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Microbial Enhanced Oil Recovery Research

Final Report
Annex V

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ABSTRACT

The objective of this project was to develop an engineering framework for the exploitation of microorganisms to enhance oil recovery. An order of magnitude analysis indicated that selective plugging and the production of biosurfactants are the two most likely mechanisms for the mobilization of oil in microbial enhanced oil recovery (MEOR). The latter, biosurfactant production, is easier to control within a reservoir environment and was investigated in some detail. An extensive literature survey indicated that the bacterium *Bacillus licheniformis* JF-2 produces a very effective surface active agent capable of increasing the capillary number to values sufficiently low for oil mobilization. In addition, earlier studies had shown that growth of this bacterium and biosurfactant production occur under conditions that are typically encountered in MEOR, namely temperatures up to 55 °C, lack of oxygen and salinities of up to 10% w/v. The chemical structure of the surfactant, its interfacial properties and its production by fermentation were characterized in some detail. In parallel, a set of experiments as conducted to measure the transport of *Bacillus licheniformis* JF-2 in sandpacks. It was shown that the determining parameters for cell transport in porous media are: cell size and degree of coagulation, presence of dispersants, injection velocity and cell concentration. The mechanisms of bacteria retention within the pores of the reservoir were analyzed based on heuristic arguments. A mathematical simulator of MEOR was developed using conservation equations in which the mechanisms of bacteria retention and the growth kinetics of the cells were incorporated. The predictions of the model agreed reasonably well with experimental results.

EXECUTIVE SUMMARY

Microbial enhanced oil recovery (MEOR) provides two important advantages: i) It is a more economical alternative to conventional chemical or thermal oil recovery methods. ii) MEOR requires small capital equipment and therefore it is particularly suitable for small oil operators. However, the use of microbial cultures is not widely accepted by the oil industry because of uncertainties regarding the engineering and environmental aspects of MEOR. There is a number of ways in which microorganisms may affect the mobilization of oil within a reservoir. Accumulation of polymers can increase the viscosity and thus positively affect the capillary number. Similarly, the capillary number may be increased by a reduction in interfacial tension due to surfactant production. Biogass formation may

cause repressurization of the reservoir whereas the formation of organic acids may cause dissolution of the reservoir rock resulting in changes in porosity. Finally the accumulation of biomass within the reservoir can cause selective plugging of the most highly permeable regions of the reservoir. In chapter 1 we present an engineering analysis of the relative significance of the above mechanisms of MEOR. It is shown that based on the known metabolic capabilities of bacteria, (such as the amount of gas or acid that can be produced per unit weight of carbohydrate nutrients and biomass) the most significant mechanisms of MEOR are the production of biosurfactants and selective plugging. Unlike other microbial products, the formation of very small amounts of biosurfactants in situ is sufficient to induce large changes in the flow of oil within the reservoir. From an engineering standpoint biosurfactant production is much easier to control than in situ microbial plugging. Consequently it is the most promising target for the rational design of MEOR and was selected for further studies.

In chapter 2 we review the recent progress in the understanding of biosurfactant structure, synthesis and surface properties. Microbial biosurfactants include a wide variety of chemical structures such as glycolipids, polysaccharide-protein complexes, phospholipids, fatty acids and lipopeptides. The latter have been reported to have the best interfacial characteristics. Little is known about the biosynthesis and regulation of lipopeptides and other biosurfactants. For well studied lipopeptides, such as surfactin from *Bacillus subtilis*, the hydrophobic domain is derived from microbial fatty acids whereas the peptide part of the compound is synthesized non-ribosomally by a large multipeptide complex. The production of surfactin is affected by the ratio of carbon to nitrogen source and the presence of trace ions. The genes responsible for the production of surfactin are found in three loci in the Bacillus chromosome. Recent studies have clearly demonstrated that the molecular mechanisms responsible for surfactin production are complicated and are not well understood currently.

Bacillus licheniformis JF-2 has been shown previously to produce a very active surfactant similar to surfactin. This bacterium grows and produces the biosurfactant under a variety of conditions that are relevant to MEOR. Biosurfactant production occurred in the temperature range 23 °C to 50°C and was optimal (i.e. minimum interfacial tension against decane) in cultures grown at 45 °C. *B. licheniformis* grew well in the presence of up to 10% NaCl; high biosurfactant production was obtained in 0.5% to 5.5% NaCl. Under aerobic conditions the major metabolic products resulting from cell growth were lactic acid and acetic acid together with small amounts of acetoin and formic acid. However, even though it was found that certain compounds in the growth medium act as a cosurfactant

(see below) none of the above major metabolic byproducts was found to reduce the interfacial tension when mixed with the purified surfactant.

The surface active compound of *B. licheniformis* JF-2 was purified to near homogeneity using acid precipitation, organic extraction, silica gel chromatography and preparative high pressure liquid chromatography. The molecular weight of the purified compound was found to be 1035 Da using Fast Atom Bombardment Mass Spectroscopy. One and two dimensional NMR spectroscopy showed that it consists of a peptide domain with structure similar to surfactin and a heterogeneous lipid domain consisting of n-, iso-, and aniso C18 fatty acids which are esterified to the peptide. The purified surfactant exhibits a CMC of 10 mg/ml and reduces the interfacial tension against decane to less than 6×10^{-3} mN/m. The surface activity of the surfactant was dependent on the concentration of NaCl and the presence of unidentified metabolite(s) in the aqueous phase. Furthermore, the surface activity showed a unique dependence on the pH. Optimal interfacial tensions against decane were obtained at pH 6.0; a one-unit change in the pH from the optimal value increased the interfacial tension by at least an order of magnitude (chapter 4).

Following the microbiological characterization of the bacteria, the identification of their metabolic products and the properties of the surfactant, it was of interest to determine how injected bacteria can be propagated within a porous reservoir. For this purpose the transport of bacteria in sandpacks was studied experimentally using a specially designed apparatus. In this system we found that bacterial retention and permeability reduction occur primarily within the first few centimeters after the entrance of the porous medium. This phenomenon was due to the formation of microagregates that were excluded from the reservoir pores and/or multiparticle hydrodynamic exclusion. The effects of (i) cell dispersant, (ii) injection velocity, (iii) cell concentration and (iv) salinity were studied experimentally. Addition of dispersants was found to be essential for the propagation of the cells within the sandpack. Furthermore, the efficiency of transport was increased at high injection velocities and in the presence of low salt concentrations.

Finally in chapter six, we describe a MEOR simulator which incorporates a quantitative description of bacteria transport, growth, reservoir permeability alterations and changes in the capillary number due to surfactant production. The retention and transport of bacteria was modelled using a fractional flow approach. In this way, the various mechanisms of bacteria retention were incorporated into the model through the selection of an appropriate shape for the fractional flow curve. The reduction in the permeability of the reservoir due to microbial plugging was modelled using the effective medium theory. The resulting equations are strongly coupled to bacteria growth kinetics (determined

experimentally) through conservation relations. The model can be used to predict in - situ transport and growth of bacteria, nutrient consumption and metabolite production. Reasonably good agreement between the model and experimental data was obtained indicating that the physical processes incorporated in the model are adequate.

Chapter 1: A Critical Evaluation of MEOR Processes

ABSTRACT

In this chapter, the status of in situ microbial enhanced oil recovery methods (MEOR) is reviewed. It is shown that the method shows considerable promise as an economical alternative to conventional EOR processes. Several major technical problems , however, still exist in the application of MEOR methods. These include strain selection and improvement, competitive growth and control of in situ microbial populations after injection of nutrients, transportability of injected bacteria into the reservoir to minimize permeability impairment and selection of suitable injection strategies. The extensive literature in these areas is reviewed.

1.1 INTRODUCTION

Up to two-thirds of the crude oil in place in petroleum reservoirs remains unrecovered at the end of primary production. Waterflooding and/or enhanced oil recovery methods then need to be initiated to recover the remainder. Enhanced oil recovery (EOR) methods that have been tried and tested in the field include steam injection, in situ combustion, CO_2 flooding, surfactant, polymer and caustic flooding or a combination of the above. All of these processes aim at either improving the sweep efficiency in the reservoir or substantially altering the capillary number N_{ca}, which is defined as the ratio of viscous to capillary forces holding the oil in place.

$$N_{ca} = \frac{\mu_w v_w}{\sigma_{ow}}$$

Chemical methods such as surfactant, micellar polymer or caustic flooding attempt to reduce the surface tension (s) thereby increasing the capillary number. Thermal methods aim at reducing the viscosity (μ) of the trapped phase, whereas polymeric additives in the injected water tend to increase the viscous pressure gradients in the flowing water phase. The injection of CO_2 or organic solvents is meant to miscibly displace the oil. At reservoir pressures CO_2 becomes partially miscible with the oil, increases the effective oil saturation and allows it to flow. Each of the methods described above have both economic and

and allows it to flow. Each of the methods described above have both economic and technical limitations on their applicability. Every candidate reservoir must be carefully evaluated before the application of a specific EOR method. Surfactant and polymer floods have high chemical costs whereas thermal methods have very high energy requirements. A review of the criteria used to evaluate the applicability of the above EOR methods to the particular conditions in an oil reservoir is given by Brown et al. (1986).

In recent years a number of studies have demonstrated the potential applicability of microbes for enhanced oil recovery (MEOR). MEOR offers two major economic advantages over more conventional EOR methods:

(i) Lower capital costs and

(ii) Substantially lower chemical/energy costs.

However, several important technological problems need to be overcome before microbial enhanced oil recovery becomes readily applicable in the field. Microbial activities useful for oil recovery can be exploited in two ways. Surfactants, polysaccharides, or other products that can facilitate oil recovery may be produced by conventional fermentation techniques and then injected into the reservoir. This *ex-situ* process is, in general, more capital and labor intensive. Alternatively, the *in-situ* process where bacteria and nutrients are injected either sequentially or simultaneously into the reservoir is less costly and therefore more promising for a wider range of conditions. This review concentrates only on the latter MEOR process with special emphasis on some of the technical problems that will have to be investigated further.

The most common MEOR process involves the simultaneous injection of bacteria and nutrients into the reservoir. Certain microbial activities affect the reservoir environment and flow properties of oil and thus facilitate its transport. The mechanism by which the growing microorganisms enhance oil recovery is not clear. It is likely that several microbial processes act cooperatively to enhance the flow of oil. Their relative importance depends on the reservoir conditions, the strain(s) of microorganisms and the strategies employed for inocula and nutrient injection. These parameters, in turn, determine the extent of microbial propagation and metabolic activities within the reservoir and consequently the efficiency of oil recovery.

Little is known about the fate of microorganisms within the oil reservoir. Bryant and Douglas (1985 a & b) have investigated the survival of injected microbes within core samples as well as their ability to compete with indigenous organisms. However, the ability of microorganisms to survive is not always indicative of metabolic activities. For example, bacterial spores can withstand adverse environmental conditions while remaining

metabolically inactive. Overall, the growth and metabolism of microorganisms within porous rocks depend on four main processes.

(i) The physiology of the microorganism, i.e. substrate utilization, growth inhibition by metabolic by-products etc.

(ii) The propagation of the microorganism within the porous matrix.

(iii) Adsorption of the cells on the solid and subsequent plugging of pores due to growth.

(iv) The transport of nutrients and metabolites in the pores.

As with any other heterogeneous system, consumption of nutrients results in the establishment of concentration gradients. In areas where the cell density is high, nutrients become depleted, affecting further growth and product formation. Diffusion-limited growth has been demonstrated in many ecologically and industrially important systems including biofilms (Atkinson, 1974), microbial pellets (Atkinson, 1974), and colonization of solid substrates (Georgiou and Shuler, 1986). Clearly the interactions which take place when bacteria grow and colonize the petroleum reservoir are very complex and cannot be understood solely from macroscopic parameters such as the number of bacteria in the effluent.

1.2. BASIC OIL RECOVERY MECHANISMS IN MEOR

1.2.1 Early Work

Beckman in 1926 was the first to recommend the introduction of microorganisms into reservoirs for the purpose of enhancing oil recovery. However, the first laboratory experiments were conducted twenty years later by ZoBell (1946,1947). He used cultures of sulfate-reducing bacteria isolated from oilfield brines, particularly *Desulfovibrio hydrocarbonoclasticus* and *D. halohydrocarbonoclastus,* and showed that treatment of oil-bearing cores with bacteria caused the release of oil. He postulated several mechanisms for the additional oil recovery.

1) The microbes reduce inorganic sulfate to H_2S. Hydrogen sulfide is slightly acidic and reacts with carbonates in the rock to increase permeability.
2) Production of carbon dioxide and other gases results in increased reservoir pressure and decreased oil viscosity, therefore promoting displacement.
3) Attachment and growth of bacteria on the surface of oil-bearing rocks displaces films of oil.

4) Microbial degradation of heavier oil components can decrease the average molecular weight of the oil and reduce its viscosity.
5) The production of surface-active agents by bacteria lowers the interfacial tension.

Updegraff and Wren (1954) repeated ZoBell's experiments but were unable to demonstrate enhancement of oil release following the injection of sulfate-reducing bacteria. Nevertheless, they developed and patented techniques involving the injection of a variety of other organisms including *Escherichia coli,* bacteria of the genera *Clostridium* and *Bacillus*, or sulphate-reducing bacteria. These organisms were used in combination with other species and were supplied with carbohydrates and mineral nutrients.

1.2.2 Current Status

The exact mechanism by which the growth of microorganisms *in -situ* facilitates the flow of oil is uncertain. In principle many microbial activities may be beneficial for oil recovery (Moses and Springham, 1982; Springham, 1984; Hitzman, 1988). Most microorganisms produce gases (CO_2, CH_4, H_2) that may cause pressurization of the reservoir. Organic acids (e.g. acetic acid, formic acid, etc.) dissolve carbonates and open additional microscopic flow paths. Biosurfactants can decrease the interfacial tension between oil and brine. Under anaerobic conditions many microorganisms produce solvents such as butanol or acetone which can act as co-surfactants. The production of biological polymers can increase the viscosity of the water phase and facilitate recovery. Finally, growth of the microorganisms and production of adhesive molecules on the cell walls may cause plugging of high permeability areas of the reservoir, resulting in better sweep efficiency.

All the above processes have been implicated in oil recovery but clearly they are not equally significant. A better understanding of the main mechanism of oil recovery is extremely useful for selecting appropriate bacterial strains and for the design of optimal operational procedures for MEOR. Unfortunately, because of the complex interactions between microorganisms, the reservoir surface, the aqueous phase and the oil, the complete elucidation of the role of microbial activities is no simple matter and will probably require intensive research efforts for several years. The complexity of the problem is further amplified by the fact that the mechanism of microbial enhanced oil recovery may very well be different for various reservoirs. Nevertheless, a better understanding of

MEOR can be obtained by using simple arguments to evaluate the relative importance of microbial activities.

The formation of gases has been stressed in nearly all field trials (Brown et al., 1986) as well as in laboratory experiments (Yarbrough and Coty, 1983). It has been proposed that gases increase the reservoir pressure and aid the release of the oil. However the amount of gas needed for pressurizing the reservoir is very large and could not be easily generated from microbial growth. In CO_2 flooding it is not uncommon to use 20% CO_2 per unit weight of oil produced (Holm, 1977). A simple stoichiometric calculation reveals that the biological production of such large amounts of gas is not realistic. As an example, consider the microbial production of CO_2 from an organic carbohydrate source. Under anaerobic conditions, typical high-CO_2-producing microorganisms (for example *Zymomonas mobilis*) form about 1 mole of CO_2 for every 12.5 grams of cells (wet weight) formed (Gottschalk, 1983). A simple calculation reveals that a large amount of cell mass (approximately 6 % biomass wet weight per unit weight of oil) would have to be generated in order to produce the amount of CO_2 needed for satisfactory oil recovery. Among other things, the generation of such high concentrations of biomass is very likely to cause serious plugging problems. Similarly, Grist (1983) conducted a simple economic analysis and concluded that *in-situ* production of biogas is not economical because of the large amount of nutrients that must be injected. Brown et al. (1986) suggested that the production of microbial gas may be important for increasing the pressure of localized areas, such as the area around the wellbore, that are not in equilibrium with the rest of the field. This explanation may be more plausible than the overall pressurization of the reservoir. The mechanisms by which such a local pressurization would occur and how it could lead to enhanced oil production on the reservoir scale is not clear.

Grist (1983) showed that the production of organic acids is probably not a primary mechanism for enhancing the flow of oil. Similarly, reduction in the viscosity of the oil because of *in-situ* degradation of higher molecular weight hydrocarbons is unlikely (Springham, 1984; also see following section). The conclusion that can be drawn from these general arguments is that three microbial activities may play a critical role in microbial enhanced oil recovery.

i) Production of surfactants.

ii) Production of biopolymers.

iii) Selective plugging of high permeability zones (Brown et al., 1986).

The significance of biosurfactants in MEOR depends on several factors including the effectiveness of the biosurfactant in lowering the interfacial tension, the amount

necessary to obtain significant surface tension reduction, and the adsorption of the surfactant on the rock surface. It has been estimated (Lake, 1988) that the interfacial tension must be lowered to 10^{-2} to 10^{-3} mN/m, corresponding to $N_{Ca}=10^{-3}$ to 10^{-4}, before significant oil recovery is achieved. Biosurfactants which are capable of reducing the interfacial tension to such low values have been reported (Brown et al., 1986). Such surfactants may be suitable for enhanced oil recovery. A wide variety of different organisms produce surface active compounds such as glycolipids, phospholipids, fatty acids, amino-acid-containing compounds (i.e. proteins and lipopeptides) and neutral lipids (Cooper and Zajic, 1980; Lang and Wagner, 1987; Parkinson, 1987). The production of biosurfactants varies depending on the nature of the compound, the bacterial strain and the fermentation conditions, e.g. nutrient composition, pH, presence of metal ions, etc. Significant surfactant yields (up to 0.18 g surfactant / g substrate consumed) have been reported (Syldatk and Wagner, 1987). Many surfactant-producing organisms require oxygen for growth and are unsuitable for *in-situ* production (see following section). Recently, however, microorganisms which produce surfactants under anaerobic conditions have been isolated (Javaheri et al. , 1985). These studies indicate that the production of relatively large amounts of biosurfactants within the reservoir is feasible. With proper design, microbial growth and surfactant production can be localized near the water-oil interface. Production of the surfactant at the location where it is most needed should minimize losses due to adsorption on the rock.

High-molecular-weight polymers are injected into the reservoir to increase the viscous pressure gradient in the water and reduce viscous fingering effects. Microbially produced polysaccharides such as xanthan gums have very favorable characteristics and have been used for years in enhanced oil recovery. Xanthan gum is synthesized aerobically and therefore cannot be produced *in situ*. However, some other polysaccharides are produced under anaerobic conditions (Springham, 1984). At present there is very little information available on the role of *in-situ* production of biopolymers for viscosity enhancement of the displacing brine.

