxidation to produce a lower carboxylic acid. Although some details of this mechanism have yet to be worked out, the most important message is that anoxic degradation of aliphatic hydrocarbons can be achieved by numerous organisms.

There are thousands of compounds present in crude oil and it is clearly unrealistic to expect to characterize the bacterial degradation of any more than a handful of them. But the works mentioned in the above paragraph are sufficient to give an idea of the ways in which the main classes of constituents in crude oil can be biodegraded.

4. BIOSURFACTANTS

Microbial enhancement of oil recovery results from the production of biosurfactants by the microbes. The two main effects of these surface-active compounds are (1) a reduction in the interfacial tension between oil and water, and (2) the formation of micelles. The first of these effects reduces the hydrostatic pressure that must be applied to the liquid in the pores of the formation to overcome the capillary effect, while the second provides a physical mechanism whereby oil can be mobilized by a moving aqueous phase. Both effects result from the presence of hydrophilic and hydrophobic structural elements, with affinities for the water phase and for the oil phase, respectively. Reviews by Banat (1995a,b) discuss techniques for their recovery

and production as well as application in *in situ* bioremediation which, together with MEOR, has provided much of the motivation for the study of these substances, since the original idea of their use as environmentally-benign biodegradable detergents (Fiechter, 1992; Kosaric, 1992). Other possible commercial applications are reviewed by Banat et al. (2000) and Singh et al. (2007). More recently, Maier (2003) considered the phenomenon of biosurfactant production from the viewpoint of evolutionary biology, and concluded that while the surfactants produced by bacterial and archaeal organisms (the latter group includes most of the so-called extremophiles) fulfil similar functions, they are very different in molecular structure.

The two main structural types of biosurfactants are lipopeptides and glycolipids; in both cases, the hydrophobic element is a fatty acid molecule with a chain length of at least 12 carbon atoms, while the hydrophilic part is an oligopeptide (a collection of amino acids joined together by amide linkages), or a sugar, respectively. The connexion between the two elements is commonly made by formation of an ester between the fatty acid and one of the hydroxyl groups on the sugar. The sugars that most commonly appear in glycolipid biosurfactants are the monosaccharide rhamnose (Banat, 1993; Robert et al., 1989; Van Dyke et al., 1993; Scheibenbogen et al., 1994; Rahman et al., 2002), and the disaccharides sophorose (Lesik et al., 1989; Hommel et al., 1994) and trehalose (Kosaric et al., 1990; Singer et al., 1990; Abu-Ruwaida et al., 1991). Rhamnolipids are often produced by *Pseudomonas* species, and sophorolipids by *Candida* species. The surfactants produced by *Arthrobacter* species are lipopeptides (Morikawa et al., 1993), as are those produced by *Bacillus* species (McInerney et al., 1990; Horowitz et al., 1990). A particularly important lipopeptide is surfactin, which is produced by *B. pumilus* (Morikawa et al., 1992) and *B. subtilis* (Georgiou et al., 1992). A similar compound is produced by several strains of *B. licheniformis* (Yakimov et al., 1997). According to Bonmatin et al. (1994, Figure 1), the structure of surfactin consists of a chain of seven amino acids with sequence *L* Glu(1)-*L* Leu(2)-*D* Leu(3)-*L* Val(4)-*L* Asp(5)-*D* Leu(6)-*L* Leu(7), formed into a loop with 3-hydroxytetradecanoic acid by an amide bond at position 1 and a lactone bond at position 7. The fatty acid chain allows it to penetrate and rupture cell walls.

While many biosurfactant-producing organisms are known, and increasing numbers of surfactants are being structurally characterized, there are comparatively few quantitative data on their effectiveness in reducing interfacial tensions between water and hydrocarbons, or even their critical micelle concentrations. Youssef et al. (2007) considered the problem of formulating optimum biosurfactant mixtures derived from *Bacillus* strains, for subsurface remediation. They found that the interfacial activity against toluene depended on the relative proportions of surfactants with 3-hydroxy fatty acids of 14, 15, 16, and 18 carbons in the hydrophobic part. The successful application of biosurfactants for enhanced oil recovery would appear to be crucially dependent on quantitative characterization of biosurfactant performance in this way. A fundamental difficulty that can be expected to arise in the use of bacteria, as opposed to their surfactants, in such investigations is that the structures of the surfactants is itself dependent on the available nutrients. The most thorough quantitative study of the interfacial tension reduction appears to be that of McInerney et al. (2004).

The cytotoxicity of some biosurfactants, such as surfactin, raises important questions relevant to the *in situ* use of the bacteria as opposed to the use of their surfactants. (Antimicrobial activity of lipopeptides is, in fact, the subject of a recent patent (Hill et al., 2005). When growing in a nutrient medium, it is known that bacterial populations do not increase indefinitely, but reach a steady state controlled by the nutrient levels. The possibilities that the bacteria might also start to be killed by accumulation of these metabolic products, or possess some feedback mechanism to regulate surfactant production, do not appear to have been investigated. A further possibility

is that these substances could be toxic to humans and other life forms in sufficiently high concentrations.

5. BACTERIAL EFFECTS ON POROUS MEDIA

In the foregoing paragraphs, the emphasis has been on the environmental controls on the growth and survival of bacteria in subsurface environments. Attention is now turned to the effects that bacterial growth can exert on their environments, in particular, hydrocarbon-bearing sedimentary formations. The significance of microbial activity in hydrocarbon reservoirs can be summarized approximately as follows. Beneficial effects include the selective degradation of large molecules (reduction in viscosity), production of surfactants (reducing interfacial tension) and gas (providing an additional pressure driving force.) Detrimental effects of microbial activity in petroleum reservoirs include corrosion of well-bore casings (by products such as hydrogen sulfide), and the consumption of the hydrocarbons by the bacteria. Permeability reduction, due to metabolic products or the bacteria themselves, can exert positive as well as negative effects, by causing secondary flow paths to become active.