Fingering effects are accentuated when permeability variations exist between oil-bearing zones in a reservoir. The high-permeability zones (thief zones) conduct most of the injected fluids and result in poor vertical sweep efficiencies. The selective plugging of the "thief zones" is a potential application of MEOR. Traditionally "selective plugging" agents or "profile control" agents include time-delayed polymer gels or inorganic precipitates. The problem with the application of these compounds is the lack of control in the location of the plugging. If the region immediately around the wellbore is plugged by a "shallow"

treatment, the fluid will invade the low-permeability zone only to the depth of the treatment. A deep treatment ensures that a larger fraction of the low-permeability zone sees the injected fluids. Microbial plugging may offer a potential advantage if the placement and growth of microorganisms can be controlled. Biopolymers play a significant role in the plugging of high-permeability regions. Microbial slimes mediate the adhesion of cells to the pore walls and the formation of cell aggregates. Experiments by Jenneman et al. (1982) have shown that, as expected, microbial growth *in situ* causes significant reductions in permeability. More recently Torbati et al. (1986) have shown that microbial growth causes preferential plugging of large pores in Berea sandstone. Bacteria selected specifically for their ability to produce biopolymers for selective plugging have been employed in at least one field trial (Jack and Stehmeier, 1987). Overall, the selective reduction of reservoir permeability is considered one of the more promising mechanisms of microbial enhanced oil recovery. With proper design, the production of relatively small amounts of biomass in the right location should be sufficient to provide the desired degree of plugging.

1.3. SELECTION OF MICROORGANISMS FOR MEOR

1.3.1 Criteria for Selecting Microbial Cultures

To a very large extent, the success of MEOR depends on the use of suitable microorganisms. If the culture injected in the oil well is inappropriate for MEOR, then it will be at best ineffective and will not facilitate recovery or, more likely, it will be detrimental to production. The selection of microorganisms for MEOR must be based on biological, geophysical and ecological considerations. Above all, there are two important criteria that must be met. First and foremost the microorganism should not be pathogenic to humans or animals. Second, growth of the microorganisms must not have any adverse effects on the quality of the oil or produce any undesirable metabolic products. Undesirable microbial activities include non-selective reservoir plugging, equipment corrosion and production of H_2S from inorganic sulphur. Interestingly, the microorganisms that were used in early MEOR experiments (cf. section 2.1) produce H_2S and are therefore unacceptable for field applications by today's standards.

The environment of the reservoir can be inhibitory for the growth of many organisms. A number of deep reservoirs are considered too inhospitable for growth primarily because of high prevailing temperatures, which may exceed 100°C. Reservoirs suitable for MEOR should typically be at temperatures lower than 80°C with salinities less

than 10%. More extreme conditions limit the growth of the large majority of microbial species. The reservoir pressure is not normally a limiting factor because the growth of most microorganisms is unaffected by pressures of up to 300 atm. Such high pressures are not encountered in the more shallow reservoirs where the temperature is permissive for microbial growth.

For all practical purposes the reservoir should be considered to be an anaerobic environment. This means that organisms with a strict aerobic requirement are not suitable for MEOR. The introduction of air in the well has been suggested as a means of stimulating the growth of aerobic cells but is clearly not practical in view of the cost and the increased risk of equipment corrosion (Shennan and Levi, 1987). Only strict anaerobic and facultative organisms (which can grow either in the presence or absence of oxygen) should be considered for injection. Strict anaerobic organisms lack an enzymatic system for protection against oxidative damage. They are poisoned by oxygen and therefore require some care in handling and for growth of the inoculum. For this reason faculative organisms which can survive either in the presence of absence of oxygen may be preferable.

Several microorganisms can metabolize hydrocarbons quite efficiently. The use of microorganisms which utilize oil-derived hydrocarbons for growth could reduce raw material costs significantly. The microorganisms would use a small fraction of the trapped hydrocarbons to facilitate the recovery of much larger quantities of oil. However, there is strong evidence that molecular oxygen is necessary for the biological degradation of hydrocarbons. There are only few and rather controversial reports that the oxidation of hydrocarbons can take place in the absence of molecular oxygen (Brown et al., 1986). In most of these studies, the rate of hydrocarbon degradation was extremely low and for this reason artifacts due to the presence of trace amounts of oxygen or the spontaneous decomposition of hydrocarbons could not be dismissed. Even if anaerobic degradation of hydrocarbons does occur, the rates appear to be too slow for practical purposes. Therefore, because of the lack of oxygen in the reservoir, it is unlikely that hydrocarbon-degrading organisms will be useful for *in-situ* oil recovery applications.

The growth of indigenous populations is stimulated when nutrient solutions are introduced in the reservoir. To manipulate the reservoir environment in the proper way, the injected cultures must be in a position to outgrow native organisms. Otherwise, the latter will prevail with unpredictable and frequently undesirable effects (Crawford, 1982; Moses and Springham, 1982). With a few exceptions, the competition between indigenous and injected microorganisms has not been studied extensively (Douglas et al., 1988; Bryant

and Douglas, 1987 b). More experimental information on microbial interactions within the reservoir is desirable. In practice it may not be possible to evaluate the competitive ability of the injected culture relative to the diverse microbial populations that exist within different reservoirs. A more realistic approach is the *a priori* selection of microbial cultures that have a high probability of outgrowing other species. To compete successfully, the injected microorganisms should have high growth rates and efficient nutrient transport system so that they can grow in nutrient-deficient areas of the reservoir. The synthesis of metabolic products having antimicrobial activities may also be desirable in order to suppress the growth of other species.

A problem which has not been addressed in detail in the past is the ability of microorganisms to grow in a heterogeneous environment. Typically, in MEOR after the injection of nutrients and cells, the well is shut in for several weeks. Within that time the microorganisms grow in the static environment of the reservoir pores. The consumption of nutrients and the production of metabolites within the pores gives rise to concentration gradients that affect metabolism and growth. The significance of heterogeneous environments for microbial growth has been studied rather extensively with cells immobilized within polymeric matrices. In these experiments the cells are first immobilized uniformly within a porous support. The cell matrix is then suspended in nutrients. After a short period of time all the viable cells are located at the surface of the matrix, in close contact with the nutrient solution. Typically the viable cell layer is less than 200 μm thick (Karel et al., 1985). The cells in the interior are either dead or metabolize extremely slowly because of mass transfer limitations. A similar situation can arise within reservoir pores. The existence of heterogeneous conditions is very important for the metabolic activities of the cells and must be taken into account in MEOR. The microorganism of choice should not be significantly affected by high cell densities or the accumulation of low to moderate concentrations of its own metabolites (i.e. should not be limited by feedback inhibition). It must also be able to remain viable for long periods of time in nutrient-depleted environments.

Finally, and most importantly, the organism to be used in MEOR must be able to facilitate the release of oil. The most plausible mechanisms of oil release as described previously should be the starting point in the selection of organisms with beneficial activities.

1.3.2 Selection of Microorganisms in the Past

Two methods have been used for the selection of microorganisms for enhanced oil recovery. The first one consists of selecting one or more previously characterized microbial strains either from culture collections or from other sources. These microorganisms must exhibit properties useful for MEOR such as production of large amounts of biosurfactants, water-soluble polymers, etc. Using characterized strains has the advantage of simplifying the identification of the inoculated species and their metabolic products in the co-produced water. As a result it is easier to evaluate the ability of the microorganisms to grow and produce metabolites within the reservoir. On the other hand, such cultures may not be adapted to the reservoir conditions or may be unable to compete very successfully with the indigenous populations. Alternatively, suitable inocula may be selected by screening microorganisms from different natural sources which resemble the reservoir environment. Lazar (1983) suggested that four sources are particularly suitable for culture isolation: formation waters with high levels of bacterial contamination, sediments from formation water purification plants, sludge from biogas operations and microbial cultures growing on the effluents from sugar refineries. Commonly used sources include the soil and water from the oil reservoir which is to be treated or from a reservoir with similar characteristics, so that the selected organisms are able to survive under these particular reservoir conditions and can compete successfully with the indigenous flora. Selected microorganisms may also be adapted to the reservoir environment prior to injection. For example the inoculum used in the Arlansk field trial in the USSR was prepared by growing specific cultures in oil and formation water in pits adjacent to the well to be treated (Hitzman, 1988).

Essentially two ways of screening microorganisms have commonly been used: screening for microbes able to release oil from sandpacks or rock cores, or screening for organisms synthesizing chemicals of interest for oil recovery such as biosurfactants (Jenneman et al., 1985) or biopolymers (e.g. Pfiffner et al.,1986). A possible strategy (shown in figure 1) combining these two methods has been proposed by Shennan et al.(1986). The first method has been successfully used by Lazar (1983) to select suitable bacterial populations. He found that the best two sources of species were formation water collected as co-produced water from the reservoir and sugar refinery effluents. He used techniques previously applied in Hungary and Poland to "adapt" the bacteria to reservoir conditions in a special type of apparatus called a collector. The bacteria were selected based on their ability to release oil and then concentrated before injection. There was evidence that these bacterial populations increased the production of oil in two out of seven reservoirs during a one to five year period. This method of selection usually gives rise to largely

undefined populations. The composition of the microbial mixture is likely to change in the reservoir. Because of the variations in microbial compositions and metabolic activities, it is almost impossible to decide by which mechanism oil recovery is enhanced. Pfiffner et al. (1986) used the second method to select biopolymer-producing bacteria. Strains isolated from environmental samples were screened for rapid anaerobic growth, viscosity increase and extracellular polysaccharide production. A bacterium able to produce, under anaerobic conditions and high salt concentrations, a polymer showing better characteristics and behavior than xanthan gum, was isolated.

1.3.3 Future Trends

The stressful and competitive environment of the oil reservoir imposes rather stringent constraints on culture selection. However, given the immense variety of microorganisms in nature, there are still plenty of choices. As discussed by Moses and Springham (1982) organisms such as protozoa, algae and fungi do not satisfy the basic requirements for MEOR. Only bacteria are suitable for in situ oil recovery. Janshekar (1983) compiled a partial list of some common bacteria which have been used in MEOR experiments and field studies. The use of a wide variety of bacterial strains in different studies makes it difficult to compare results and draw conclusions about the relative significance of different mechanisms of oil recovery. In more mature industrial fermentation processes it is common practice to concentrate research and development efforts on a small number of strains that appear most promising based on past experience and common sense. Eventually, MEOR studies should also be conducted with appropriate model organisms. The selected strains could be modified and improved by using standard microbiological techniques. Strain improvement has proved extremely useful in industrial fermentations and waste water applications. There is no reason why standard chemical or mutagenesis or U.V. irradiation followed by selection should not be used in MEOR to obtain cells with desirable characteristics. Recent advances in genetic engineering have led to the development of metabolic engineering, i.e. the rerouting of the cell's metabolism to increase the production and yield of desired chemicals. This very powerful approach can be used to develop improved bacteria for biosurfactant and biopolymer production. However, within the current regulatory environment, genetically engineered organisms are not yet acceptable for field applications.

Early MEOR experiments were conducted with mixed microbial cultures consisting of a variety of microbial species. Inocula consisting of different bacteria are capable of a

wider range of metabolic activities compared to pure cultures. In addition symbiotic relations between species may ensure the stability of the culture against external perturbations. Consider for example a microbial population consisting of two symbiotic organisms. The first organism degrades a compound which cannot be degraded by the other. In turn, the second organism uses the metabolic by-products generated by the first culture. In the absence of the second organism the metabolic by-products from the first would accumulate and eventually inhibit growth. Mixed cultures exhibit very complex responses to environmental parameters. It is almost impossible to reproduce the exact microbial composition of a mixed culture (Bailey and Ollis, 1986). Therefore large variations in the culture characteristics and consequently the release of oil should be expected. Currently MEOR is still in the development stage and it is important to establish a careful record of successful field trials with reproducible results. In contrast, pure cultures, consisting of a single bacterial species, can be prepared very easily and reproducibly. For this reason, pure cultures have been preferred in more recent laboratory experiments as well as in some field trials.

1.4. TRANSPORT OF MICROORGANISMS IN RESERVOIRS

The growth of indigenous bacteria is known to have a deleterious effect on reservoir permeability. For this reason there is a very real concern in MEOR that the injected bacteria will form an external or internal filter cake around the wellbore and cause irreversible permeability impairment. Bactericides are used to inhibit growth and filtration equipment is routinely employed to remove bacteria and other particulates in the injection of brines into reservoirs. It follows that the deliberate introduction of potentially problematic "plugging agents" must be done with extreme caution. Indeed, in the eyes of skeptics this should not be done at all. Until the transportability of the bacteria in reservoir rocks can be accomplished and clearly demonstrated, MEOR processes have little chance of becoming commercially successful.

One of the most comprehensive experimental studies on bacterial transport in sandstones was conducted by Kalish et al. (1964). Seven bacterial species with different shapes, sizes and aggregation tendencies were used. It was found that the injection of high concentrations of bacteria (10^7/ml) resulted in a smaller reduction in permeability for the same cumulative cell population injected. This is counter intuitive, as one might expect more coagulation at higher cell concentrations. A likely explanation may be that immobilized cells on the face of the core act as better collectors of incoming cells than the

rock grains. Higher permeability rocks(~300 md) had a smaller rate of reduction of permeability ($k/k_i = 0.8$) as opposed to lower-permeability cores ($k/k_i = 0.1$). However, the stabilized k/k_i values were higher for the low-permeability cores and the depth of the permeability impairment was also less. This indicates the formation of an external filter cake of bacteria in the low-permeability cores. As expected, the larger cells of *Bacillus cereus* (average length 6 μm), which have a tendency to aggregate, were shown to cause more damage than the non-aggregating smaller cells such as *Pseudomonas aeruginosa* (length 1.5 μm). The smaller bacteria (~2 μm) could easily penetrate consolidated rocks of high permeability (~300 md). This study was conducted with dead bacteria which may behave differently than the metabolically active organisms.

In a similar study Raleigh and Flock (1965) and Hart et al. (1960) showed that the pore geometry can be correlated well with the depth of plugging. However, for the relatively low-permeability rocks (from 5 md to 139 md) used in these experiments, no simple correlation could be established with the rate of plugging. Myers and McCready (1966) demonstrated that *Seratia marcescens* organisms can penetrate Berea cores up to 14 inches long over a period of a few days. Although no data were presented, they claimed penetration of up to 3 inches in limestones cores of very low permeability (0.1 millidarcy). Updegraff and Wren (1954) showed that sulfate-reducing bacteria could propagate through highly permeable sandpacks without any problem.

More recently, Jang et al. (1982,1983) showed that strains of *Bacillus subtilis*, *Pseudomonas* and *Clostridium* can be transported through high-permeability cores (>400 md). In such cases, retention of the bacteria seems to be controlled by surface adsorption on the rock grains rather than by size exclusion or sieving. The presence of a residual oil saturation in the core enhanced bacterial movement. Surface charges on the bacteria and the rock grains as well as the ionic composition of the brine influence the adsorption or adhesion of bacteria. Sharma et al. (1985) showed that the addition of anionic polyelectrolytes significantly increased the transportability of the bacteria. Effluent bacteria counts increased by two or three orders of magnitude with the addition of polyphosphates and heparin. The use of EDTA as a chelating agent for polyvalent cations also increased the electrostatic repulsion between bacteria and substrate causing less retention. Surface charge modification of the bacteria and the substrate can provide a useful method for reducing the retention and plugging ability of the bacteria.

Jenneman et al. (1982a, 1982b) provided experimental evidence that the ability of bacteria to plug pores in low- to medium-permeability rocks can be useful in selective plugging treatments. This was also the strategy adopted by Jack et al. (1982), Updegraff

(1982) and Crawford (1982) to enhance oil recovery. For low- to medium-permeability reservoirs (k<300 to 400 md) this may be the strategy of choice. However, as suggested by other studies, the application of other MEOR strategies to high-permeability reservoirs should not be limited by the transportability of microbes.

The selective plugging of high-permeability streaks needs to extend several feet away from the wellbore for it to be effective. A new strategy to achieve this has recently been demonstrated by MacLeod et al. (1988) and Lappin-Scott et al. (1988). They have shown that starved cells of *Klebsiella pneumoniae* isolated from oilfield brines could penetrate sandstone cores from 200 md to 400 md in permeability without causing a substantial reduction in permeability. This observation was attributed to size reduction of the bacteria (down to 0.5 to 0.25 μm) and the absence of polymer slime on the cell walls. Both these factors minimized bacterial retention so that the bacteria were evenly distributed across a 5 cm long core. These results demonstrated clearly the ability of smaller sized bacteria to penetrate reservoir cores without causing damage. Injection of "nutrients" to resuscitate the cells caused growth and immediate plugging throughout the length of the core. The bacteria became encapsulated by glycocalyx and their size increased up to 1.7 mm. These experiments demonstrated a more effective approach for using microbes in selective plugging processes.

Despite the experimental effort directed at studying microbial transport, no consensus exists as to the mechanisms responsible for bacterial retention. Without such a consistent framework it is difficult to stimulate bacterial transport. Five basic mechanisms can be identified to be responsible for the retention of bacteria in porous rocks. These are briefly discussed below.

1) Size exclusion or sieving. In general the injection of bacteria of size comparable to or larger than the mean pore size will result in straining the bacteria at the formation face in the form of an external cake. Studies in filtration suggest that the ratio of particle to pore size (r_s/r_p) must be no more than 1/3 to 1/5 if external filter cake formation is to be minimized. For typical bacteria sizes (without coagulation) of ~ 2 μm, this translates into a minimum acceptable pore size of ~ 6 μm to 10 μm. The ratio r_s/r_p also depends on the concentration of bacteria injected. Consequently, $r_s/r_p < 1/3$ does not preclude the formation of an external filter cake, which could form over time because of buildup of bacterial deposits on the grain surfaces.

Chauhan (1988) reported that the average pore throat sizes are in the range from 1-10 μm for Berea cores having permeabilities between 50- 500 md. A large fraction of the

pore throats are of the same size as the bacteria (0.5-2.0 μm) for cores having permeabilities in the range of 1 to 60 md.

2) Retention on rock grains because of surface forces. The retention of bacteria (or other colloidal particles) can occur because of attractive surface forces between the sand grains and the bacteria. These forces include van der Waals and electrostatic forces (Sharma and Yortsos, 1987; Payatakes and Tien, 1973). Cell walls for most bacteria are negatively charged at neutral pH, as are the surfaces of the sand grains. However, the overall potential energy of interaction between them is attractive since the electrostatic term is not sufficiently large to overcome the van der Waals attraction. By appropriate manipulation of the charges on bacterial surfaces and sand grains, it is possible to substantially reduce the extent of bacterial retention (Sharma et al, 1985). This has been experimentally demonstrated by Sharma et al. (1985). It was shown that the addition of polyphosphates increases the effluent concentration of bacteria by two or three orders of magnitude. As pointed out later, not all the increase can be attributed to changes in the interactions between cells and pore walls. Reductions in the extent of coagulation may also be significant.

3) Adhesion on rock surfaces because of polymeric binding. Many bacteria are surrounded by an extracellular polymeric matrix consisting of polysaccharides and/or glycoproteins. The amount and composition of the coating varies with the growth cycle and environmental parameters such as nutrient composition, temperature and culture pH. This polymeric material has adhesive properties and mediates the attachment of cells to each other (coagulation) or to the pore walls. While this is a short-range interaction (by comparison with the electrostatic and van der Waals interaction, i.e. DLVO interaction), it can cause the irreversible attachment of cells and is mechanistically quite different compared to the physical, partially reversible adhesion due to DLVO forces.

4) Coagulation and entrapment. Bacterial cells may have a higher propensity to coagulate than inert colloidal particles. This is partly due to the presence of extracellular adhesive polymers. In dilute cell suspensions the composition of the suspending medium is of prime importance in determining the extent of coagulation. In more concentrated suspensions, where cell-cell interactions are relatively close range, the composition of the cell wall and the presence of extracellular polymers are equally important. Coagulation affects the transportability of cells primarily by increasing the effective particle size and enhancing straining or capture at pore constrictions.

5) Gravity settling. An order-of-magnitude comparison of the gravitational forces and the surface forces on an almost neutrally buoyant Brownian particle clearly shows that the surface forces are several orders of magnitude larger (Sharma and Yortsos, 1987). Gravity should therefore play a relatively minor role in determining the extent of bacterial retention.

With so many factors controlling the mobility of the bacteria it is not surprising that no general conclusions can be reached for a given species. Definition of the above mechanisms, however, now allows us to devise methods to enhance the transportability of bacteria in reservoir rocks. Such methods are essential for placing the microorganisms deep in the reservoir where the production of metabolites is most beneficial.

1.5. ECOLOGICAL AND ENVIRONMENTAL ASPECTS OF MEOR

MEOR is based on the injection of relatively large quantities of nutrients in the reservoir. These nutrients accelerate the growth of both the injected cultures as well as the indigenous microorganisms. The environmental consequences of generating large amounts of biomass within a natural underground system have not yet been fully studied. From the available information on MEOR there does not seem to be any reason for concern. However, there are three issues that will have to be addressed in more detail:

1) The addition of nutrients can stimulate indigenous microorganisms, leading to the proliferation of possible pathogens. Fortunately, the indigenous microorganisms that have been isolated from reservoirs are generally non-pathogenic. The injected bacteria should pose no danger since they are specifically screened to exclude any undesirable consequences.
2) Leakage of the microorganisms from the oil reservoir into an underlying aquifer could lead to contamination of the water. However, no such incidents have been reported in over 200 field trials conducted so far.
3) The water effluent from MEOR is rich in organic material (mostly biomass) and cannot be disposed directly because it will contaminate surface fresh waters, leading to eutrophication. Removal of organics can be achieved by conventional treatment methods (Sundstrom and Klei, 1979), for general overview of wastewater treatment).

At present there are no specific regulations regarding MEOR. The use and disposal of microbial cultures must comply with current state and federal regulations. Douglas et al. (1988) concluded that MEOR is safe provided that it is properly designed and monitored. However, environmental concerns cannot yet be completely dismissed, partly because the

impact of MEOR has not been sufficiently addressed. Careful studies will have to be conducted before MEOR can be applied extensively, especially if the oil field is near population centers and sources of drinking water.

1.6. MEOR INJECTION STRATEGIES AND FIELD TRIALS

1.6.1 Early Field Trials

Since the mid-1950's, numerous trials have been conducted in eastern Europe, the U.S.S.R, the Netherlands and the U.S. Extensive reviews have been published by Hitzman (1982, 1988). Two types of injection strategies have commonly been used: a cyclic process and a microbial flooding process. Both treatments involve three major steps.

1) Injection - A solution of microorganisms and nutrients is introduced into the reservoir, sometimes after preliminary well cleaning.
2) Incubation - The well is shut in for a certain period to allow sufficient microbial growth and metabolite production.
3) Production - In the case of a cyclic process, the well used for injection is reopened for the release of oil and by-products resulting from the treatment. In case of a microbial flooding process, the released oil and products are usually displaced through the reservoir toward a production well by injection of water.

Table 1.2 summarizes some of the techniques used to inoculate and treat wells. It is apparent that a variety of operational strategies have been used in different countries.

It is difficult to draw any final conclusion from early trials, for a number of reasons:

1) Essential data on methods and conditions are frequently omitted and the results are often given in vague terms.
2) Even when a full set of data is available, the extent to which oil production has increased (if at all) is not easy to estimate because of the absence of proper controls, such as untreated wells comparable to those receiving the treatment and remaining unaffected by the bacterial inoculation.
3) Generally, stimulation of oil production has to be estimated by extrapolating the production curves (cumulative oil production rate as a function of time). However, accurate extrapolation of the production curves is often very difficult.
4) Most field trials have been conducted on wells that produced very little oil to start with. The incremental oil production due to MEOR is often so small (a few barrels a day) that

the increase could well be due to just shutting the well in for a few days or conducting a workover.

5) Finally, in most cases, it is almost impossible to decide on the mechanism responsible for the increase in oil release. This is because the inocula used in early studies consisted largely of undefined mixed populations isolated from the environment.

1.6.2 Recent Research

The main impetus for recent research in MEOR is to obtain a clear understanding of important parameters before attempting a field trial. A series of laboratory experiments, each designed for the specific study of one part (if possible) of the overall MEOR treatment (e.g., transport of bacteria in a porous medium, study of the effects of useful chemicals produced by bacteria) must first be conducted. It is now widely recognized that scaling-up microbial oil recovery methods from the laboratory to the field is a challenging task (Moses, 1987).

1.6.2.1 Laboratory Experiments

Sand-packs and reservoir rock cores have been used to simulate the reservoir. A wide variety of cores have been used in laboratory experiments. Berea sandstone cores have been used most often because of their fairly uniform intergranular porosity and the availability of samples with different permeabilities. Sterilization of cores has been difficult to achieve. Jenneman et al. (1986) found evidence of surviving indigenous bacteria even after the cores had been treated with steam for two weeks, autoclaved for 12 hours and dried at 121°C.

In a typical experiment (e.g. Bryant and Douglas 1987) cores or sandpacks are first flooded with brine. Crude oil is then injected until no more brine is displaced. Subsequently, several pore volumes of brine are injected to simulate the effect of water-flooding. The core or sandpack is now ready for bacterial inoculation. Preliminary experiments to determine the rate of transport of the microorganisms in the core can be very useful for the interpretation of subsequent studies. The rate of bacterial penetration is measured as a function of permeability and bacterial concentration. Optimal injection concentrations are determined based upon good penetration and low plugging of the rock matrix around the injection port. Low to intermediate (around 10^6/ml) bacterial counts and/or the use of bacterial spores are recommended in order to obtain good penetration in

Berea sandstone cores (Jang et al., 1983). The additional oil recovered during or after injections of the bacterial culture is measured. This is a direct measure of the effectiveness of a culture in recovering oil. There are significant problems in scaling up the observations made in such a bench scale experiment to a reservoir scale. Such problems include reservoir heterogeneity, the linear geometry of the experimental apparatus versus the radial geometry in the field, and scaling characteristic residence times.

1.6.2.2 Recent Field Trials

Considerable attention is now given to a careful design of a MEOR field project. Many laboratory tests and at least one field trial (single well) are performed before injection over the entire reservoir. This preliminary work is necessary to test the compatibility of the MEOR system with the reservoir conditions and its ability to enhance oil recovery under field conditions. Some of the tests that are actually used to select good microbial formulations for MEOR injection are listed below.

1) The compatibility of the microbial culture with reservoir fluids. The compatibility of microbes with injected water, produced water, nutrients, crude oil and reservoir rock is investigated (e.g. Bryant et al., 1985). Normally, the ability of microbes to withstand reservoir conditions (temperature, pressure, salinities, etc.) is usually satisfied since most microorganisms have been originally isolated from oil reservoirs.
2) Optimization of the microbial cultures. The microbial cultures are optimized using rock cores to duplicate the permeability and mineralogy of the target reservoir. The necessity for this step has been shown by Bryant et al. (1988). They found that some microorganisms grow very well and produce potentially useful metabolites in liquid cultures but are unable to mobilize residual oil in porous media studies. Microbial coreflooding can give a first estimate of the maximum oil recovery efficiency. It must be kept in mind that the actual recovery in the field is usually lower because of differences in sweep efficiency.
3) Compatibility with indigenous bacteria. Single-well injection has been used to check the ability of the microbial system to compete successfully with indigenous bacteria. Backflush water samples are analyzed to determine if the injected bacteria survived and metabolized sufficiently for significant oil recovery. From a single-well study, Douglas et al. (1987) showed that a microbial formulation tested and optimized in the laboratory could not only survive reservoir conditions but also outgrow indigenous organisms.

1.7. PROSPECTS AND PROBLEMS IN THE APPLICATION OF MEOR TO RESERVOIRS

MEOR can offer significant economic advantages over conventional EOR processes. However, several very complex questions must be answered before it becomes widely applicable. Additional information is necessary to demonstrate clearly the technical feasibility of microbial oil recovery methods in the field. In our opinion, research in the following areas will be critical for the commercialization of MEOR processes.

1) A better understanding of the mechanisms of oil recovery. As discussed earlier, the mechanism for the release of oil in MEOR is uncertain. The interpretation of laboratory and field studies is complicated by the complexity of microbial responses and the heterogeneity of the geological, chemical and microbiological composition of the reservoir. It is very likely that the mechanism of MEOR is not unique even for the same reservoir. In other words, treatment of the well with different cultures, each exhibiting its own pattern of metabolic activities, could be equally effective in enhancing the flow of oil.
2) The ability of bacteria to plug reservoirs is of great concern to the oilfield operator who has spent a lifetime avoiding bacterial fouling of the well. This concern is well founded. It is the responsibility of MEOR researchers to demonstrate that some strains of bacteria can be successfully propagated through rocks at high enough concentrations and can benefit oil recovery through the generation of metabolites. The studies referred in the section on transportability of bacteria have focused on these issues. It is not yet clear whether the relatively low concentrations at which the bacteria will penetrate the reservoir rock will be adequate to generate sufficient quantities of metabolites.

The potential for damage to the near wellbore region arises not only from injected species but also from the indigenous organisms that grow when nutrients are injected. The growth of indigenous bacteria can outpace that of the injected species. Localized large increases in biomass without the concomitant production of metabolites useful for oil release can arise. Furthermore, the accumulation of undesirable products such as H_2S may be accelerated. The control and management of subsurface populations is very important for the successful application of MEOR.

3) Numerical simulations should be developed to guide the application of MEOR in the field. Some very important concerns, such as undesired microbial plugging and low penetration, can be circumvented by applying the appropriate injection strategy. At present, there are no methods for the proper evaluation of the response of the reservoir

consuming and difficult to scale up. A realistic simulation of the rates of microbial transport, growth and metabolism as well as the displacement of oil and water can provide valuable guidance in the selection of suitable injection strategies.

Despite several sporadic efforts spanning four decades, the technology of microbial enhanced oil recovery is still in its infancy. The rational use of microorganisms in the field requires a thorough understanding of their patterns of growth and metabolism, their distribution and impact within the reservoir and finally, the engineering expertise to exploit their beneficial effects. These questions can only be addressed through a number of careful and well controlled studies both in the laboratory and in the field. The need for further studies becomes immediately obvious when one considers the enormous economic potential of MEOR. Microbial processes are perhaps unique among tertiary oil recovery methods in that they require low capital and operating costs. Thus, they can provide significant economic advantages and can be applied easily to small oil operations. MEOR research conducted at the University of Texas at Austin over the past four years is summarized in this report. Chapter 2 reviews our understanding of biosurfactants, Chapters 3 and 4 present results of research conducted on the production and isolation of biosurfactants. Chapter 5 presents experimental data on bacteria transport in sand columns. Chapter 6 presents a model developed for simulating MEOR processes.

Nomenclature

N_{ca} Capillary number, dimensionless.

μ_w Viscosity of displacing phase.

v_w Velocity of displacing phase.

σ_{ow} Interfacial tension between displacing and displaced fluids.

k_i Initial permeability of the porous medium.

k Final permeability of the porous medium.

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TABLE 1.1: Microorganisms Used in Past Field Trials

Organism	Expected Mechanism	Result	Remarks	Place, Date	Reference
Sulfate-reducing ++hydrocarbon bacteria	acidification oil decomposition		-oil recovery increases parallel microbial counts increases	Czechoslovakia 1954	Hitzman 1982
Sulfate-reducing +*Pseudonomas*	acidification oil decomposition	viscosity of the oil decreased in one well	-good growth of the sulfate-reducing bacteria -oil recovery increases parallel microbial counts increases	Czechoslovakia 1955	Hitzman 1982
Desulfovibrio +*Pseudonomas*	acidification oil decomposition		-good growth of sulfate-reducing bacteria -no trace of Pseudomonas	Czechoslovakia 1957-58	Hitzman 1982
Betacoccus dextranicus	increase in water viscosity		-recovery attributed to water viscosity increase	The Netherlands 1957	Hitzman 1987
Clostridium acetobutylicum	production of CO_2 solvents,emulsifiers	production of CO_2 and volatile acids	-C.acetobutylicum were never isolated -underground fermentation was established	Mobil field test U.S.A 1954	Hitzman 1982
Desulfovibrio hydrocarbonoclastus	acidification		-microorganisms in fracturing fluids increased oil production	U.S.A 1957	Hitzman 1982
Bacillus +*Clostridium*	production of gases acids,solvents and surfactants		-tests were performed on 150 wells -increase in oil production up to 350 percent	A.C.Johnson process U.S. 1977-81	Hitzman 1982
Clostridium	production of CO_2 acids, solvents surfactants	production of CO_2 acids and solvents		Oklahoma State University U.S.A 1983	Hitzman 1987
Leucomostoc mesenteroides	selective plugging	pH decreased, surface tension slightly decreased	-number of indigenous polymer forming bacteria increased -at least two populations were present	Bioplug well test Canada 1985	Hitzman 1987

TABLE 1.2 : MEOR Injection Strategies of Different Countries

Country	Injection Strategy	
Poland	1-inject formation water (l)	50000
	+molasses (kg)	2000
	+culture (l)	500
	+oil (kg)	150
	2-shut in well	3-4months
	3-well fracturing sometime done following treatment	
Czechoslovakia	1-clean well	
	2-inject culture (l)	40-60
	+molasses (kg)	30-100
	3-increase pressure	
Hungary	1-inject formation water (m^3)	20
	2-inject the nutrient containing:	
	molasses (t)	2
	KNO3 (kg)	70
	Na phosphate (kg)	10-20
	sucrose (kg)	50
	formation water (m^3)	100
	3-inject culture (l)	100
	4-inject remaining half of nutrient	
	5-close the well exept to add more nutrient	
U.S.S.R	1-inject bioreagent containing a mixture of aerobic and anaerobic organisms with hydrolizable subsstances from peat biomass and salt	
	2-waterflooding with fresh water (m^3/day)	650
The Netherlands	1-inject inoculated sucrose-molasses medium (l)	3000
	2-inject same medium but uninoculated or inject molasses solution	
	3-shut in	
U.S.A (Mobil)	1-inject culture in 2% molasses in 18 injections over 5 months (gal)	4000
	2-waterflood (bbl/day)	100-500
U.S.A (Pure Oil Co.)	1-inject fracturing fluid (lactate with chelating agent), + inoculum, gal	5000
	2-apply pressure	
	3-shut-in for 3 months	

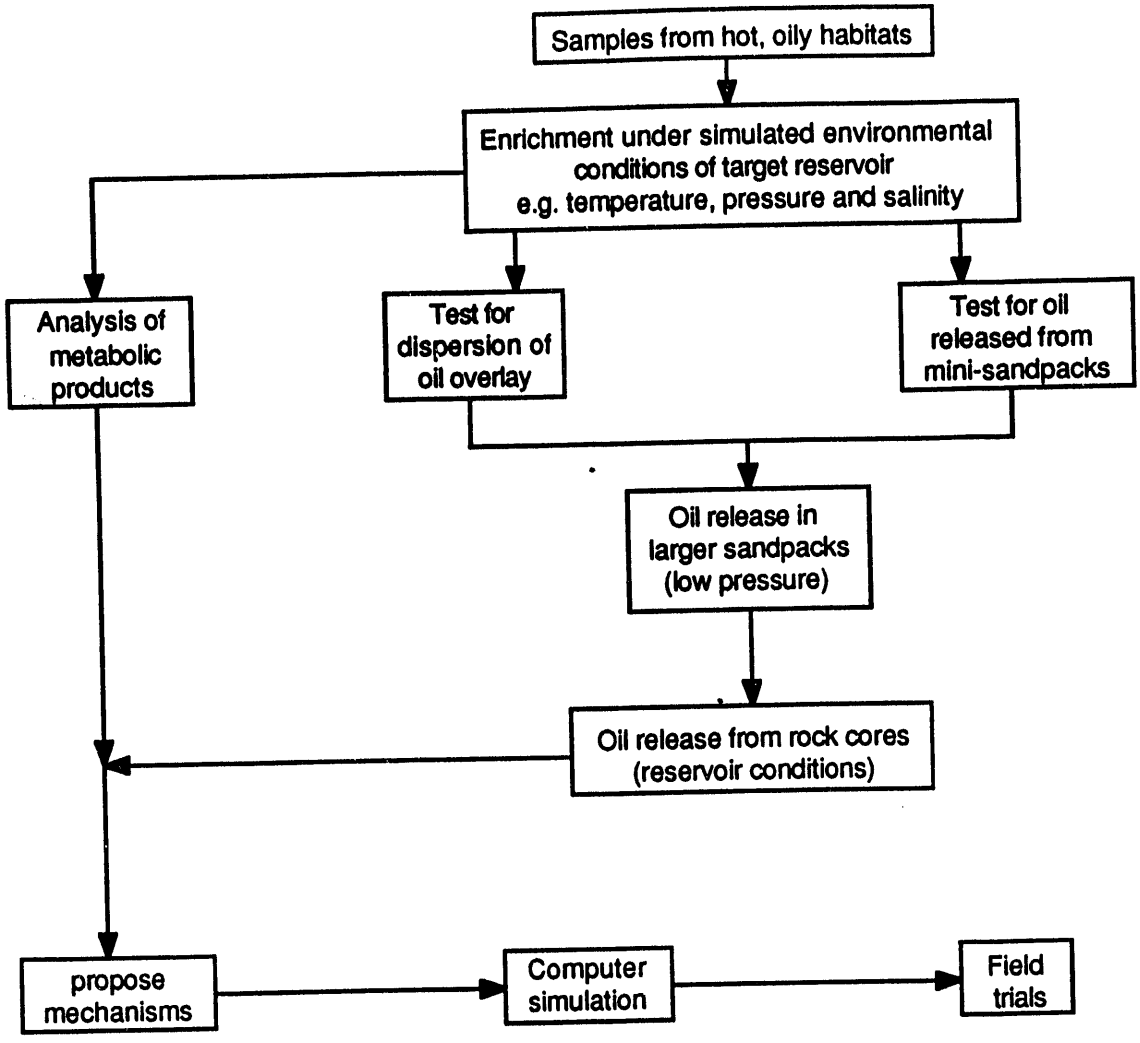

Figure 1.1: Strategy for Screening of Microorganisms Suitable for MEOR (Shennan, et.al., 1987)

Chapter 2: Surface-active Compounds from Microorganisms: A Review

ABSTRACT

Microbial surfactants are a structurally diverse group of compounds which are consist of a hydrophilic and a hydrophobic domain and partition preferentially at interfaces. Biosurfactants are of increasing interest commercially as substitutes for synthetic surfactants particularly for environmental applications. This article discusses recent progress in the genetic and biochemical analysis of biosurfactant synthesis as well as the current status of fermentation technologies.