5.1 Field Studies

Bailey et al. (2001) present a useful overview of the performance of MEOR and related technologies in the field. Numerous field applications of MEOR have been described, to oilfields in Argentina (Buciak et al., 1995; Strappa et al., 2004), China (Zhang et al., 1999; Deng et al., 1999; Chen et al., 1999; Li et al., 2002; Nagase et al., 2002; Feng et al., 2002), Indonesia (Sugihardjo et al., 1999; Kadarwati et al., 2001; Sabut et al., 2003; Ibrahim et al., 2004), Iran (Abtahi et al., 2003), and the United States (Vadie et al., 1996; Lee et al., 1998; Gullapalli et al., 2000; Brown et al., 2002).

It is difficult to draw general conclusions from the above-cited field studies, since the physical and mineralogical characteristics of the reservoirs concerned are all different. In particular, reported enhancements of oil recovery vary very widely, but the rigorous control experiments needed to substantiate such claims are lacking, and are in any event are arguably impossible, in view of the irreversible changes in reservoir properties that typically accompany the removal of crude oil. For example, Dietrich et al. (1996) describe several case studies of successful applications of MEOR, in dolomite and sandstone reservoirs with permeabilities between 1.7 and 300 md; production rate enhancements varied widely, between 10 and 500%. There is, on the other hand, a broader consensus that microbes can cause beneficial modifications of relevant fluid properties (reduction of viscosity by preferential degradation of large molecules, reduction of interfacial tension by biosurfactant production), as well as usually deleterious effects on the flow properties of the reservoir (reduction in permeability by biomass or mineral deposition).

5.2 Effects on Fluid Properties

Many laboratory-scale experimental studies directed quantifying the bacterial effect on fluid properties have been described. Bryant et al. (1989) showed that higher oil recovery from Berea sandstone cores could be achieved by use of solutions containing suspended bacteria, than by use of solutions contain microbial byproducts (such as surfactants) from which cells had been filtered. Kianipey and Donaldson (1986) described effects of bacteria on capillary pressure hysteresis loops determined by performing immiscible displacements of oil by water, and water by oil. The wetting behavior of immiscible fluids on solid surfaces was investigated by Zekri et al. (2003) through measurements of contact angles, and how this depends on factors such as

salinity, temperature, and the presence of bacteria. Up to a certain concentration, increasing bacterial concentration results in a decrease in contact angle, and beyond this concentration, there is no effect. Mei et al. (2003) carried out core-flooding experiments in presence of bacteria that can use crude oil and polymer as carbon source. Decreases in viscosity, average molecular weight, and paraffin content were observed for the strains of bacteria studied (these were not identified). Zhang et al. (1999) reported that inoculation of Daqing crude oil samples in the laboratory reduced the oil viscosity. Results of field application of the same microbes were described. Deng et al. (1999) performed experiments on the effects of microbes on other properties of crude oil, such as the composition of gas produced, and the organic acid content in produced water. Chen et al. (1999) described use of bacteria to control wax, gum and bitumen content of crude oil. An alternative strategy to the use of bacteria is to use their byproducts, as in the work of Maudgalya et al. (2004) and Maudgalya et al. (2005), who used a mixture of bacterially-produced biosurfactant, 2,3-butanediol, and partially hydrolyzed polyacrylamide.

5.3 Effects on Reservoir Properties

The metabolic byproducts of microbes can exert either positive or negative effects on the flow properties of reservoirs. This is well illustrated by the work of Udegbumam et al. (1991), who studied the effects of bacterial metabolites on pore structures of sandstone and carbonate reservoir materials. The species studied were *Clostridium acetobutylicum*, which produces polymers, and an unidentified halophile that produces acids and gases. Studies of conductivity, permeability, porosity and capillary pressure showed that the the former species causes pore-throat reduction due to biomass plugging, and the latter causes pore-throat enlargement by acid dissolution of carbonates. On the other hand, formation of insoluble mineral products, such as carbonates and sulfates, can reduce both porosity and permeability. Thus, Zhong and Islam (1995) studied the precipitation of calcium carbonate by *Bacillus pasteurii* in sand columns. This mineral precipitation was increased when the pH was increased, but can be mitigated by production of other bacterial metabolites that act as chelating agents (Bailey et al., 2001). Dissolution of carbonates could be promoted indirectly by high pressures. The work of Jennemann and Clark (1992) on the effects of pressure on microbial activity suggests that indirect mitigation of this phenomenon might be possible. These authors observed that at pressures between 0.1 and 8.8 MPa, hydrogen ion concentration, carbohydrate utilization rates, and methanol production were observed to increase with increasing pressures.

A variety of techniques have been devised to permit direct observation of microbes in porous media. Bryant et al. (1992) described a program of experiments on Berea sandstone cores, including CT imaging experiments to detect microbial gas production and determine the gas saturation, in addition to more conventional measurements of relative permeability, microbial retention, and pressure profiles. Hoskins et al. (1999) described a magnetic resonance imaging technique for noninvasive imaging of biofilms of bacteria in porous media. A paper by Paulsen et al. (1994) described a novel experimental setup for investigation of microbial behavior in porous materials, known as AMORS (Automated Microscope Observation Reaction System). This consisted of glass plates with an irregularly-etched pattern mimicing the pore profiles of a porous medium, between which fluid was made to flow. The use of glass permitted direct observation of bacterial attachment and behavior. In this way, it was found that attachment of bacteria and development of slime and extracellular polymeric material was most pronounced in channels of preferential water flow. This observation is germane to the use of bacteria for selective reductions in permeability of highly permeable regions referred to as 'thief zones', to increase sweep efficiency, as described by Stepp et al. (1996), Vadie et al. (1996), and Lee et al. (1998). Gullapalli et al. (2000) described a microbial profile modification process consisting of sequential injection of spores and nutrients, and tests in a carbonate reservoir. The experiment

resulted in the selective plugging of the thief zones, and the biofilm remained stable for 8 months (compare Lee et al. (1998)). The work of Kadarwati et al. (2001) was directed towards finding a cost-effective nutrient to stimulate microbes and plug the thief zones. The nutrients contained molasses combined with nitrogen and phosphorus sources. The use of bacteria for this type of selective fluid diversion represents an alternative to the use of foams (Nguyen et al., 2005a,b), *in situ* formation of gels (Castelijns et al., 2005), and acid treatment of carbonate reservoirs (Kumar et al., 2005).

A novel approach to the analysis of bacterial effects on porous media is that of Han et al. (2001), who performed a stochastic simulation of the reduction of the effective permeability of fractured rocks due to *in situ* bacterial growth. The fracture length distribution is assumed to follow a power law characterized by a length exponent a , according to which the number of fractures with lengths in the interval $[l, l + dl]$ is $n(l)dl$, where

$$n(l) \propto l^{-a}. \quad (5-1)$$

This representation is referred to papers by Pickering et al. (1995) and Renshaw (1999). The distribution of fracture apertures b is described by fractional Brownian motion (fBm), which is a stochastic process with the properties

$$\langle b(\mathbf{r}) - b(\mathbf{r}_0) \rangle = 0 \quad (5-2)$$

$$\langle [b(\mathbf{r}) - b(\mathbf{r}_0)]^2 \rangle \propto |\mathbf{r} - \mathbf{r}_0|^{2H}, \quad (5-3)$$

where H is known as the Hurst exponent. The references given for this are a paper by Hewett (1988), and a book by Feder (1988). These statistical distributions are used to generate discrete fracture networks, within which the transport equations are solved for bacteria and their nutrients and byproducts.

5.4 Gas Generation

Effects of gaseous product generation are commonly assumed to be positive, contributing to the pressure differential driving the movement of the crude oil. But as pointed out by Bryant and Lockhart (2002), production of carbon dioxide by aerobic bacteria is severely constrained by the low solubility of oxygen in the water, and production of large volumes of methane by the anaerobic degradation of crude oil will be limited by the much higher solubility of methane in the oil, especially at elevated pressures. A particularly important gaseous product is hydrogen sulfide, which, in addition to being associated with corrosion of well casings and pore plugging by iron sulfides, is highly toxic. Hitzman et al. (2004) devised a 'biocompetitive exclusion' MEOR process that aims to achieve enhanced oil recovery at the same time as suppressing the biogenic production of hydrogen sulfide. On the basis of a simulation study, Shedid and Abed (2004) reported that increased oil recovery could result from injection of water, hydrogen sulfide, or a combination of both into an oil reservoir. This enhancement is attributed to the higher molecular weight of hydrogen sulfide in relation to methane. However, this idea appears to be far too dangerous to have even a remote chance of success in the field.

6. EXPERIMENTAL STUDIES OF MICROBIAL BEHAVIOR

Since some species of bacteria produce polymeric material, models for the behavior of bacteria in porous media need to consider the occupation of pore space by this biopolymer as well as by the bacteria themselves. Kim and Fogler (2000) studied the formation of biofilm by *Leuconostoc mesenteroides* in the pores of a two-dimensional glass micromodel of a porous medium, by examining the changes in permeability resulting from growth of bacteria as measured by the pressure drop corresponding to a given flow rate, taking into account the erosion of biofilm due to the higher shear stresses associated with constricted flow channels (according to the first-order expression proposed by Rittmann (1982).) They also developed a dynamic model for the process, involving spatial discretization of the differential balance equations, and numerical solution of the resulting ordinary differential equations subject to a periodic boundary condition. They found the dynamic behavior of the model to be in qualitative agreement with experiment. With a continuous supply of nutrient, the bacterial growth resulted in a decrease in permeability (referred to as phase I). Bacterial growth slowed when supply of nutrient ceased (phase II), and permeability went through a minimum. Constriction of flow channels causes an increase in shear stress until the critical value at which erosion of biofilm commences and permeability starts to increase (phase III), until a limiting state is reached in which the shear stress is below the critical value needed for erosion. Stewart and Fogler (2001) carried out further micromodel studies with *Leuconostoc mesenteroides* and concluded that exopolymer production by the bacteria was the dominant mechanism of plugging.

Lanning and Ford (2001) examined the dispersion of *E. coli* NR50 bacteria in glass micromodels representing a spatially periodic network of cylinders and a network of interconnecting capillaries. The distribution of bacteria was determined by direct measurement of turbidity in the pores and by constructing breakthrough curves, and the dispersivities in the two media were determined by fitting the breakthrough curves to the analytical solution of the one-dimensional advection/dispersion equation (Brenner, 1962). Chemotaxis was observed under stagnant flow conditions.

The influence of water flow on the spatial distribution of microbial growth (*Pseudomonas* strain PS⁺) in a two-dimensional bed of glass beads was investigated by Thullner et al. (2002). These authors found that microbial activity was limited by transverse mixing of the electron donor- (glucose) and acceptor- (nitrate) nutrient streams, as evidenced by the high biological activities in the mixing zone. For this species, extracellular polymeric material was the dominant contributor to the bio-clogging process. These authors found that microbial activity was limited by transverse mixing of the electron donor- (glucose) and acceptor- (nitrate) nutrient streams, as evidenced by the high biological activities in the mixing zone.

The changes in the flow properties of a two-dimensional saturated porous medium due to biomass accumulation were also studied experimentally and theoretically by Thullner et al. (2004). The theoretical model developed by these authors involved solution of partial differential equations for the piezometric head and the concentration of solutes, and assumed that concentrations in the stationary bio-phase were constant and that bacterial growth kinetics were described by the Monod equation. The authors found that the best representation of their data was provided by a model assuming bacterial growth in discrete colonies rather than in a continuous biofilm. These two models for the distribution of biomass result in very different relationships between the hydraulic conductivity and the porosity.

Jordan et al. (2004) investigated the influence of indigenous bacteria on the dispersion of *Pseudomonas putida* in sterilized sand, sterilized loamy sand, and two unsterilized loamy sand soils with their indigenous microbial populations. The sand-based system behaved ideally with

respect to cell and substrate transport, the sterilized loamy sand behaved ideally with respect to substrate transport but gave variable behavior for cell transport, and the unsterilized loamy soils were nonideal with respect to both substrate and cell transport. What this indicates is that the indigenous bacterial populations exert a profound influence on the fate of introduced bacteria; in view of this, it seems that any attempt to perform MEOR is unlikely to succeed without careful characterization of the indigenous microflora and experiments of the type described in this paper.