2.1 INTRODUCTION

Surfactants are amphiphilic molecules consisting of a hydrophilic and a hydrophobic domain.

Usually the hydrophobic domain is a hydrocarbon whereas the hydrophilic group can be non-ionic, positively or negatively charged, or amphoteric. The most common non-ionic surfactants are ethoxylates, ethylene and propylene oxide copolymers and sorbitan esters. Examples of commercially available ionic surfactants include fatty acids, ester sulfonates or sulfates (anionic) and quartenary ammonium salts (cationic). Because of the presence of hydrophilic and hydrophobic groups within the same molecule surfactants partition preferentially at the interface between fluid phases of different degrees of polarity and hydrogen bonding (such as oil/water or air/water interfaces). The formation of an ordered molecular film at the interface lowers the interfacial energy (interfacial tension, IFT) and is responsible for the unique properties of surfactant molecules. In addition to lowering the IFT, the molecular layer can also dominate the interfacial rheological behavior and mass transfer. Surfactants find applications in an extremely wide variety of industrial applications involving emulsification, foaming, detergency, wetting and phase dispersion or solubilization.

The critical micelle concentration (CMC) is in effect the solubility of a surfactant within an aqueous phase. At concentrations above the CMC, amphiphilic molecules associate readily to form supramo-lecular structures such as micelles, bilayers and vesicles.

The forces that hold these structures together include hydrophobic, van der Waals, electrostatic and hydrogen bonding interactions. Since no chemical bonds are formed, these structures are fluid-like and are easily transformed from one state to another as conditions such as electrolyte concentration and temperature are changed. Lipids can form micelles (spherical or cylindrical) or bilayers based mainly on the area of the hydrophilic head group and the chain length of the hydrophobic tail. Molecules with small chain lengths and large head groups generally form spherical micelles. Those with smaller head groups tend to associate into cylindrical micelles, while those with long hydrophobic chains form bilayers which, in turn, under certain conditions form vesicles[1].

The interfacial tension between the aqueous and oleic phases changes very little above the critical micelle concentration because all additional surfactant molecules form micellar structures since the oil/water interface already has a monomolecular layer of amphiphiles. Mixtures of oil, brine and surfactant at concentrations above the CMC give rise to complicated phase behavior. The formation of micelles can result in the solubilization of oil or water in the other phase giving rise to a microemulsion. Microemulsions can be water continuous (i.e. with oil dispersed within micelles in the water) or oil continuous [2]. Much more complicated phase behavior is possible in such systems through changes in electrolyte, surfactant concentrations, temperature, the additions of co-surfactants and the presence of polymeric molecules in the brine.

Many biological molecules are amphiphilic and partition preferentially at interfaces. Microbial compounds which exhibit particularly high surface activity are classified as biosurfactants[3,4,5]. In terms of physicochemical properties such as decrease in interfacial tension, heat and pH stability, many biosurfactants are comparable to synthetic surfactants. In addition, the chemical diversity of naturally produced amphiphiles offers a wider selection of surface active agents with properties closely tailored to specific applications. However, because of technical and/or economic reasons, biosurfactants have not yet been employed extensively in industry. This is beginning to change as environmental compatibility is becoming an increasingly important factor for the selection of industrial chemicals. Unlike synthetic surfactants, microbially produced compounds are easily biodegradable and thus particularly suited for environmental applications such as bioremediation and the dispersion of oil spills.

2.2 STRUCTURES AND PROPERTIES OF BIOSURFACTANTS

A very wide spectrum of biological molecules partition at interfaces. Perhaps the most common amphiphilic structure is the peptide α-helix in which hydrophobic and

hydrophilic amino acids are arranged at opposite faces. Many proteins are surface active and cause foaming in aqueous solutions[6]. Some polypeptides are even synthesized specifically to serve a biological function as surfactants. The most notable example is the pulmonary surfactants which are essential for normal respiration and serve to reduce the surface tension of the alveolar film in the lungs. However, the surface activity of proteins *per se* is not of practical interest, except perhaps in the food industry.

Microbial biosurfactants include a wide variety of chemical structures, such as glycolipids[7-19], lipopeptides[20-31], polysaccharide-protein complexes[32-34], phospholipids[35-36], fatty acids and neutral lipids[37-39]. Most biosurfactants consist of distinct hydrophilic and hydrophobic moieties. The former can be either ionic or non-ionic and consist of mono-, di-, or polysaccharides, carboxylic acids, amino acids, or peptides. The hydrophobic moieties are usually saturated, unsaturated or hydroxylated fatty acids. For some high molecular weight surfactant molecules, such as protein-polysaccharide complexes, the hydrophobic and hydrophilic moieties are contributed by different molecules. The most widespread microbial surfactants are glycolipids. Among the best studied biosurfactants are the rhamnolipids of *Pseudomonas aeruginosa*[40] and the trehalose lipids of *Rhodococcus erythropolis*[41] (Figure 2.1). For both compounds the chain length of the hydrophobic domain and the structure of the carbohydrate head group exhibit considerable variability depending on the growth conditions[42]. The structure of surfactin, a representative lipopeptide biosurfactant produced by *Bacillus subtilis* is also shown in Figure 2.1. Surfactin consists of 3-hydroxyl-13-methyl-tetradecanoic acid amidated to the N-terminal amine of a heptapeptide. The carboxy terminal end of the peptide is further esterified to the hydroxyl of the fatty acid[43]. The ionizable side chains of glutamic and aspartic acid probably contribute significantly to the excellent surface active properties of the molecule.

The proper characterization of the activity of biosurfactants requires detailed information on the thermodynamic properties of aqueous/non-aqueous/surfactant mixtures. This information is experimentally difficult to obtain and is not available for any natural products. As a result the most frequently used indices for the performance of biosurfactants are the surface and interfacial tension and the critical micelle concentrations. Representative values for the most promising and extensively studied microbial surfactants are listed in Table 2.1. Care should be taken in comparing the interfacial properties in Table 2.1 because the experimental conditions used in different reports vary significantly. Several biosurfactants show low CMC values, reduce the surface tension of the fermentation broth to less than 30 mN/m and the interfacial tension against n-alkanes to

mN/m[5-11,10-24,44,45]. In particular the glycolipids produced by *Rhodococcus sp.* H13[8] and the major surfactant from *Bacillus licheniformis* strain JF-2[20-23] (S. C. Lin, unpublished results) have been shown to reduce the surface tension of aqueous solutions to 26-27 mN/m and the interfacial tension against decane or octane to 10^{-2} mN/m. These values compare favorably to those obtained with commercial synthetic surfactants. It is important to note that in addition to the major biosurfactants both *B. licheniformis* JF-2 and *Rhodococcus sp.* H13A produce several additional surface active agents which appear to act synergistically [22,8]. Furthermore, some biosurfactants also exhibit good thermal and chemical stability characteristics. For example, the lipopeptides from *B. licheniformis* JF-2 are stable at a temperature up to 75 °C for at least 140 hours [23]. The surfactant is stable at pH values between 5.5 and 12 but slowly loses activity under more acidic conditions.

The interfacial properties of surfactants depend on the ionic composition of the aqueous phase. High NaCl concentrations inactivate the glycolipids of *T. apicola*. On the other hand, the interfacial tension of the fermentation broth of *B. licheniformis* JF-2 decreases by more than an order of magnitude in the presence of 10% NaCl but is not affected by calcium salts[22,23]. Interestingly, this microorganism grows and produces surfactant under both aerobic and anaerobic conditions and in the presence of up to 8% NaCl[46].

2.3 BIOSYNTHESIS OF BIOSURFACTANTS

As may be expected from the wide variety of biosurfactant structures, their formation involves an equally diverse range of biosynthetic pathways. For simplicity, three classes of pathways can be distinguished depending on whether the hydrophobic domain, the hydrophilic domain, or both, are synthesized *de novo* [47]. Obviously, this classification does not reflect the many different biosynthetic routes that are involved in the formation of the lipid and hydrophilic domains. Those components that are not synthesized *de novo* are produced by modification of the carbon source, i.e.sugars, alkanes etc. Often a variety of different carbon substrates can be incorporated into the surfactant giving rise to a family of related molecules.

In lipopeptides such as herbicolin A and surfactin, both the lipid and the peptide domains are directly synthesized from carbohydrates. Addition of amino acids or fatty acids in the growth medium can affect the yield but not the structure of the product [48]. The trehalose lipids formed by *Rhodococcus erythropolis* are typical of compounds in which the hydrophilic component, in this case the disaccharide trehalose, is not affected by the carbon substrate, whereas the fatty acid domain depends on the chain length of the alkane

feed [49]. In an enzymatic step that is probably rate limiting, the fatty acid (corynomycolic acid) is esterified to trehalose-6-phosphatase which is subsequently subject to dephosphorylation and further modification. Finally, the surfactants produced by *Arthrobacter paraffineus* represent an example of the class of compounds in which the hydrophilic (sugar) moiety is influenced by the carbon source. Fructose lipids are produced when this microorganism is grown on fructose as the carbon source whereas glucose and sucrose lipids predominate in sucrose grown cultures[50,51].

The fatty acid components of biosurfactants are synthesized by the rather well characterized pathways of lipid metabolism[52]. The hydrophilic moieties, on the other hand, exhibit a greater degree of structural complexity which is the outcome of a wide variety of biosynthetic mechanisms. Recent studies have begun to shed light on the formation of the amino acyl part of lipopeptide antibiotics, many of which display interesting surface active characteristics[53-55]. Lipopeptides are synthesized non-ribosomally by large multifunctional enzyme complexes exemplified by gramicidin S synthe-tase. The first step in the formation of the decapeptide antibiotic gramicidin S is the activation of amino acids via adenylation by ATP. The activated intermediates are attached to specific sites on the gramicidin S synthetase complex by thioether linkages. The amino acid intermediates are arranged on the enzyme in a linear fashion corresponding to the sequence with which they will be incorporated into the growing peptide. Assembly of the peptide involves a pantetheine cofactor having a reactive -SH group. The cofactor functions as an internal swinging arm to mediate the transport of the growing peptide between the sites of attachment of the activated amino acids. This mode of synthesis is called the thiotemplate mechanism. The antibiotic tyrocidine is formed by a similar process except that the tyrocidine synthetase complex consists of three rather than two enzymes.

The synthesis of the surface active lipopeptide surfactin, which also has antibiotic properties, has been investigated in detail. Genetic evidence has indicated that two putative components of the surfactin synthesizing enzyme from *Bacillus subtilis* complex share homology with tyrocidine synthetase 1 and gramicidin S synthetase 1[56]. Recent biochemical studies demonstrated that surfactin synthesis occurs via a thiotemplate-based process. In vitro, the synthesis of surfactin by a cell free system requires ATP, Mg^{+2}, precursors and sucrose, the latter presumably because of the need to stabilize the enzyme complex. Even though the peptide contains D-Leu, only the L-isomer of the amino acid can serve as a precursor. The fatty acid component is incorporated only as a acetyl-CoA derivative[57,58]. Further elucidation of the biosynthetic pathway of surfactin could open the

way for the specific modification of the molecular structure to tailor the surface active properties.

The physiological significance of biosurfactants stems from their amphiphilic character. Solubilization of hydrocarbons by surfactants is an important step in nutrient uptake and utilization for a variety of microorganisms[59]. The significance of emulsification for the biodegradation of non-polar substrates and the enhancement of microbial growth has been demonstrated in numerous studies[60-62]. Cell-bound biosurfactants are involved in adhesion to surfaces and the transport of hydrocarbons[63-65]. Finally, certain biosurfactants, mainly lipopeptides and glycolipids, have antibiotic and/or biostatic activities[66-68]. The antibiotic function of biosurfactants is related to their ability to bind to hydrophobic molecules and to partition within membranes. For example rhamnolipids and surfactin act in solubilization of cell envelope components of competing microorganisms[67,68].

2.4 GENETICS AND REGULATION

Although the genetic analysis of biosurfactant production is currently at an early stage, the use of recombinant DNA techniques for the manipulation of biosurfactant production is slowly gaining ground and can become instrumental in future efforts for commercial development. Metabolic engineering, i.e. the application of genetic engineering to improve the synthesis of non-ribosomal products[69,70], has been exploited effectively in the antibiotics area. So far the only example of metabolic engineering for biosurfactant production is the expression of the lactose utilization genes in *Pseudomonas aeruginosa* to allow growth and rhamnolipid production on lactose or cheese whey [71].

DNA transfer systems including shuttle vectors and transducing phages are available for many biosurfactant-producing microorganisms such as *Bacillus* and *Rhodococcus* sp. The main problem in genetic studies has been the lack of suitable plate assays for the screening of mutants, since a change in surface tension by itself is not an easily detectable property *in situ*. Matsuyama et al.[72] have been able to identify cells which produce surface wetting agents by direct thin layer chromatography from a single microbial colony. However, in most cases the amount of biosurfactant is too small to be detectable[73]. Mulligan et al.[74] have exploited the hemolytic property of some biosurfactants to develop a simple assay based on the formation of a clearance zone around colonies grown on blood agar. They were able to isolate a *B. subtilis* mutant which produced three times higher levels of surfactin than the wild type[75]. Using a similar approach Nakano and Zuber isolated null mutants for surfactin production[76]. Koch et

al.[73] screened a Tn5 mutagenized population of *Pseudomonas aeruginosa* for defective growth on hydrocarbon minimal media and characterized two mutants in detail. One strain was found to be defective for surfactant production whereas the second exhibited a two-fold higher production when grown in minimal media with glucose as the carbon source.

The studies of Nakano, Zuber and their coworkers on the organization of the surfactin biosynthesis genes have shed light on the complexities involved in the molecular-level regulation of biosurfactant synthesis. Three chromosomal loci in *B. subtilis* are required for surfactin production[55, 76]. The *srfA* locus is a large operon of more than 25 kb of DNA. Two open reading frames that may correspond to subunits of surfactin synthetase have been found in *srfA* [55]. Mutations in *srfA* not only abolish the synthesis of the lipopeptide but also affect the cell's ability to take up foreign DNA and sporulation, indicating that the proteins expressed from this operon are important for cell specialization and differentiation. Nakano et al.[77] proposed that the *srfA* operon is involved in the production of peptides which function as developmental signals. Similar pheromone-like peptide factors have been implicated in the initiation of sporulation in *Bacillus* [78]. The synthesis of these regulatory peptides and surfactin appears to involve the same intermediates.

The modification of the common intermediate to yield surfactin depends partly on the closely linked *sfp* locus, which codes for a 224 amino acid polypeptide with strong homology to a protein transcribed from the gramicidin S operon [56]. Overproduction of the Sfp protein lowers transcription from the *srfA* operon indicating that *sfp* has a regulatory function in addition to its direct role in surfactin biosynthesis. Surfactin production is also affected by the regulatory genes *comP*, *spoOK* and *comA* (or *sfrB*). An independently isolated mutation which increases surfactin production maps in the *srfB* region of the *B. subtilis* chromosome[75]. The ComA and ComP proteins probably form a complex which acts as a positive regulator of transcription from *srfA* in response to the levels of glutamine and glucose in the growth medium[77,79]. Since the production of most biosurfactants depends on the ratio of carbon to nitrogen source in the growth medium this mechanism of regulation may be very general. For practical applications it is interesting to note that the *srfA* genes have been placed under the control of an inducible promoter so that the production of surfactin is only dependent on the addition of the inducer isopropyl-β-galactoside (IPTG) in the growth medium (Nakano and Zuber, submitted).

2.5 BIOSURFACTANT FERMENTATION AND RECOVERY

As with other microbial fermentations the goal in the production of biosurfactants is to maximize the productivity (i.e. grams/liter/hr), to increase the yield of biosurfactant from the carbon source and to achieve high final concentrations. In addition it is important to reduce the accumulation of other metabolic products that may interfere with the physical properties or the recovery of the surface active agent.

Biosurfactants are a diverse group of compounds produced by a variety of microbial species. It is therefore difficult to draw general guidelines for process development and fermentations must be optimized on a case by case basis. Most biosurfactants are released into the culture medium throughout the exponential phase but some can also be produced by resting cells or by immobilized biocatalysts[10,16]. In certain cases the biosurfactant is produced during a part of the growth cycle and are then either inactivated or incorporated into other metabolites. For example *Corynebacterium lepus* produces two distinct surface-active compounds during the course of the fermentation[37]. Surface-active corynomycolic acids produced at the early stage of growth are rapidly incorporated into lipopeptides, the major surfactants in the later phase of the fermentation. An interesting pattern of biosurfactant production is illustrated by the change in the interfacial tension of the fermentation broth of *Bacillus licheniformis* JF-2 grown in minimal media (Figure 2)[46]. The accumulation of the active agent reaches a maximum in mid-exponential phase and subsequently decreases rapidly, presumably because it is converted into a metabolite with no surface activity.

The choice of carbon source plays an important role on the yield and structure of microbial surfactants. There are microorganisms which produce biosurfactants only when grown on hydrocrabons[2,16,62] and others which require simple, water soluble substrates such as carbohydrates and amino acids. Some bacteria such as *Pseudomonas aeruginosa* produce surface- active agents whether grown with water-soluble or water-insoluble substrates, although in the latter case production is higher [13]. The chain length of hydrocarbon substrates often has a significant effect on the final concentration of the product in the fermentation broth[13]. In addition, as was discussed above, the chemical structure of the surfactant, particularly the hydrophobic tail, is often determined by the carbon source.