The generation of gas pressure by microbial activity and its possibly beneficial effect on oil recovery is germane to carbon dioxide-enhanced oil recovery, which is identified by the US Geological Survey (Anonymous, 2000) as one of the most successful enhanced oil-recovery technologies. This is described in a recent technical report by Advanced Resources International (Anonymous, 2005), concerned specifically with the large volume (some 57 billion barrels) of so-called 'stranded oil,' which is not economically recoverable by conventional methods. Two main mechanisms of CO₂-EOR have been identified. In miscible CO₂-EOR, contact of injected carbon dioxide with the oil results in vaporization of the light hydrocarbons into the gas phase and condensation of carbon dioxide into the liquid phase, which can eventually result in the coalescence of the two phases, depending on the conditions. The minimum miscibility pressure is found to be proportional to a fractional power of the temperature (Fahrenheit), where the exponent varies linearly with the molecular weight of pentanes and heavier fractions in the reservoir's oil. In immiscible CO₂-EOR, saturation of the oil with carbon dioxide leads to swelling and reduction of the viscosity of the liquid phase, and mobilization by extra driving pressure.

Use of microbes for the enhancement of crude oil recovery is closely related to the problem of bioremediation of areas, such as the shoreline of the Gulf of Mexico, which are frequently contaminated by spills of petroleum products. Simon et al. (2004) examined the efficacy of two commercial bioaugmentation products (containing bacteria of genera *Acetivibrio*, *Bacillus*, and *Pseudomonas*) in a small-scale controlled oil spill at the San Jacinto River Wetlands Facility. Although the products studied had been shown to be capable of degrading hydrocarbons under laboratory conditions, neither was found to result in a statistically-significant increase in first-order biodegradation rate coefficients. On the other hand, a study by Owens et al. (2003) in an Arctic setting found that the biodegradation by indigenous microbes could be enhanced by application of nitrogen- and phosphorus-containing nutrients and mechanical mixing of oiled sediments.

Nan et al. (2000) and Nan and Tan (2002) investigated the growth kinetics of bacteria isolated from oil reservoirs, in relation to temperature and sodium chloride concentration, by measurements of thermal power. A noteworthy feature of this study is its use of the Verhulst (linear logistic) model for bacterial growth kinetics, in contrast to the more common use of the Monod equation. The Verhulst equation is more accurate, and consistent with more general theories of population dynamics.

Nemati et al. (2005) carried out experiments on the modification of permeability profiles in porous media by microbial precipitation of calcium carbonate from the hydrolysis of urea in the presence of calcium chloride. (The microorganism used for this purpose was not formally identified, but stated to be closely related to *Proteus vulgaris*.) The objective was to compare the performance of the bacteria in this task with that achieved by use of the urease enzyme. The authors found that the yield of carbonate obtained by the enzymatic route was much higher, since the enzyme could handle concentrations of urea that would inhibit the activity of the bacteria. The advantages offered by this finding would, however, very likely be offset by the much higher cost of using the enzyme than the bacterium.

7. MATHEMATICAL MODELING

Development of detailed mathematical models for MEOR is a uniquely challenging task, not only as a result of the inherent complexity of the microbes, but also because of the variety of physical and chemical variables that control their behavior in subsurface porous media. Specific or general goals can be envisaged for modeling studies. In specific cases, it is desired to use the models to maximize the yield and minimize the costs of the MEOR process. In a more general sense, a mathematical model can be used to identify the most important parameters and their functional relationships.

While the specific models invariably require intensive numerical computation, some important physical insights can be produced by quite simple analytical models. An example of such an analytical approach is the engineering analysis of MEOR carried out by Bryant and Lockhart (2002), involving examination of the quantitative relationships between microbial performance, reservoir characteristics, and operating conditions (such as well spacing, injection rates and residual oil saturation.) The most important point made by the authors is that the chemical reaction engineering of the microbial process imposes quite severe constraints. These are expressed by the relation between the residence time of the bacteria in a cylindrical reaction region of radius r_m and depth h and porosity ϕ , which is

$$\tau_{res} = \pi r_m^2 h \phi (1 - S_{or}) / Q, \quad (7-1)$$

where Q is the volumetric flow rate and S_{or} is the residual oil saturation, and the time τ_{rxn} required for the microbial reaction to produce a desired concentration c_{req} of some metabolite C from nutrient N, according to the stoichiometric relationship



To estimate the reaction time, the authors assumed isothermal plug flow through the reactor, that consumption of N is first order and irreversible, and that it is injected at initial concentration n_0 . The rate equation is

$$\frac{dc}{dt} = -\nu_N \frac{dn}{dt} = k_1 \nu_N n \quad (7-3)$$

where the stoichiometric coefficient ν_N defines the conversion efficiency of nutrient into product. When integrated subject to the initial condition $n(0) = n_0$,

$$n = n_0 e^{-k_1 t} \Rightarrow \frac{dn}{dt} = -k_1 n_0 e^{-k_1 t}. \quad (7-4)$$

The kinetic equation for c is therefore

$$\frac{dc}{dt} = \nu_N k_1 n_0 e^{-k_1 t} \quad (7-5)$$

which, when integrated subject to the initial condition $c(0) = 0$, gives

$$c = v_N k_1 n_0 \left[-\frac{e^{-k_1 t}}{k_1} \right]_0^t = v_N n_0 [1 - e^{-k_1 t}]. \quad (7-6)$$

The limiting state implied by this equation is complete consumption of the nutrient, and from this result the reaction time needed to establish the desired concentration c_{req} is

$$c_{req} = v_N n_0 [1 - e^{-k_1 \tau_{rxn}}] \Rightarrow \tau_{rxn} = -\frac{1}{k_1} \ln \left[1 - \frac{c_{req}}{v_N n_0} \right]. \quad (7-7)$$

A fundamental design criterion identified by the authors is that $\tau_{rxn} < \tau_{res}$; since τ_{rxn} can be changed only through the nutrient concentration, this condition is satisfied for large values of n_0 , for large values of r_m , and for small values of Q .