Other components of the growth medium can have a pronounced effect on biosurfactant production. For example, the ratio of nitrogen to carbon source determines the yield of glycolipids from *Pseudomonas* [80]. Production of surfactin is enhanced in the presence of increase concentrations of iron or manganese in the growth medium[29].

Addition of antibiotics, such as penicillin or chloramphenicol, can either improve or inhibit the production of various biosurfactants[81,82]. The synthesis of glycolipids by *Torulopsis* sp. is strongly affected by the concentration of yeast extract[9,15]. In certain cases the yield of biosurfactants can be improved by continuous removal of the desired product or other inhibitory metabolites through on-line product recovery[29]. Finally, as with any other fermentation, the growth temperature, pH and rate of oxygen transfer exert an important effect on the final concentration and the volumetric productivity of the biosurfactant[10,38,46]. By optimizing the fermentation conditions, Cooper has obtained a final concentration of 70 grams of sophorose lipids per liter and a 30% yield on the carbon substrate[3,15]. These are the highest values reported so far for biosurfactant fermentations.

The recovery and concentration of biosurfactants from the fermentation broth can account for a large fraction of the total production costs. Often, the low concentration and amphiphilic character of these compounds present serious impediments to efficient separation. Fortunately, for most applications the final degree of product purity is not very important as long as the final preparation exhibits the desired properties. As a result, the separation of biosurfactants for preparative purposes typically involves only a few simple steps such as precipitation, organic extraction or adsorption chromatography. Water-insoluble compounds, such as the glycolipids produced by *Torulopsis bombicola* [15], can be separated from the broth as heavy oils following centrifugation. Surfactin and other charged biosurfactants are isolated by acid-precipitation at pH 2.0[20,24,28]. Ammonium sulfate[83] has also been used to precipitate biosurfactants from the fermentation broth. Organic extraction is used as an alternative or in addition to precipitation. A high partition coefficient in the extracting phase is obtained by judicious selection of an organic solvent and adjustment of the ionic strength and pH of the aqueous solution. Adsorption chromatography on ion-exchange resins[84], activated carbon[9,25], or Amberlite XAD-2[12] have been used to obtain a higher purity product. Also, ultrafiltration has been used successfully to recover biosurfactants from the fermentation broth[89,90]. Finally, for analytical purposes high resolution purification is obtained by a combination of thin layer, gel filtration and, most importantly, reverse phase chromatography[24,25,28].

2.6 MEOR AND OTHER APPLICATIONS

The mobilization, by surfactant solutions, of oil trapped after water flooding has long been practiced in the petroleum industry. For efficient oil recovery the surfactant must be able to reduce the interfacial tension between oil and water to less than 10^{-2} mN/m. A few biosurfactants can give such low IFT values (Table 2.1). However, surfactant

flooding is not economical at the current oil prices and therefore it is not likely to become a major market for biosurfactants. Another enhanced oil recovery method involves the injection and subsequent *in situ* growth of microorganisms within the reservoir (microbial enhanced oil recovery)[88,89]. Microbial enhanced oil recovery is economically attractive but there are many technical hurdles that need to be overcome before it becomes widely accepted in the petroleum industry.

Biosurfactants are ideally suited for environmental applications. The dispersion and/or solubilization of water insoluble pollutants is an important step in bioremediation. Surfactants are required to remove organic compounds from soil and for the formation of emulsions that facilitate assimilation by microorganisms. Compared to synthetic compounds, biosurfactants offer the advantages of little or no environmental impact and the possibility of *in situ* production. Recent studies have demonstrated the successful use of biosurfactants for facilitating the degradation of organic pollutants in soil and for the dispersion of oil from the Exxon-Valdez oil spill [62,90].

The commercial application of biosurfactants is determined by their cost and properties in relation to competing synthetic compounds. At present, the use of biosurfactants in the food and chemical industries is not generally competitive because of their higher production costs[86]. However, environmental concerns with synthetic surfactants are beginning to weigh the economic balance in favor of microbially produced compounds. In addition, the chemical diversity of biosurfactants results in a wide variety of physicochemical properties with some compounds particularly suited for specific properties. For example Horowitz and Currie[87] recently found that the biosurfactant BL-86 is an excellent dispersant for ceramics processing. Such specialty uses are likely to increase rapidly as more biosurfactants become available in commercial quantities and can be employed for testing purposes.

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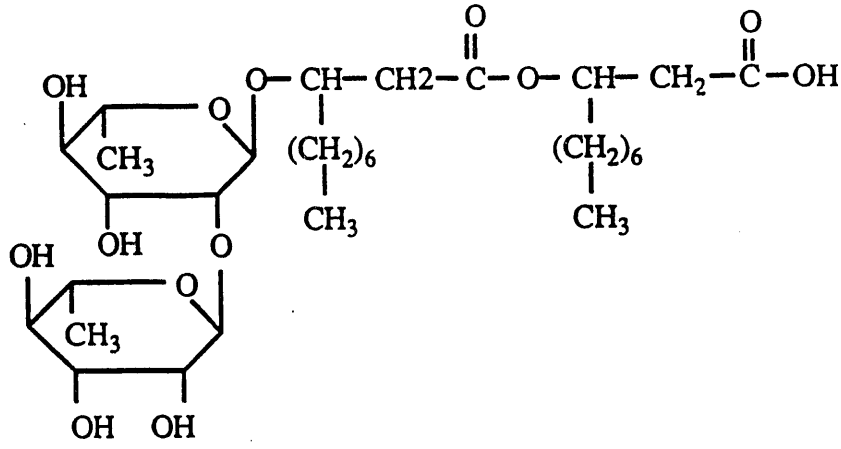
Table 2.1 Structures, interfacial properties and production levels of typical biosurfactants.

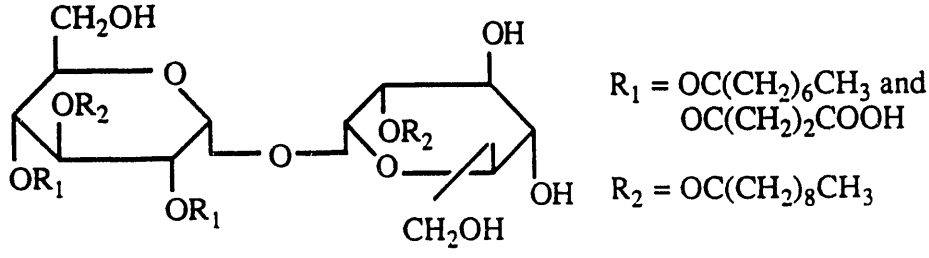
BIOSURFACTANT	MICROORGANISM	C-SOURCE	SURFACE TENSION (mN/m)	INTERFACIAL TENSION (mN/m)	CMC	Concentration in Medium / Yield	Ref.
Glycolipids							
Glycolipids	*Rhodococcus aurantiacus*	n-alkanes	26	0.35	na	400 X CMC	7
Glycolipids	*Rhodococcus sp. Strain H13A*	Hexadecane	na	0.02[b]	1.5 g/l	660 mg/l	8
Glycolipids	*Torulopsis apicola*	alkane/carbohydrate	30	< 0.9[b]	na	4.1 X CMC	9
Pentasaccharide lipid	*Nocardia corynebacteroides*	n-alkane	26	< 1[a]	na	2.5 g/l	19
Rhamnolipid	*Pseudomonos aeruginosa*	glucose	29	0.25	na	2.5 g/l	11-13
Rubiwettins	*Serratia rubidaea*	glycerol	25.5-25.8	na*	10 mg/l	na	14
Sophorolipids	*Torulopsis bombicola*	glucose/oleic acid	33	1.8	na	38 - 77g/l	15-17
Trehalose-mono-, di-corynomycolates	*Nocardia corynebecteroides* *Rhodococcus erythropolis*	n-alkanes	32-36	14 - 17	na	0.25-3.2 g/l	18
Lipopeptides / Aminolipids							
Lipopeptides	*Bacillus licheniformis JF2*	glucose	27	0.016[b]	0.02 mg/l	0.1 - 0.7 g/l	20-23
Lipopeptides	*Bacillus licheniformis 86*	glucose	27	0.36[a]	10 mg/l	18 g/l	24
Viscosin	*Pseudomonas fluorescens*	glycerol	26.5	na	150 mg/l	na	25
Serrawettin	*Serratia marcescens*	glycerol	28.8 - 33.9	na	na	na	26,27
Surfactin	*Bacillus subtilis*	glucose	27 - 32	1[a]	23 - 160 mg/l	60 - 240 X CMC	28-31
Fatty Acids / Neutral Lipids							
Fatty Acids	*Corynebacterium lepus*	kerosene / alkanes	< 30	2[c]	150 mg/l	150 mg/l	37,38
Fatty acid + neutral lipids	*Nocardia erythropolis*	hexadecane	32	<3	na	160 X CMC	39
Others							
Protein-Carbohydrate Complex	*Pseudomonas fluorescens 378*	sucorse	27	na	< 10 mg/l	210 X CMC	32
Phosphatidylethanol-amines	*Rhodococcus erythropolis*	n-alkanes	30	< 1	30 mg/l	na	44
na	*Corynebacterium insidiosum*	hexadecane	28.5	0.55[a]	na	25 X CMC	45

a. Interfacial tension measured against hexadecane.
b. Interfacial tension measured against decane.
c. Interfacial tension measured against kerosene.
*. Not available.

A



B



C

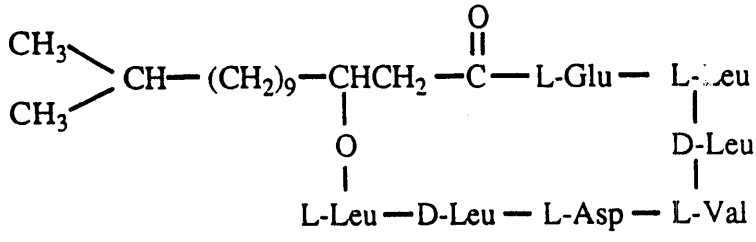


Figure 2.1 Structures of typical biosurfactants. (A) Rhamnolipid R3 produced by *Pseudomonas aeruginosa*. (B) Trehalose-2,3,4,2'-tetraester produced by *Thodococcus erythropolis*. (C) Surfactin produced by *Bacillus subtilis*.

Chapter 3: Production of Biosurfactant by *Bacillus licheniformis* JF-2

ABSTRACT

Bacillus licheniformis strain JF-2 produces a very effective surfactant under conditions typical of oil reservoirs. We have conducted a systematic investigation of the effects of environmental parameters (temperature, medium pH and NaCl) on: i) the production of the biosurfactant, ii) formation of fermentation end-products and iii) growth of *B. licheniformis* in batch cultures. The partially purified biosurfactant from JF-2 was shown to be the most active microbial surfactant found so far and gave an interfacial tension against decane of 0.016 mN/m. Optimal production of the surfactant was obtained in cultures grown in the presence of 5% NaCl at a temperature of 45°C and pH 7.0. The major fermentation end-products were lactic acid and acetic acid with smaller amount of formic acid and acetoin. Growth and biosurfactant formation were also observed in anaerobic cultures supplemented with a suitable electron acceptor such as $NaNO_3$.

3.1 Introduction

In a recent review (Sarkar et al., 1989), we have shown that in situ microbial enhanced oil recovery offers some advantages compared to the conventional EOR processes. Although the exact mechanism by which the growth of microorganisms in situ facilitates the flow of oil is uncertain, the biosurfactant and biopolymer production and the selective plugging of high permeability zones (Brown et al. 1986) are expected to play critical roles in microbial enhanced oil recovery. It has been estimated (Lake, 1988) that the interfacial tension between oil and brine must be lowered to 10^{-2} to 10^{-3} mN/m before significant oil recovery can be achieved. Biosurfactants which are capable of reducing the interfacial tension to such low values have been reported (Cooper and Zajic, 1980; Brown et al, 1986). However, microorganisms producing these biosurfactants require oxygen for growth and are therefore unsuitable for in situ applications. Recently, Knapp and co-workers (Javaheri et al., 1985) isolated *Bacillus licheniformis* strain JF-2 from water injection brine. They were able to demonstrate that strain JF-2 grew and produced biosurfactant anaerobically in medium E supplemented with glucose and 0.1% $NaNO_3$. It was also shown that *B. licheniformis* JF-2 grew in medium with NaCl concentrations up to 10% and over a wide range of temperatures (up to 50°C) and pH values (4.6 to 9.0). Furthermore, growth was not inhibited by the presence of crude oil. The biosurfactant produced was not affected by the pH, temperature, or the NaCl and calcium concentrations

typical of many reservoirs. These result indicate that *B. licheniformis* JF-2 is a good candidate for in situ microbial enhanced oil recovery processes. As a first step in understanding the kinetics of growth in porous media and the mechanisms of MEOR, we systematically investigated the effect of environmental parameters on the growth, biosurfactant production and formation of fermentation end-products. The accumulation of fermentation end-products can have an adverse effect on cell growth and thus, it may be a serious limitation of in situ MEOR. On the other hand some microbially produced organic acids and alcohols can facilitate oil recovery by acting as co-surfactants.

3.2 MATERIALS AND METHODS

3.2.1. *Batch fermentations*

Batch fermentations were carried out in a 2-liter Multigen fermentor (New Brunswick Scientific Co., New Brunswick, NJ). The working volume was 1 liter. The fermentor was inoculated with 25 ml of fresh culture, which was grown in medium E: 0.1% $(NH_4)_2SO_4$, 0.025% $MgSO_4$, 1% glucose in 100mM phosphate buffer consisting of KH_2PO_4 and K_2HPO_4 supplemented with a 1% (v/v) trace salt solution. The trace salt solution contained the following (grams/l): EDTA, 1.0; $MnSO_4$, 3.0; $FeSO_4$, 0.1; $CaCl_2$, 0.1; $CoCl_2$, 0.1; $ZnSO_4$, 0.1; $CuSO_4$, 0.01; $AlK(SO_4)_2$, 0.01; H_3BO_4, 0.01 and Na_2MoO_4, 0.01. Unless otherwise specified the growth medium also contained 5% NaCl. The medium pH was maintained constant by the addition of 1M NaOH or 1M HCl using an external pH controller. For anaerobic growth cultures were supplemented with 1% $NaNO_3$ as electron acceptor. Oxygen-free nitrogen (Linde National Specialty Gas Office, Somerest, NJ) was sparged throughout the fermentation to ensure anaerobic conditions.

Bacterial concentrations were monitored by taking samples and measuring the optical density at 600 nm with Ultraspec II (LKB Biochrom Ltd., Cambridge, England) periodically. The calibration curve for the bacterial dry weight as a function of the optical density was obtained as :

Dry weight of *B. licheniformis* JF-2 per ml of culture = 0.83 x OD at 600 nm

3.2.2. *Product analysis*

The production of biosurfactant was monitored by the decrease in the interfacial tension between the growth medium and decane by the spinning-drop technique (Cayias et al. 1975). Glucose concentration was determined by an enzymatic glucose analyzer

(Yellow Spring Instrument Co., Inc., Yellow Springs, OH). A Waters HPLC instrument was employed for the analysis of fermentation end-products. A PRP-X300 column (7 μm, 250x4.1 mm I.D., Hamilton, Reno, NV), maintained at 50°C with a water bath was used for analysis. The flow rate of the mobile phase (1 mM sulfuric acid) was 1.5 ml/min. The elution from the column was monitored at 196 nm. Each sample was filtered with 0.45μm HV filter (Nihon Millipore Kogyo K.K., Yonezawa, Japan) prior to injection. The standard calibration curve between the peak areas and concentrations of each compound was constructed by injecting standard solutions with known concentrations. The concentrations of the fermentation end-products were determined from the integration areas of the corresponding peaks.

3.2.3. *Biosurfactant characterization*

The surfactant was isolated from the growth medium by acid-precipitation following the removal of cells by centrifugation at 10,000 g for 10 minutes. For the acid-precipitation process, the fermentation medium was first acidified to pH 2.0 with concentrated HCl. The pellet, which was collected by centrifugation at 30,000 g for 45 minutes, was then extracted with methanol. Upon the evaporation of the methanol, the crude biosurfactant sample was obtained.

3.3 Results

3.3.1. *Batch fermentations*

The optimal condition for the bacterial growth was found to be at a temperature of 45°C and pH 7.0 both aerobically and anaerobically (data not shown). A typical fermentation profile is shown in Figure 3.1. A lag phase of approximately 4-5 hours was observed before the beginning of exponential growth under aerobic conditions, while a 2-3 days lag phase was typical for anaerobic fermentations (data not shown). A maximum bacterial concentration of 1-1.5 g/l was obtained before growth ceased and the optical density began to decrease.

3.3.2. *Product analysis*

As shown in Figure 3.1, the interfacial tension of the medium reached the lowest value at the middle of the exponential phase and increased significantly during early stationary phase. The effects of growth temperature, medium pH and NaCl concentration on the interfacial tension are shown in Table 3.1, Table 3.2 and Figure 3.2, respectively. It was

observed that both the aerobic and anaerobic cultures grown with a medium supplemented with 5% NaCl (wt/vol) at a temperature of 45°C and pH 7.0 gave the lowest interfacial tension values.

The major fermentation end-products were lactic acid and acetic acid with small amount of acetoin and formic acid. For the culture grown with a medium supplemented with 5% NaCl at 45°C and pH 7.0, the fractions of the carbon source utilized for the production of lactic acid and acetic acid were approximately 38% and 10%, respectively. About two percent of the carbon consumed was used for the production of acetoin and fomic acid. The biomass yield for both the aerobic and anaerobic fermentations was in the range of 0.05 to 0.13 g/l of cell dry weight /g glucose consumed.

3.3.3. *Biosurfactant characterization*

About 0.1 g of crude biosurfactant per liter of fermentation broth was obtained. TLC analysis on cellulose plates followed by staining with rhodamine B showed that the major component of the samples from aerobic and aerobic fermentations has the same retention factor value (Rf = 0.55). This suggested that the same biosurfactant was produced under aerobic and anaerobic conditions. The solution of the partially purified biosurfactant in 5% NaCl gave an interfacial tension against decane of 0.016 mN/m. The effects of pH and NaCl concentration on the ability of the surfactant to reduce the interfacial tension between the fermentation broth and decane are shown in Figures 3.3 and 3.4, respectively. The interfacial tension was independent of pH in the range of 5.5 to 12.0; however, it increased significantly at a pH of 5.0 or lower. Upon re-neutralization of the acidified medium, the activity was regained. The interfacial tension also depended on the NaCl concentration in the medium, consistent with earlier results (Javaheri et al., 1985). The interfacial tension of the culture in the presence of 10% NaCl was 12 times lower than with 0.5% NaCl. It was also found that the surfactant was both acid and heat stable. Incubation at pH 2.0 at 4°C for up to two days did not affect the interfacial tension. However, more prolonged exposure to acid did result in slightly higher interfacial tension values. Similarly, the partially purified surfactant was stable at 75°C for up to 140 hours.

3.4 Discussion

In this study we have demonstrated that *Bacillus licheniformis* JF-2 grows and produces biosurfactant under a variety of conditions typical of many oil reservoirs. The biosurfactant was shown to be one of the most active microbial surfactants known and gave minimal interfacial tension values in the range of 10^{-2} mN/m. Such interfacial tension

values are considered significant for oil mobilization. This result supports the argument that the production of surface active agents has an important role in microbial enhanced oil recovery.

For all the fermentations conducted in this study the interfacial tension of the broth reached minimal values around the end of the exponential phase and subsequently increased by an order of magnitude or more. Since the partially purified surfactant was shown to be stable under prolonged incubation at rather extreme conditions of temperature and pH, the increase in interfacial tension is not likely to be due to the spontaneous degradation of the surface active compound. A more plausible explanation is that during stationary phase the cells produce extracellular enzymes which affect the surfactant. Alternatively, it is possible that the inactivation of the surfactant is not the result of degradation, but is caused by the binding of other molecules which reduce its amphiphilic character and its ability to lower the interfacial tension. The interfacial tension of the medium was also reduced at low pH probably because of surfactant precipitation. NaCl exerts an important effect on interfacial tension. Similarly, most synthetic surfactants are strongly affected by the ionic strength and composition of the aqueous phase.

The optimal conditions for bacterial growth and biosurfactant production are 45°C, pH 7.0 and 5% NaCl. The biomass yield for both the aerobic and anaerobic fermentation are low compared to the values obtained with other *Bacillus* species (Snay, 1989). A likely explanation for the observed low biomass yields is that most of the carbon consumed is diverted for the production of metabolic by-products. This argument is supported by the presence of high concentrations of lactic acid and acetic acid in the fermentation broth.

Although the measurement of the interfacial tension of the fermentation broth gives a rough idea about the concentration of the biosurfactant in the broth, many factors, such as ionic strength, affect the ability of the surfactant to reduce the interfacial tension. Furthermore, the time required for the precise measurement of the interfacial tension is fairly long, typically several hours. These facts make the utilization of the interfacial tension measurement for the quantitative monitoring of surfactant concentration and fermentation control inappropriate. Therefore, a simple assay for the biosurfactant needs to be developed before further optimization of biosurfactant production can be carried out. The development of an assay for the biosurfactant as well as the elucidation of its structure are the subjects addressed in the next Chapter.

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TABLE 3.1

The effect of temperature on the growth and biosurfactant production of *Bacillus licheniformis* JF-2 in the presence of 5% NaCl at pH 7.0 under aerobic and anaerobic conditions.

T (°C)	Aerobic Fermentation		Anaerobic Fermentation	
	Minimal IFT* (mN/m)	Final Bacterial Concentration**	Minimal IFT (mN/m)	Final Bacterial Concentration
30	0.43	1.521	5.81	0.714
45	0.31	1.021	0.51	0.835
50	0.28	1.164	8.06	0.200

* IFT : The interfacial tension against decane.
** Bacterial concentration in mg/ml.

TABLE 3.2

The effect of medium pH on the growth and biosurfactant production of *Bacillus licheniformis* JF-2 in the presence of 5% NaCl at 45°C under aerobic conditions.

Medium pH	Minimal IFT (mN/m)	Final Bacterial Concentration (mg/ml)
6.0	0.50	1.243
7.0	0.30	1.162
8.0	3.65	0.830

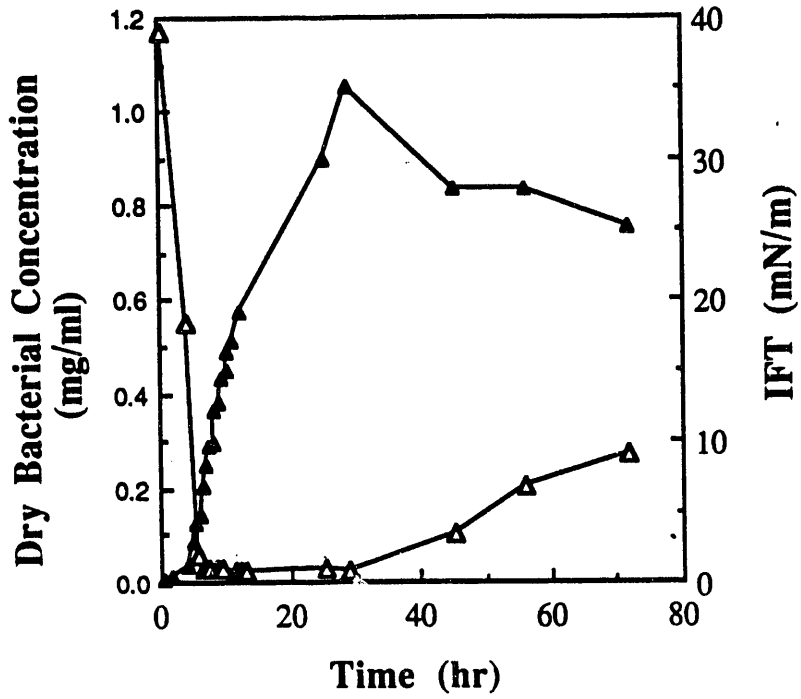

Figure 3.1 Typical fermentation profile of *Bacillus licheniformis* JF-2 grown under aerobic conditions at constant pH.

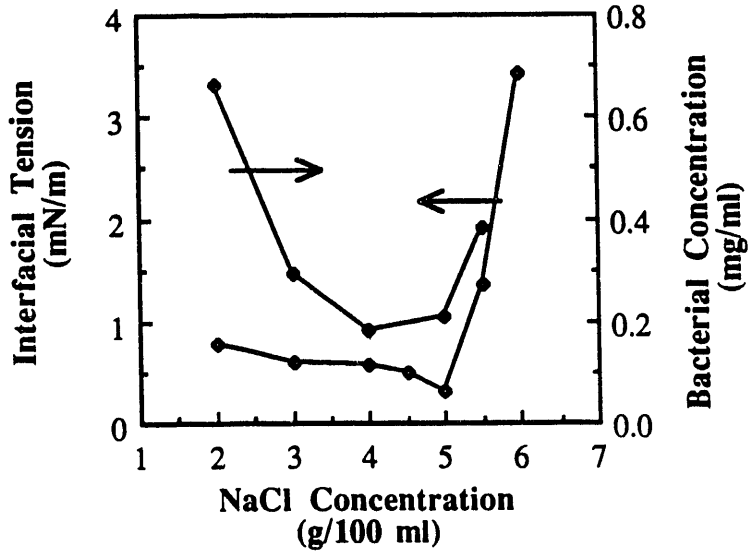

Figure 3.2 The effect of NaCl concentration on the growth and biosurfactant production of *Bacillus licheniformis* JF-2 under aerobic conditions.

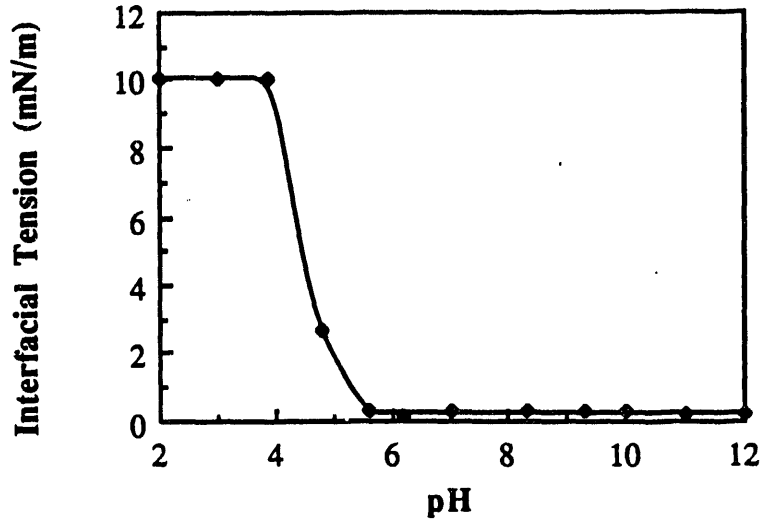

Figure 3.3 The effect of pH on the ability of the surfactant to reduce the interfacial tension.

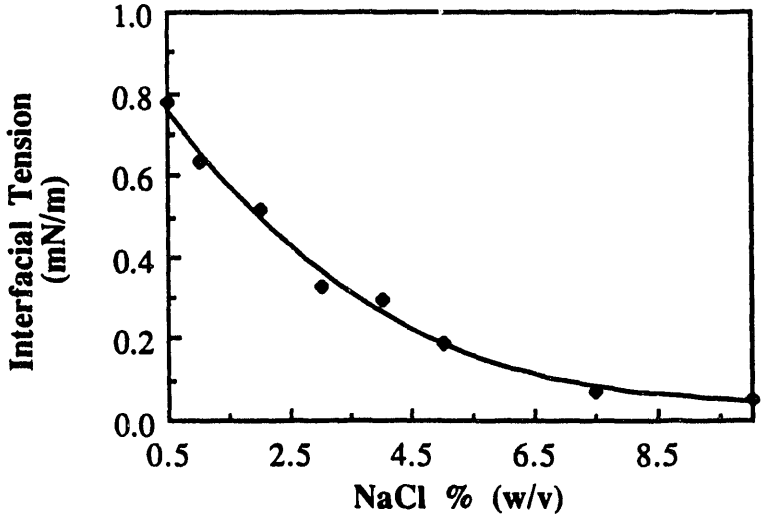

Figure 3.4 The effect of NaCl concentration on the ability of the surfactant to reduce the interfacial tension.

Chapter 4: Purification, and Characterization of Biosurfactant Produced by *Bacillus licheniformis* JF-2

ABSTRACT

Bacillus licheniformis strain JF-2 has been shown to be able to grow and produce a very active biosurfactant under both aerobic and anaerobic conditions and, therefore is a promising candidate for application in microbial enhanced oil recovery. The surface active compound was purified to homogeneity using reverse phase C_{18} HPLC. This compound was found to be a lipopeptide with a molecular weight of 1035 daltons. 1H, 13C and 2-D NMR spectrocopies showed that the biosurfactant has a structure very similar to surfactin produced by *Bacillus subtilis*. The *B. licheniformis* JF-2 surfactant exhibits a CMC of 10mg/l and reduces the interfacial tension against decane to 6 $X10^{-3}$ mN/m. The surface activity of JF-2 surfactant depends on the presence of fermentation products and is strongly dependent of the pH. Under optimal conditions the supernatant of *B. licheniformis* fermentation broth results in the lowest interfacial tension against decane that has been reported so far.

4.1 INTRODUCTION

Microbial compounds which exhibit particularly high surface activity are classified as biosurfactants. Microbial biosurfactants include a wide variety of surface and interfacially active compounds, such as glycolipids, lipopeptides polysaccharide-protein complexes, phospholipids , fatty acids and neutral lipids (1). Most biosurfactants consist of distinct hydrophilic and hydrophobic moieties. The former can be either ionic or non-ionic and consist of mono-, di-, or polysaccharides, carboxylic acids, amino acids, or peptides. The hydrophobic moieties are usually saturated, unsaturated or hydroxylated fatty acids. Biosurfactants are easily biodegradable and thus are particularly suited for environmental applications such as bioremediation and the dispersion of oil spills (2-4).

Among the many classes of biosurfactants, lipopeptides are particularly interesting due to their high surface activities and therapeutical values. For example, surfactin, a well studied lipopeptide antibiotic produced by *Bacillus subtilis* , is not only a very effective biosurfactant (5), but also an inhibitor of fibrin clotting (6,7) and cAMP phosphodiesterase(8).

Bacillus licheniformis strain JF-2, isolated from oil-field injection brine (9), has been shown to be able to grow and produce a very effective biosurfactant under both aerobic and anaerobic conditions at a very wide range of temperatures in the presence of high concentrations of salts (10,11), and therefore is a promising candidate for application in microbial enhanced oil recovery (MEOR) (12). The effects of various environmental parameters on the production of the biosurfactant, the formation of fermentation end-products, and the growth of *B. licheniformis* JF-2 in batch cultures have been reported(11). In this study a procedure for the isolation and purification of biosurfactant was developed, and the chemical structure and interfacial properties of biosurfactant produced by *Bacillus licheniformis* JF-2 were characterized.

4.2 MATERIALS AND METHODS

Microorganism and growth conditions. Bacillus licheniformis strain JF-2 ATCC # 39307 was purchased from the ATCC (Rockville, MD). The bacteria were grown aerobically in a mineral salt medium (9, solution I), containing 0.1 % $(NH_4)_2SO_4$, 0.025% $MgSO_4$, 1% glucose, 0.5% NaCl in 100 mM phosphate buffer (pH 7.0), and 1.0% (v/v) trace metals solution, in 2-litter Erlenmeyer flasks with a working volume of 1 litter at 42°C for 15 hours. The trace metals solution contained 0.1 % (w/v) EDTA, 0.3 % $MnSO_4$, 0.01% $FeSO_4$, 0.01% $CaCl_2$, 0.01% $CoCl_2$, 0.01% $ZnSO_4$, 0.001% $CuSO_4$, 0.001% $AlK(SO_4)_2$, 0.001% H_3BO_4, and 0.001% Na_2MoO_4 (13).

4.2.1 *Isolation and purification.*

The procedure employed for the isolation and purification of the biosurfactant is outlined in Figure 4.1. The cells were removed from the culture by centrifugation. Surface-active compounds were then isolated from the clear broth either by acid-precipitation with concentrated HCl at pH 2.0 (6) or XAD-2 (Sigma, St. Louis, MO) adsorption chromatography. For the former, the surfactant containing precipitate was collected by centrifugation and resuspended in 15 ml of water at pH 6.0 and subsequently lyophilized. The lyophilized material was then extracted with 5 ml of a mixture of chloroform and methanol (v:v = 1:2). For XAD-2 adsorption chromatography, the fermentation broth supernatant was loaded onto a 16x500 mm column at a flow rate of 1 ml/min. The column was eluted with 1.5 bed volume of methanol following 1.5 bed volume of water wash. Non-volatile material in the eluent was concentrated by evaporation in a Müchi rotary evaporator (Fisher Scientific, Fair Lawn, NJ) at 45°C. Samples obtained

by this procedure were designated as the crude biosurfactant preparation. 62, (Mallinckrodt, Paris, KY) column (28 x 500 mm) eluted with 5% methanol in chloroform at a flow rate of 2 ml/min. The fraction containing the surface active component was identified by interfacial tension measurements, as described below. The active compound was eventually purified to appropriate homogeneity by preparative reverse phase liquid chromatography at room temperature with a Waters HPLC system (Milford, MA) equipped with Waters C_{18} μ Bondapak column (19X300 mm). The solvent system consisted of mobile phase A : 10 mM KH_2PO_4 buffer at pH6.0 and mobile phase B: 20 % tetrahydrofuran in acetonitrile (HPLC grade, Fisher Scientific, Fair Lawn, NJ). The biosurfactant preparation was eluted with 53% B isocratically at a flow rate of 2 ml/min. Fractions collected were lyophilized and extracted with methanol to remove salt. For analytical reverse phase C_{18} HPLC analysis, a 7.8X300 mm μBondapak C_{18} column was used at a flow rate of 0.5 ml/min. The absorbance of the eluent was monitored at 210 nm.

4.2.2 *Characterization.*

FTIR was performed on a Nicolet 60SXR FT-IR spectrometer. The spectrum was measured in a sample compartment purged for at least half an hour with dry nitrogen before acquiring data, which were measured at 4 cm^{-1} resolution and were averaged over 500 scans. Base lines were electronically adjusted to zero absorbance for the measurement of spectral intensities.

For amino acid analysis, the purified biosurfactant was hydrolyzed in 6 M HCl at 105°C for 24 hours. The hydrolysate was subsequently subjected to an Applied Biosystem 420H Derivatizer/Analyzer with on-line 130A Separation System and 920A Data Analysis Module (Foster City, CA). 500 pM of normal-leucine was also included as an internal standard.

FAB/MS, fast atom bombardment mass spectroscopy, analysis was performed on a Finnigan TSQ 70 mass spectrometer with a NBA matrix. The mass spectrometer was scanned from 100 to 1200 amu. Positive ions were detected.

NMR analysis (^1H, ^{13}C, 2-D COSY, and 2-D TOCSY NMR) of JF-2 biosurfactant was performed in a Bruker high-field (11.9T) nuclear magnetic resonance spectroscopy, using 1, 2-dideuteriotetrachloroethane ($CDCl_2CDCl_2$) (Norell, Landisville, NJ) as solvent at 348°K.

4.2.3 *Interfacial Properties.*

Interfacial tension measurement against decane was performed in a spinning drop interfacial tensiometer (Model 300, University of Texas, Austin, TX). The critical micelle concentration (CMC) was determined by measuring the interfacial tensions of the biosurfactant solution following serial dilution. The effects of pH and microbial metabolites on the interfacial activity of the biosurfactant were also investigated with interfacial tension measurement. All interfacial tensions were measured against decane.

4.3 RESULTS

4.3.1 *Isolation and purification.*

Interfacial tension measurement indicated that the biosurfactant can be effectively isolated from cell-free culture by either acid-precipitation or XAD-2 adsorption chromatography. The interfacial tension increased from 0.085 mN/m for cell-free culture to more than 25 mN/m for the acid-precipitation supernatant of the culture and XAD-2 column eluent. Upon evaporation, approximately 65 mg per liter of culture was obtained from the XAD-2 column. For acid-precipitation, about 250 mg of acid-precipitate was obtained per liter of culture after lyophilization. Following organic extraction, 110 mg of crude biosurfactant preparation was obtained. This crude biosurfactant preparation was further separated into five fractions by silica gel chromatography. Only one fraction, eluted after 1.8 bed volume, contained surface active material. Analytical reverse phase C_{18} HPLC analysis indicated that there were at least three major compounds in this fraction, Figure 4.2. This fraction was further separated into three fractions by preparative reverse phase C_{18} HPLC. Interfacial tension measurement indicated that fraction C contained the most surface active compound. At a concentration of 100 mg/l in solution I with 5% NaCl and adjusted to pH 6.0, the material obtained from fraction A, B, and C gave interfacial tensions against decane of 1.317, 1.873, and 0.060 mN/cm, respectively. Material from fraction C was eventually purified by organic extraction to remove salt originated from HPLC mobile phase. Approximately 35 mg pure biosurfactant was obtained.

4.3.2 *Characterization*

Bands characteristic of peptides (wavenumber 3430, NH; wavenumber 1655, CO; and wavenumber 1534, CN) and aliphatic chains (wavenumber 3000 - 2800, CH_2 and CH_3) were observed in the infrared spectrum of the purified biosurfactant, indicating that

this compound is a lipopeptide. Also observed was a band corresponding to an ester carbonyl group (wavenumber 1730, CO). The result of amino acid analysis indicated the presence of four different amino acid residues in the peptide portion of the biosurfactant. The composition was determined to be glutamic acid: aspartic acid: valine: leucine = 1: 1: 1: 4. FAB/MS spectrum. Figure 4.4, indicated the biosurfactant has a molecular weight of 1035 daltons.