It can of course be argued that the physical model on which the above argument is based is overly simplistic, but the analysis draws attention to the important issue of reaction kinetics that has to be addressed by more sophisticated treatments. It is in principle possible to write a balanced equation for the production of a given metabolite (biosurfactant, for example), but the overall rate of production can only be determined experimentally, and must be controlled for bacterial growth rates. An interesting discussion of ‘chemostat models,’ in which nutrient levels and organism densities are determined by solving coupled differential equations expressing the laws of mass action, is given by Kot (2001, pages 161—180). None of the individual species of bacteria proposed as candidates for use in MEOR appear to have been characterized in this way, and the dynamics of populations of different microbes competing for the same food supply have not been considered at all.

Most of the published mathematical models for behavior of bacteria and viruses in porous media were originally motivated by problems arising in water filtration and wastewater treatment (Corapcioglu and Haridas, 1984; Stevik et al., 2004). Such models have three main components:

7.1 Transport Properties

The first is an estimate of the transport properties of the bacteria in the fluid. In the treatment given by Corapcioglu and Haridas (1984), the diffusivity of the bacteria is obtained by application of the Stokes-Einstein equation, which effectively treats the microbe as if it were a particle undergoing Brownian motion. Thus, the diffusivity of a spherical bacterium of diameter d moving through a liquid of viscosity η at temperature T is

$$D = \frac{kT}{3\pi\eta d}, \quad (7-8)$$

where k is Boltzmann's constant. Since the diameter of a bacterium is about 4 orders of magnitude greater than that of a small molecule, the diffusivity due to Brownian motion should be approximately 4 orders of magnitude smaller than the typical molecular diffusivity of $10^{-9} \text{ m}^2 \text{ s}^{-1}$. Many bacteria differ from other Brownian particles in being able to move

independently in the direction of nutrient concentration gradients, but in MEOR, this effect is likely to be insignificant compared to advection by the fluid. The remaining parameters needed to characterize the transport of the bacteria are the longitudinal and transverse dispersivities of the porous medium. These can in principle be determined from the grain size according to experimentally based correlations (Delgado, 2007) in terms of the Péclet number $d\langle v\rangle/D$, where $\langle v\rangle$ is the average fluid velocity, which is the ratio of the rate of advection to the rate of diffusion. They are typically between 100 and 1000 times greater than D . In practice, the heterogeneity of the ground can cause dispersivities inferred from field measurements to differ by orders of magnitude from those measured in a laboratory under carefully-controlled conditions.

7.2 Conservation Law

With the neglect of chemotaxis, the concentration of bacteria in the fluid phase of a small element of the porous medium is defined by a partial differential equation expressing the rate of change of the concentration as the sum of terms resulting from diffusion (or dispersion), advection, and transfer between the fluid phase and the surface of the solid grains. Numerical solution of systems of equations of this general type is at the heart of computational hydrology and simulation of oil wells.

Application of such analysis to bacterial transport requires an additional assumption about how the local concentration of ‘adsorbed’ bacteria on the solid grains is related to the concentration in the liquid phase; this is intimately linked with considerations of the origin and strength of the physical interactions between the bacteria and the solid. The attachment or filtration process can be assumed to be irreversible or reversible. Treatment of the latter case can be based on explicit consideration of forward and reverse reaction rates, or the assumption of an equilibrium isotherm such as the Langmuir, Freundlich, or linear isotherm. The equation for the concentration is amenable to exact solution only for the last case, and forms the basis of colloid filtration theory, reviewed by Harvey and Garabedian (1991), Ryan and Elimelech (1996), and most recently by Tufenkji (2007), who identifies several notable deficiencies of the classical treatments based on colloid filtration theory. These are as follows:

7.2.1 Local Equilibrium

A detailed theoretical analysis of the local equilibrium assumption for the one dimensional advection-dispersion equation was presented by Valocchi (1991), who found that the assumption is reasonable if the kinetic parameter $F = k_r L/\langle v\rangle$ (where k_r is the rate constant for desorption and L is the column length) is at least 100. It seems unlikely that this condition would be satisfied by bacteria, considering their large size relative to chemical solutes. [It is also worth remarking that even for this fairly simple model, the analytical solution obtained by Valocchi (1991) requires numerical inverse Laplace Transformation.]

7.2.2 Breakdown of Filtration Theory

The classical theory of colloid filtration performs poorly where there are repulsive electrostatic forces between the suspended particles and the substrate grains. In a review of the energetics of bacterial adhesion, van Loosdrecht and Zehnder (1990) compare treatments based on surface Gibbs energy with those based on the DLVO (Derjaguin - Landau - Verwey - Overbeek) theory of colloids. According to the second approach, adhesion can correspond either to a primary minimum in the energy of interaction between the colloid and the substrate, or a secondary minimum in which the surface and the colloidal particle are separated by a thin layer of water.

More recent experimental measurements of retained particle concentration profiles by Tufenkji and Elimelech (2004) and Tufenkji and Elimelech (2005) show pronounced deviations from the predictions of the classical models. The application of kinetic theory to the calculation of the collection efficiency of the porous medium (Hahn and O'Melia, 2004) indicates that taking into account the existence of the secondary energy minimum gives substantially better agreement with experiment.

7.2.3 Physical Straining

Physical straining is important for larger microorganisms, where the ratio of the particle diameter to the median grain diameter is greater than about 0.05. From geometrical considerations, Herzig et al. (1970) derived a simple formula expressing the volume fraction of retained spherical bacteria in terms of the ratio of bacterial diameter to average grain size. Weiss et al. (1995) examined the filtration behavior of 14 strains of bacteria of different sizes and shapes, and found that the more spherical bacteria (as measured by the width to length ratio) were preferentially eluted. These findings have been confirmed in very recent work by Xu et al. (2007), who also observed a linear relationship between straining rate of spherical bacteria and the ratio of bacterial diameter to average grain size for values of this ratio up to about 0.06. Rod- or peanut-shaped bacteria tend to adopt a preferred orientation as they approach pore-space constrictions, in which the major axis aligns with the direction of the flow.

The above list of factors is by no means exhaustive. Biomolecules embedded in the surfaces of the cells could potentially be responsible for highly specific interactions with solid surfaces, in addition to the nonspecific effects that can be expected to arise from ionization of embedded protein side chains. Furthermore, the kinetics of microbial growth, death, deactivation, or detachment are not adequately characterized. Tufenkji (2007) identifies three main areas in which classical filtration theory needs to be improved: (1) allowing for a distribution of adhesion efficiencies; (2) inclusion of the combined effects of physical straining and surface adhesion; (3) accounting for chemotaxis and microbe motility. While some mathematical treatments of chemotaxis have been proposed (Barton and Ford, 1997; Duffy et al., 1997), these do not include the effects of superimposed fluid motion that are of interest in MEOR. Bonilla et al. (2007) explored the dynamics of a single microbe in a pore, with particular reference to reversible attachment/detachment. The authors concluded that the average number of collisions against the solid walls is controlled by the relative magnitude of the bacterial motility to advection. The flow distribution in the pores was determined by the computational fluid dynamics code FLUENT.

Measurements of the physical interactions between bacteria and solid surfaces can be made. Camesano et al. (2007) provide a synopsis of techniques that are currently in use for characterizing interactions between bacterial cells and solid surfaces. These include atomic force microscopy, quartz-crystal microbalance measurements, total internal reflection microscopy, and total internal reflection fluorescence. The operating principles, advantages, and limitations of each technique are reviewed. As a general rule, the pH of the solution can be expected to exert a strong effect, since this determines the surface charge resulting from ionization of the protein side-chains embedded in cell walls. In addition, the strength of attachment of some bacteria can be affected by starvation. For example, Sanin et al. (2003) showed that carbon starvation of *Pseudomonas* bacteria reduces their hydrophobicity (and hence, the tendency to attach to hydrocarbons), and causes significant reduction in size and shape - from $2.5 \times 0.6 \mu\text{m}$ rods to spheres with diameter less than $0.6 \mu\text{m}$. Similar results were obtained in experiments by Cunningham et al. (2007) on *Klebsiella oxytoca*, who found that the adhesion of bacteria to surfaces in the immediate vicinity of the injection point can be reduced by long term nutrient starvation. The starved bacteria were shown to penetrate further and distribute more uniformly

in the porous medium. Although no explanation for this effect was advanced by the authors, this result is potentially significant for the MEOR strategies involving injection of ‘foreign’ bacteria into oil wells.

7.3 Biofilm Clogging and Related Phenomena

In addition to biosurfactants, many bacteria generate ‘biofilm,’ extracellular polymeric material that may serve as a growth medium and provide a means of attachment to solid substrates. As pointed out by Mostafa and Van Geel (2007) clogging of the pores of a porous medium can be caused chemically (by formation of a precipitate from dissolved electrolytes), physically (by entrainment of suspended particles), or biologically (formation of biomass by microbes); all these mechanisms are potentially relevant to enhanced oil recovery. Thus, deliberate formation of calcium carbonate has been used for selective blocking of high-permeability zones in a reservoir (Nemati et al., 2005), and porous media can be clogged by suspended bacteria or other particles whose diameters are comparable with the average pore diameters. Biological approaches to selective permeability reduction have been described by several authors (Stepp et al., 1996; Vadie et al., 1996; Lee et al., 1998; Gullalpalli et al., 2000).

Theoretical description of the biological clogging of pores is complicated because the rate of production of the clogging agent is coupled nonlinearly not only to the growth of the bacteria, but also to the flux of nutrients transported by the fluid. The basis of the earliest approaches to the development of models of this phenomenon is the idea that the the medium can be represented as a bundle of independent (that is, not interconnected) capillary tubes (Taylor and Jaffé 1990; Taylor et al., 1990; Vandevivere and Baveye, 1992; Vandevivere et al., 1995). Comparisons of the predictions of this type of theory with experiment have led to the current view that the underlying physical model is fundamentally incapable of accounting for the reductions in hydraulic conductivity that result from microbial growth. This deficiency in performance has been attributed to the underlying assumption that the biofilm forms continuous layers of uniform thickness. More recent treatments (Suchomel et al., 1998a,b; Thullner et al., 2002a) have been based on the more physically plausible assumption of a pore network rather than a bundle of parallel, independent capillaries, which is found to lead to consistently better agreement with experiment (Thullner et al., 2002b, 2004). It is desirable to consider the pore network models in some detail, not only because this general approach is of current interest, but also because this illustrates important interactions. The physical system described by Thullner et al. (2002b) consisted of a two-dimensional rectangular network of cylindrical pores, identical in length, but with different radii derived from a lognormal distribution. Within each pore i , fully-developed laminar flow is assumed to occur, so that the flow rate q_i is proportional to the pressure gradient,

$$q_i = -k_i \frac{\Delta p_i}{l} \quad (7-9)$$

where the hydraulic conductivity is proportional to the fourth power of the radius r_i according to the Hagen-Poiseuille formula:

$$k_i = \frac{\pi r_i^4}{8\eta}. \quad (7-10)$$

A potentially important simplification is the neglect of the complicated flow pattern that can be expected in the regions formed by the intersections of the cylindrical conduits - the volumes of

these intersection points are set equal to zero. Now if one pair of opposite boundaries is assumed to be impenetrable and the other pair correspond to different (but constant) pressures, the requirement that the net flows into each node should be zero leads to a system of linear equations for the corresponding pressures. Numerical solution of these equations then leads to q_i and to the total flow from one side to the other, from which the hydraulic conductivity of the network can be determined. This whole calculation is repeated for several thousand random assignments of pore radii from the lognormal distribution, and the relation between the hydraulic conductivity and the volume fraction occupied by biomass can be represented by an empirical function.

The other half of the problem is the calculation of the nutrient distribution, which is achieved by numerically integrating a spatially-discretized approximation to a transport equation. The growth of the biofilm, which results in reduction of the radii of the cylindrical pores, is coupled to the distribution of dissolved nutrients by a kinetic expression defining the rate of biomass production for a given concentration. A further effect that could make a potentially significant contribution to the local rate of biofilm growth (or nutrient consumption) is the ablation of biofilm by the increase in flow velocities due to pore constriction. This was studied experimentally by Rittmann (1982), who developed an empirical formula for the rate of biofilm loss in terms of shear stress in one-dimensional flows.