Proton NMR, Figure 4.5, showed seven NH signals (δ 7.0 - 7.7) and seven corresponding CH signals (δ 3.9 - 4.9) for the α-amino acids of a peptide. These were readily correlated with one another as well as with the signals of the corresponding alkyl residues via 2-D COSY and TOCSY spectrua, Figures 6. Since there were no signals for free CONH2, eliminating asparagine and glutamine as possibilities, the identities of the amino acids were confirmed as aspartic acid, glutamic acid, leucine, and valine. An additional low field signal at δ 5.3 consistent with CHO of the alcohol moiety of an ester (or lactone) was also observed.

Attempts to obtain the sequence of the amino acids using 2-D NOE (ROESY) were moderately successful, yielding the following partial sequences (not in particular order) :

fatty acid-Glu-Leu Asp-Leu Leu-Leu

These partial sequences are the same as those found in surfactin (14), and it seems likely that JF-2 biosurfactant and surfactin have the same complete sequence.

Although the chain lengths of the fatty acids could not be determined, it was obvious that a mixture of normal, anteiso, and iso branched forms were present (CH_3 at δ 13.4, 10.7 and 18.7 and ca. 22, respectively), with the latter predominating as seen in the proton NMR.

All these data indicate a structure, shown in Figure 4.7, closely related to surfactin.

4.3.3 *Interfacial activity*

The effect of pH on the interfacial activity of JF-2 biosurfactant was investigated. As shown in Figure 4.8, the crude biosurfactant preparation exhibited an optimal activity at pH 6.0. The interfacial tensions of the solutions at pH 6.0 (0.006 and 0.023 mN/m in the presence of 5% and 0.5% of NaCl, respectively) were 10 times lower than those at pH 7.0 (0.060 and 0.024 mN/m in the presence of 5% and 0.5% of NaCl, respectively). At pH 6.0 the interfacial tension in the presence of 5% NaCl (0.006 mN/m) was 4 times lower than that in the presence of 0.5% NaCl (0.023 mN/m). Interfacial tension measurement of each dilution of biosurfactant in freshly prepared medium with 0.5% NaCl at pH 6.0 indicated that the biosurfactant has a CMC of 10 mg/l. At CMC the interfacial tension

against was 0.028 mN/m. Even at a concentration as low as 5mg/l, the interfacial tension was less than 1.0 mN/m.

The effects of various additives on the surface activity of the biosurfactant are shown in Figure 4.9. The surface activity of biosurfactant was arbitrarily defined as the reciprocal of interfacial tension against decane. The activity of biosurfactant in the supernatant of acid-precipitation with 5% NaCl was at least 6 times higher than that of the biosurfactant in freshly prepared medium with 0.5% NaCl. The interfacial tension of solution D, contained 50 mg of crude biosurfactant in the supernatant of acid-precipitation with 5% NaCl at pH 6.0, against decane was as low as 0.006 mN/m.

4.4 DISCUSSION

The isolation and purification procedure developed in this study is proven effective for the isolation and purification of *Bacillus licheniformis* JF-2 surfactant. Furthermore, the HPLC system developed for biosurfactant purification may be adopted as an *in-vitro* assay of biosurfactant. It has been suggested that the biosurfactant produced by *Bacillus licheniformis* strain JF-2 is similar to surfactin produced by *Bacillus subtilis*. In this study a systematic characterization experiments were conducted to show that the amino acid compositions and sequences are the same for the JF-2 surfactant and surfactin. They also had same retention time in analytical HPLC analysis (data not shown). Although the exact conformations of the amino acid residues in JF-2 biosurfactant are not determined in this study, these results suggested that the chemical structures of surfactin and JF-2 biosurfactant are the same. However, some differences between these two biosurfactants were also observed. First of all, the FAB/MS spectrum of the JF-2 biosurfactant indicated the purified surfactant is a homogeneous preparation. The presence of a family of lipopetitdes with same peptide domains but different chain lengths of lipid tails, which has been frequently observed for lipopeptides produced by other microorganisms (15, 16), was not observed for the biosurfactant by JF-2. Secondly, ^{13}C NMR analysis indicated that the lipid tail of JF-2 biosurfactant is presence in three different configurations, namely, normal-, iso-, and anteiso-forms.

The biosurfactant produced by *Bacillus licheniformis* JF-2 has an CMC of 10 mg/l and can reduced the interfacial tension against decane to as low as 0.006 mN/m. The biosurfactant exhibits an optimal interfacial activity at pH 6.0. NaCl and microbial metabolites act synergistically with the biosurfactant to reach maximum interfacial activitv. In addition to high NaCl concentration, the presence of metabolites, such as organic acids and alcohols, in the supernatant of acid-precipitation also increases the interfacial activity of

the biosurfactant. These results suggested that the biosurfactant produced by *B. licheniformis* JF-2 was the most effective biological surfactant found so far.

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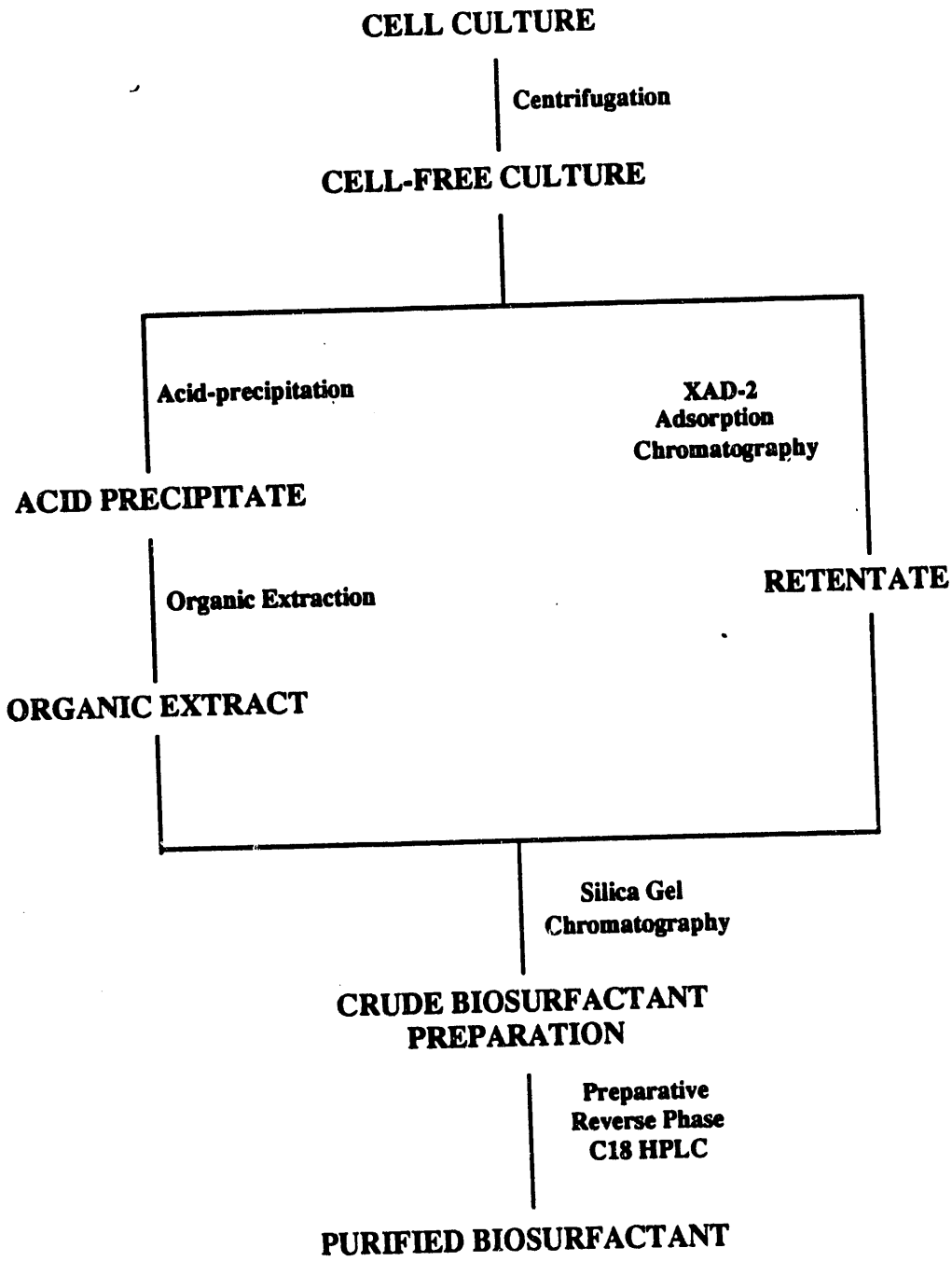

Figure 4.1 The procedure employed for the isolation and purification of biosurfactant produced by *Bacillus licheniformis* JF-2.

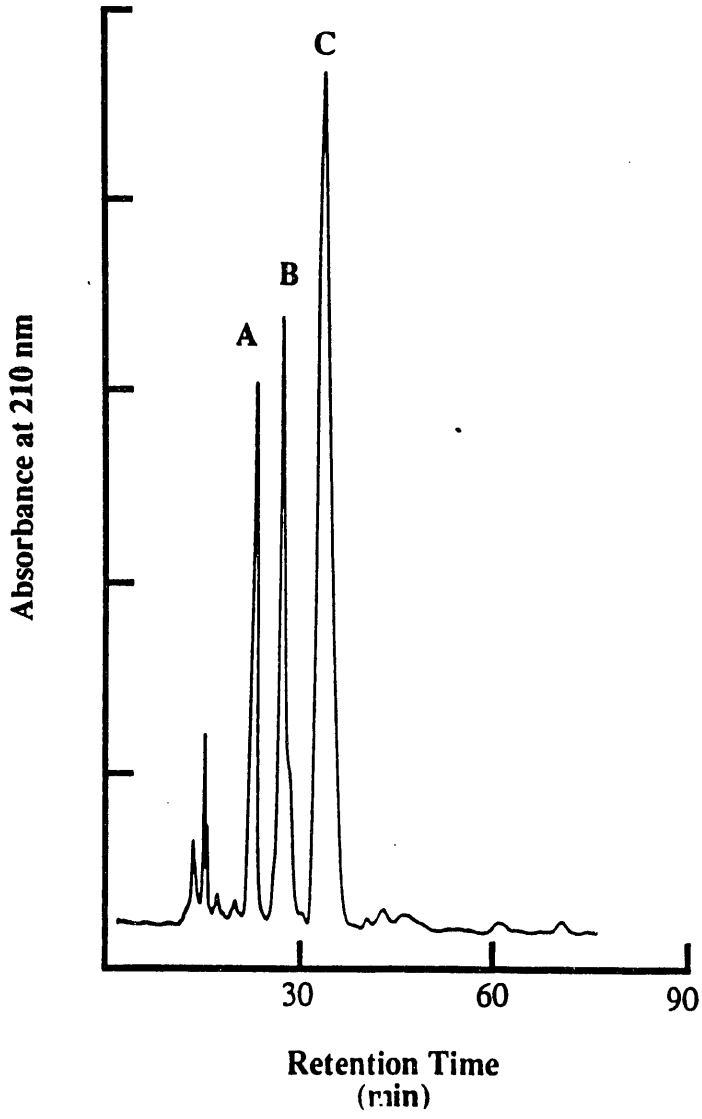

Figure 4.2 The reverse phase HPLC chromatogram of the active fraction from silica gel column. Three major components were observed. Fraction C contained the most surface active material.

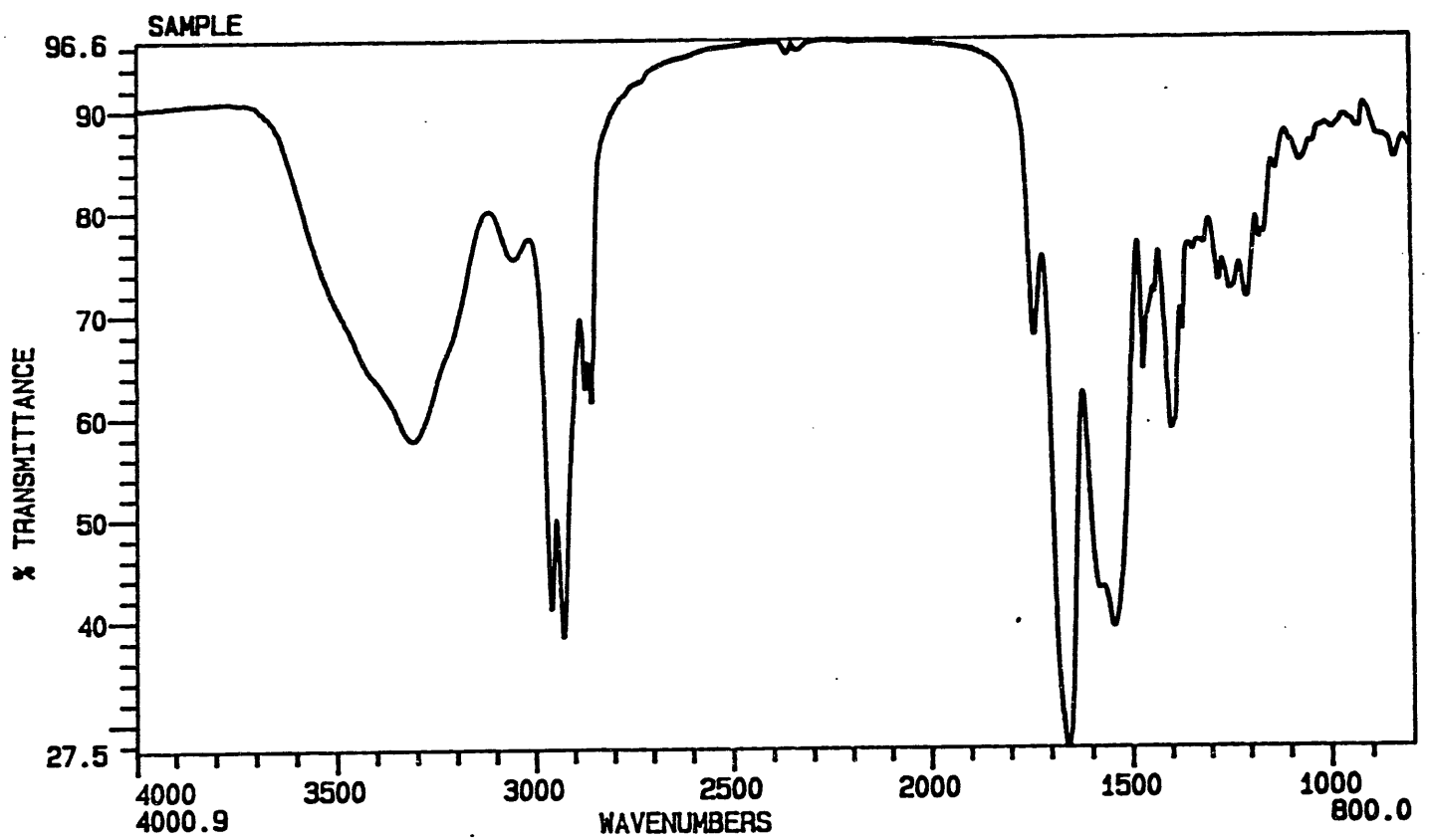

Figure 4.3 The Infrared spectrum of JF-2 biosurfactant. Bands corresponding to a lipopeptide were observd.

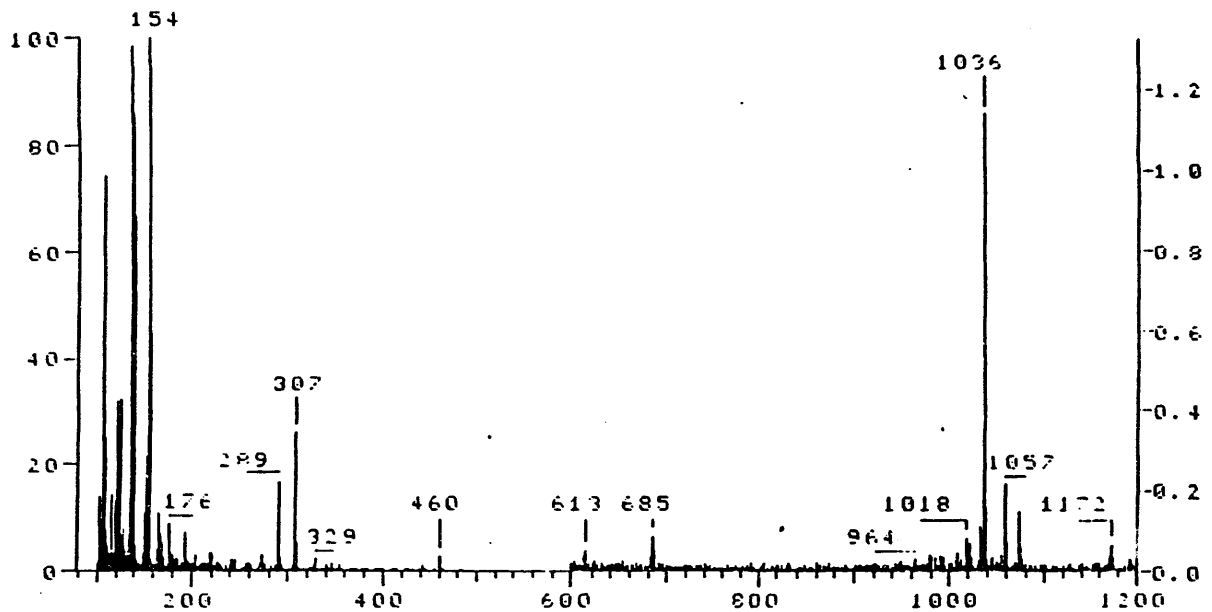

Figure 4.4 The FAB/MS spectrum of JF-2 biosurfactant. Positive ions were detected. The molecular weight of JF-2 biosurfactant is shown to be 1035.