Thullner et al. (2002b) used this approach to investigate the hydraulic effects of two modes of bacterial growth: in a biofilm spread over the surfaces of the pores, and in discrete colonies that result in complete plugging of the pores. They concluded that a given extent of hydraulic conductivity reduction could be achieved with a lot less biomass in the colony-growth model than in the biofilm model, and that the former scenario gave better agreement with published experimental data.

Recent pore-network models have involved more rigorous treatment of the fluid velocity distributions, which for two-dimensional models are well within the capability of readily available computational fluid dynamics software. Knutson et al. (2007) compared predictions of continuum and pore-scale models for a biodegradation process. The pore scale model assumes that biomass is created by the bacteria within the pores, affecting the flow distribution, which is determined by the Lattice Boltzmann Method (Hou et al., 1995; Chen and Doolen, 1998) under steady state conditions, for periodic arrays of cells that were parallel or staggered. The steady-state concentration profiles of electron-donor and electron-acceptor nutrients are determined as a function of the local flow field and concentration of bacteria by numerically solving the coupled advection-dispersion equations. In the continuum approach, on the other hand, the flow field is assumed to be one-dimensional and uniform, and biomass fills all the pore spaces in the system uniformly. The authors concluded that the classical continuum reactive transport models overpredict the amount of degradation because they fail to distinguish between the spreading and mixing of the solutes.

7.4 Fully-Numerical Approaches

The earliest published attempts (Islam 1990; Chang et al., 1991; Desouky et al., 1996) to develop mathematical models for MEOR involved solution of coupled nonlinear parabolic partial differential equations, which are typically at the heart of reservoir simulators. For this purpose, existing computer codes have to be augmented by the addition of an equation for the rate of diffusion of bacteria and their capture by the porous medium, including expressions for the effect of bacterial deposition on the permeability; differential balance equation(s) for the transport of nutrient(s), including the possible effects of adsorption; and the assumption of a

kinetic model for the bacterial growth kinetics (typically assumed to be described by the empirical Monod equation.) Zhang et al. (1992) described a one-dimensional mathematical model for MEOR in which the bacteria are partitioned into a mobile phase containing planktonic bacteria, and a stationary phase containing sessile bacteria adhering to the pore surface. Anaerobic microbial growth and metabolism are assumed to occur in both groups, and are limited by both glucose and ammonium nitrate as nutrients. Exchange between the mobile and sessile bacterial phases is also assumed to occur, and growth kinetics of the bacteria were described by an extension of the Monod equation to two growth-limiting nutrients. Delshad et al. (2002) described the application of UTCHEM to the simulation of MEOR. More recently, other members of that group (John et al., 2004) developed a version of UTCHEM optimized for parallel computation, and presented results of a large calculation involving about 100000 grid blocks. An exception to the widespread use of the Monod equation to describe the kinetics is the semi-analytical model described by Sitnikov et al. (1994), which makes use of the logistic differential equation to describe the bacterial growth kinetics.

From the engineering analysis given by Bryant and Lockhart (2002), as well as the subsequent discussion of the biofilm model of Thullner et al. (2002b), it is clear that chemical kinetics plays a vital role in coupling the production of bio-products to the fluxes of aqueous species and suspended bacteria. But the indiscriminate reliance of nearly all simulators on the Monod equation implies limiting behavior that is inconsistent with the laws of mass action that form the basis for the kinetic characterization of bacterial growth. Some potentially valuable insights regarding this point can be realized by considering the special limiting case where the concentrations of nutrients and bacteria are spatially uniform. The only changes that occur in this situation are the growth of the bacterial population and the consumption of nutrient. In the following two sections, the different kinetic laws that govern the nutrient-limited growth of bacteria and the enzyme-mediated chemical reactions that they carry out are contrasted.

7.4.1 Logistic Growth of Bacteria

Assuming that the consumption of nutrient is proportional to the product of the bacterial and nutrient concentrations (which is the law of mass action), and that the growth rate of the bacteria is also proportional to the product of nutrient and bacterial concentrations, it is possible to solve the coupled differential equations for both concentrations. In particular, it is possible to show that the sum of these concentrations is constant. Thus, if B and N are the respective concentrations of bacteria and nutrients, k is the growth rate of bacteria per unit concentration of nutrient, and the initial concentrations are $B_0 = B(0)$ and $N_0 = N(0)$, the law of mass action leads to the differential equations

$$\frac{dB}{dt} = kBN, \quad \frac{dN}{dt} = -kBN, \quad (7-11)$$

from which it is obvious that

$$\frac{dB}{dt} + \frac{dN}{dt} = 0, \quad (7-12)$$

or

$$B + N = B_0 + N_0 \equiv C. \quad (7-13)$$

This last relation can be used to express N in terms of B , leaving the single differential equation

$$\frac{dB}{dt} = kB(C - B). \quad (7-14)$$

Separating variables,

$$\int_{B_0}^B \frac{dB}{B(C - B)} = kt, \quad (7-15)$$

and since

$$\int_{B_0}^B \frac{1}{B(C - B)} dB = \frac{1}{C} \int_{B_0}^B \left[\frac{1}{B} + \frac{1}{C - B} \right] dB = \frac{1}{C} \left[\ln \frac{B}{C - B} - \ln \frac{B_0}{C - B_0} \right], \quad (7-16)$$

simple algebra leads to the solution

$$B = C \frac{1}{1 + (N_0/B_0)e^{-kCt}}, \quad N = C \frac{(N_0/B_0)e^{-kCt}}{1 + (N_0/B_0)e^{-kCt}}. \quad (7-17)$$

Thus, the bacterial concentration is limited by the nutrient concentration, which is the essence of the logistic growth law. This solution can be verified by differentiation:

$$\frac{dB}{dt} = C \cdot \frac{-1}{[1 + (N_0/B_0)e^{-kCt}]^2} \cdot -kC(N_0/B_0)e^{-kCt} = kBN. \quad (7-18)$$

7.4.2 Monod Equation

Simulators of MEOR nearly always rely on the Monod equation, which expresses the rate of bacterial growth in terms of the substrate or nutrient concentration. In terms of the above notation this is

$$\frac{dB}{dt} = \left(\frac{dB}{dt} \right)_{max} \frac{N}{K + N}, \quad (7-19)$$

where K is a constant, which is essentially the same as the Michaelis-Menten equation for enzyme kinetics. In order to get an expression for either the nutrient or the bacteria concentration as a function of time, it is necessary to impose the condition that the sum of the bacteria and nutrient concentrations is constant. But the above derivation shows that this result is an immediate consequence of the law of mass action. One practical limitation of the Monod equation is that the usual procedure for the estimation of the maximum growth rate and the constant K relies on estimation of the reaction rate, which must be obtained from the reaction extent (derived by chemical analysis) by some form of numerical differentiation. A further limitation is that its integrated form is an implicit, rather than explicit expression for the reaction extent. This is best demonstrated by the classical derivation by Briggs and Haldane (1925) of the Michaelis-Menten equation for the kinetics of the conversion of substrate S to product P ,

catalyzed by enzyme E. Denoting the respective concentrations of these species by S , P , and E , the forward and reverse rate constants for the enzyme-substrate combination



as k_1 and k_{-1} , respectively, and the rate constant for the irreversible decomposition of the adduct ES as k_2 , and the concentration of this adduct as A , application of the law of mass action gives

$$\frac{dP}{dt} = k_2 A, \quad \frac{dA}{dt} = k_1 ES - (k_{-1} + k_2) A. \quad (7-21)$$

If the steady state hypothesis is valid, the derivative of A can be set equal to zero, resulting in

$$A = \frac{k_1}{k_{-1} + k_2} ES \equiv \frac{1}{K_m} ES, \quad (7-22)$$

and since the total concentration of enzyme in the system is the sum of A and E ,

$$E_0 = E + \frac{1}{K_m} ES \rightarrow E = E_0 \left(1 + \frac{S}{K_m} \right) = E_0 \frac{K_m + S}{K_m}. \quad (7-23)$$

The rate of production of P is therefore

$$\frac{dP}{dt} = k_2 A = \frac{k_2 E_0 S}{K_m + S}, \quad (7-24)$$

and since the equation for the overall process is $S \rightarrow P$, this is equivalent to the differential equation

$$\frac{dS}{dt} = -\frac{k_2 E_0 S}{K_m + S} \equiv -\frac{k_2' S}{K_m + S}. \quad (7-25)$$

This is clearly of the same form as the Monod equation 19, but the above derivation shows that this analytical form results from mechanistic considerations that might not necessarily apply to bacterial growth. Now when this equation is integrated from the initial condition $S(0) = S_0$, the result is the transcendental equation

$$\int_{S_0}^S \left(1 + \frac{K_m}{S} \right) dS = S - S_0 + K_m \ln \frac{S}{S_0} = -k_2' t. \quad (7-26)$$

Although this equation cannot be solved analytically, its numerical solution presents no difficulties. It can be visualized as the point of intersection of the graph of the logarithmic function $K_m \ln(S_0/S)$ (which crosses the S axis at $S = S_0$), with the family of parallel lines $S - S_0 + k_2' t$ (which also passes through the point $(S_0, 0)$ for $t = 0$.) For higher values of t ,

the intersection point becomes closer to the vertical axis $S = 0$, which corresponds to complete transformation of the substrate at $t = \infty$.

In the most general case, one can expect both types of kinetic laws to be important. On one hand, application of the law of mass action to microbial populations results in the linear logistic equation, and on the other, application of the law of mass action (and the crucial steady-state approximation) to an enzyme-catalyzed process results in the Michaelis-Menten (Monod) equation. The relation between these two kinetic models becomes important when considering the production of metabolites - such as biosurfactants - by bacteria. If one assumes that the steady-state hypothesis applies to the rate-limiting step in the enzymic biosynthesis of the surfactant, the kinetics can be expected to follow the Michaelis-Menten equation. But since the bacteria can be expected to multiply at the same time, this means that the total enzyme concentration E_0 - which was regarded as constant in the above derivation - will vary with time. If the nutrient is growth-limiting and the generation of the byproduct is fast compared with the multiplication rate of the bacteria, the rate of the former process can be expected to increase as the total concentration of enzyme in the system increases. In the light of these considerations, it is clear that the design of an MEOR process relying on *in situ* biosurfactant production will require carefully controlled experimentation to determine the specific growth rate as well as the Michaelis-Menten parameters of the rate-limiting enzyme reaction.

8. CONCLUSIONS

The works referred to in this report show that impressive advances have been made in characterizing the structure and activity of biosurfactants produced by a variety of bacteria, and in understanding the theoretical aspects of phenomena related to MEOR. Of particular importance in the latter regard are the modeling studies for the clogging of pores by bacteria or biofilm, which have brought to light the fundamental deficiencies in the early 'parallel-pore' models. But a potentially significant limitation of these pore-network models is that they have so far been two-dimensional. Although the differences between the predicted behavior of three- and two-dimensional networks are unlikely to be as pronounced as those between two- and one-dimensional models - because of their allowance for interconnections between pores - the applicability of the empirical results obtained from the two-dimensional studies to three-dimensional studies remains to be determined. From a more practical viewpoint, it is also unclear how such modeling approaches could be incorporated into the oilfield simulation software in common use.

The conclusions reached by Bryant and Lockhart (2002) on the constraints imposed by chemical reaction kinetics deserve to be reiterated, not only with specific regard to the putatively beneficial effects of microbial gas production, but more generally because chemical kinetics provides an important mechanism of coupling between the fluxes of nutrients, metabolites and the bacteria themselves. Moreover, the foregoing discussion of bacterial population dynamics reveals complexities that might not be amenable to representation in terms of the widely-used Monod equation. Unfortunately, essentially nothing is known about the kinetic characterization of the bacteria that are of interest, in terms of either overall growth rates or yields of metabolic byproducts for unit input of nutrients. The obvious importance of the rate of *in situ* biosurfactant production to the practical feasibility of MEOR suggests that chemostat-type kinetic experiments on the candidate micro-organisms would be a highly desirable direction of future research.

ACKNOWLEDGEMENTS

This report was supported by the CSIRO MEOR project of the Wealth from Oceans National Research Flagship.

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