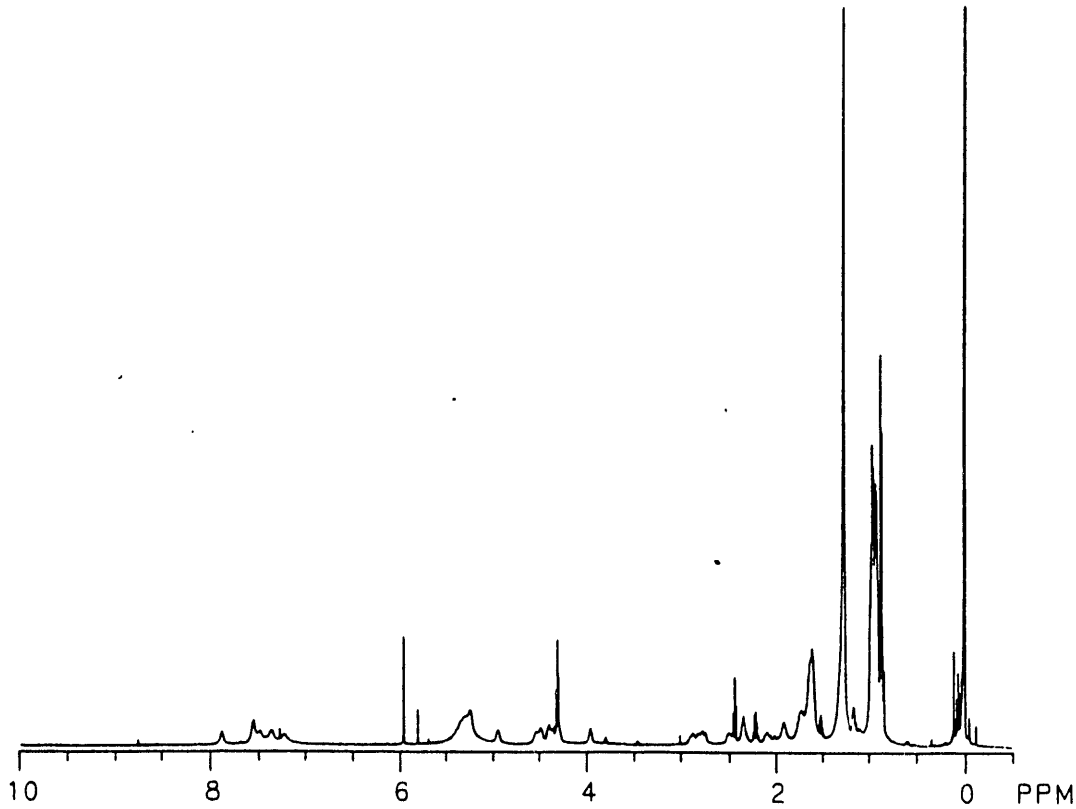

Figure 4.5 The proton NMR spectrum of JF-2 biosurfactant.

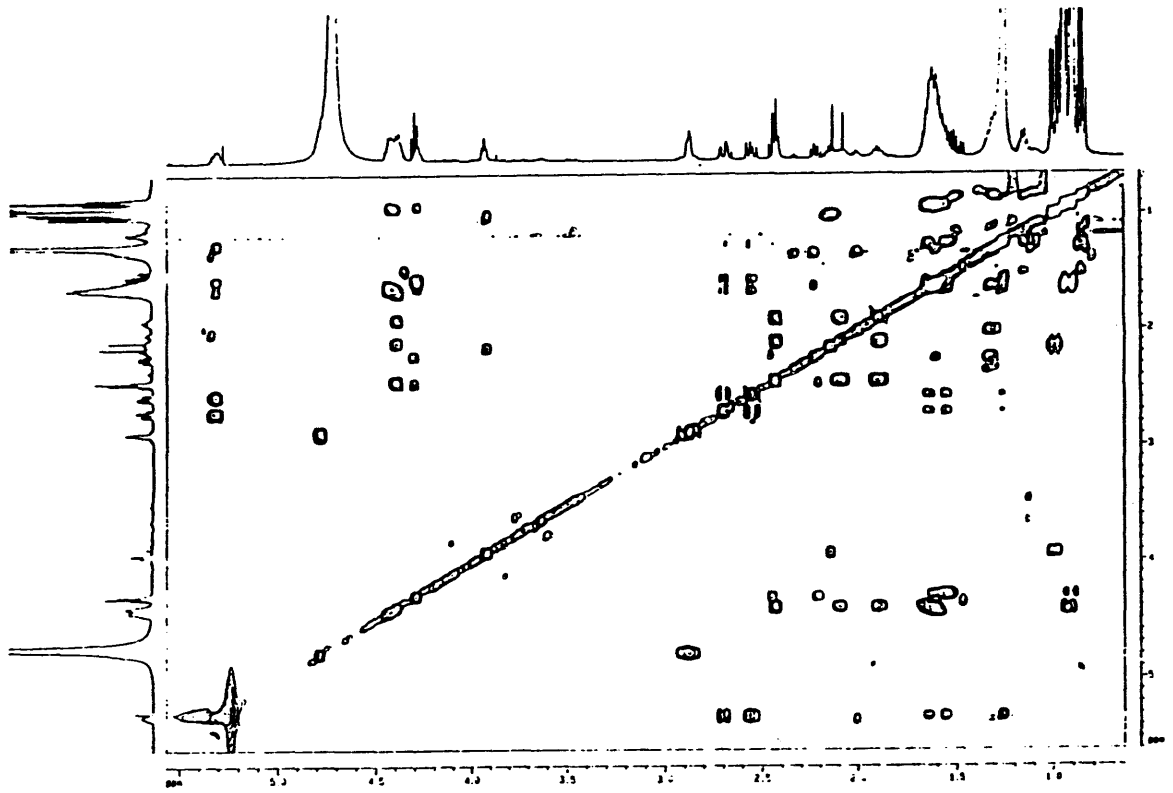

Figure 4.6 The 2-D COSY NMR spectrum of JF-2 biosurfactant.

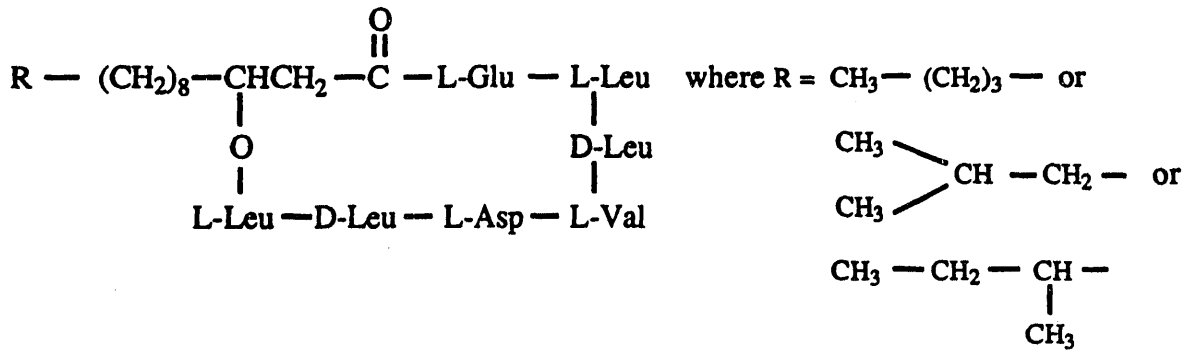

Figure 4.7 The chemical structure of JF-2 biosurfactant.

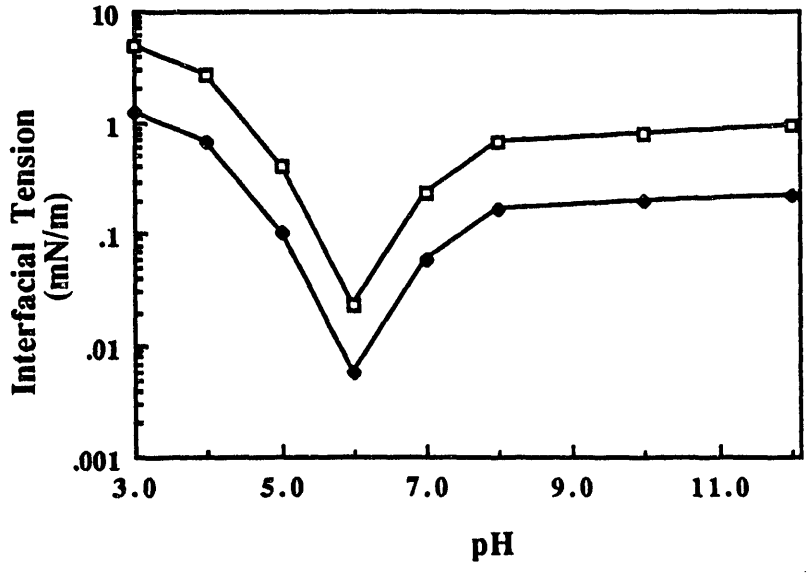

Figure 4.8 The effects of NaCl and pH on the surface activity of JF-2 biosurfactant. Crude biosurfactant was disolved at a concentration of 50 mg/l in (q) acid-precipitation supernatant with 0.5% NaCl and (u) acid-precipitation supernatant with 5% NaCl.

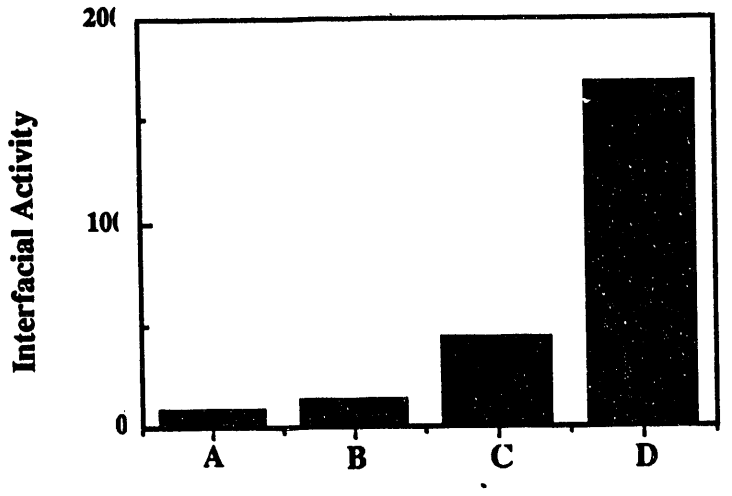

Figure 4.9 The effects of additives on the interfacial activity of JF-2 biosurfactant. Interfacial activity was arbitrarily defined as the reciprocal of interfacial tension (mN/m) measured against decane. Crude biosurfactant was dissolved in (A) fresh medium with 0.5% NaCl at pH 6.0, (B) fresh medium with 5% NaCl at pH 6.0, (C) supernatant of acid-precipitation with 0.5% NaCl at pH 6.0, and (D) supernatant of acid-precipitation with 5% NaCl at pH 6.0 at a concentration of 50 mg/l.

Chapter 5: An Experimental Investigation of Bacteria Transport in Porous Media

ABSTRACT

The convective transport of concentrated suspensions of bacteria in porous media is of interest for several processes including microbial enhanced oil recovery and in situ bioremediation. The parameters which effect the migration of the bacterium *Bacillus licheniformis* JF-2, a candidate microorganism for microbial enhanced oil recovery, were investigated experimentally in sandpacks. Bacterial retention and permeability reduction occurred primarily in the first few centimeters upon entering the porous medium. Microscopic observations, experimental measurements at different linear velocities and in the presence or absence of dispersants indicated that size exclusion of cell microaggregates and multiparticle hydrodynamic exclusion are the primary mechanisms for bacterial retention and permeability reduction. In downstream sections of the sandpack, the permeability reduction was low even in cases where high cell concentrations (10^8 cfu/ml) were detected in the effluent.

The effects of the : (1) degree of dispersion / aggregation of the bacteria, (ii) linear velocity of injection, (ii) cell concentration, (iv) salinity (v) temperature and (vi) presence of a residual oleic phase were determined. A lower reduction in permeability and a higher effluent bacteria concentration was obtained in the presence of dispersant, high injection velocities, low salinities and at higher temperature.

5.1 INTRODUCTION

The ability to transport bacteria through porous media is crucial to the implementation of microbial enhanced oil recovery processes and to the removal of environmental pollutants in groundwater through bioaugmentation. For these processes enhanced transport is desirable so as to have a uniform distribution of significant quantities of bacteria in the entire area being treated.

The primary motivation for this work is the use of microorganisms in MEOR. The experimental conditions used in this study are chosen to simulate the injection of cells in an MEOR process. The microorganisms used are known to produce the most active biosurfactant known to date (Lin et al., 1990). It is envisioned that the in situ production

of biosurfactant will increase the capillary number (defined as the ratio of viscous to capillary forces) sufficiently to enhance oil recovery (Sarkar et al., 1991). It should be noted that the experimental conditions chosen here are substantially different than those commonly used in soil science and bioremediation studies (Taylor and Jaffe, 1990). The focus of these studies was to investigate the in situ growth of indigenous populations of cells or the chemotactic movement of cell populations. For this purpose, low concentrations of cells are typically injected in a pulse and the distribution of the biomass is determined primarily by the growth of the microorganisms within the porous matrix. In contrast, the physical reality of microbial enhanced oil recovery processes is strikingly different. In this case, the ability to propagate cell population around the wellbore region is crucial. Microbial growth and biofilm formation must be avoided since they can result in plugging of the wellbore face which, of course, is detrimental. To understand the parameters that determine microbial transport under these conditions, it was necessary to study experimentally the convective transport of moderate to high concentrations of non-growing cells.

The relative rates of convection, diffusion and growth are described by the Peclet and Dahnkohler numbers defined as:

$$Pe = \frac{uL}{D} \quad ; \quad Da = \frac{k^{*}L\phi}{u}$$

where u is the superficial fluid velocity, L is the length of the porous medium, D is the diffusivity of the cells, k* is a first order kinetic rate constant and ϕ is the porosity. In our studies, experiments are conducted under conditions where Pe >>1 and Da<<1, so that diffusion and kinetic effects can be neglected.

Vandevivere and Bareye (1992) studied the effect of bacteria transport, growth and clogging efficiency. They showed that the strain that had a lower clogging efficiency also had a lower yield coefficient and was more easily eluted from the column. Their attention was, however, focused on the progressive clogging of the column with biomass production in situ.

Studies on in situ biofilm growth and the resulting changes in the properties of the porous medium have been conducted by Taylor and Jaffe(1990). Pulse injection of resting cell suspensions onto sand columns was conducted by Fontes et al.,(1991). They presented results that showed the importance of sand grain size on bacteria effluent breakthrough profiles in layered sands. Cell size and ionic strength were also shown to

play a role. Even though retention of cells was high, high cell concentration were observed in the effluent. This suggests that high concentrations of cells can be propagated significant distances into sand bodies with medium to large grain size. No permeability reduction data were reported.

Kalish et al. (1964) conducted an experimental investigation of bacteria transport in single - phase flow in consolidated porous media. It was conjectured that the important factors that should affect transport are: (i) initial permeability of the medium; (ii) characteristics of the bacterial species, such as, size, shape, aggregating tendency; (iii) the presence of extracellular polymers ; (iv) injected concentration; and (v) flow velocity/ pressure gradient. Formation of internal and external filter cakes was observed for flow through high- (~300 mD) and low- (~30 mD) permeability media, respectively. After injection over an extended period of time, it was found that the final permeability ratios (based on initial permeability) were limited to 0.05 and 0.1 for high- and low -permeability samples. The penetration distances were limited to approximately 4 inches for high permeability samples and 1 inch for the low permeability samples. Observations of high effluent concentrations were reported but no quantitative results were shown.

Sharma et al. (1985) have shown that the transport of bacteria through unconsolidated porous media can be facilitated by orders of magnitude by adding polyanionic electrolytes such as sodium pyrophosphate, to the cell suspension. An increase in the negative surface charge of both the bacteria and the sand grains was proposed to be responsible for this effect. The higher repulsive double layer force in the presence of polyelectrolytes resulted in decreased adhesion on pore surfaces and reduced flocculation of the bacteria.

Stehmeier and Jack (1987) have presented evidence indicating that the major mechanism of the bacteria retention in porous media is a velocity-dependent retention process. Retention was reduced by using a sacrificial agent (a strongly adsorbing compound that partially blocked adsorption sites on the clay). They found that pulsed flow (repeatedly starting and stopping flow) enhanced transport. It was proposed that when flow is stopped the particle bridges plugging pores are relaxed and subsequent flow results in partial disruption of the relaxed bridges. Cusak et al. (1990) reported that the transport of bacteria, in this case a *Pseudomonas* species, can be enhanced by washing the cells in a phosphate-buffered salt solution to eliminate extracellular polymers and by prolonged starvation which causes a reduction in size. Silver et al. (1989) have reported that bacterial spores, being smaller in size and free of adhesive polymers on the cell wall, can be used to facilitate transport.

Jang et al. (1982) have shown that Bacilli can migrate through nutrient saturated rock samples at a rate of approximately 0.1 cm/hr due to the combined effects of bacterial diffusion, growth, and motility. Much higher rates of penetration (0.5 cm/hr) as a result of growth and chemotaxis were reported by Reynolds et al. (1989). In both cases, the rates of transport by chemotaxis and growth were slower than those resulting from convection. Consequently, the chemotactic flux had little significance during the injection of a bacterial suspension, but it affected the local distribution of bacteria during static bacterial growth inside a porous medium.

The studies mentioned above have helped to identify some of the important factors affecting bacteria transport. None of these studies, however, has presented a complete set of results consisting of effluent concentration and permeability histories as well as permeability profiles. To fully elucidate the mechanisms of bacteria transport a complete data set is essential. To predict bacterial movement in the region away from the wellbore the importance of fluid velocity in the transport process must be evaluated because in a radial geometry velocity decreases as the inverse of radial distance from the well.

The objective of this study was to identify the important parameters that control the transport of *Bacillus licheniformis* JF-2 through porous media. Furthermore, the mechanisms of bacteria retention and permeability reduction are discussed. The effect of flow velocity, injected concentration, degree of dispersion, salinity, temperature, and the presence of a residual oil phase on bacteria transport is investigated. These effects are interpreted in terms of potential retention and permeability reduction mechanisms.

5.2 MATERIALS AND METHODS

5.2.1 Bacterial Strains and Growth Conditions

The microorganism used in this work is *Bacillus licheniformis* JF-2 (Jenneman et al., 1983). It is a facultative, Gram positive bacterium that produces a highly effective lipopeptide surfactant under both aerobic and anaerobic conditions. The dimensions of vegetative *Bacillus licheniformis* JF-2 cells were 4.0 x 1.5 μm, Sporulation was not detected under the conditions used.

Cultures were grown aerobically in a mineral salt medium (Jenneman et al., 1983) containing 0.1 % NH_4SO_4, 0.025% $MgSO_4$, 1% glucose, 0.5% NaCl in 100 mM phosphate buffer (pH 7.0, and 1.0% (v/v) trace metals solution, in 2-liter Erlenmeyer flasks with a working volume of 1 liter at 42°C for 15 hours. The trace metals solution

contained 0.1% (w/v) EDTA, 0.3% $MnSO_4$, 0.01% $FeSO_4$, 0.01% $CoCl_2$, 0.01% $ZnSO_4$, 0.001% $CuSO_4$, 0.001 $AlK(SO_4)_2$, 0.001% Na_2MoO_4 (Clark et al., 1981).

5.2.2 Analytical Techniques

The number of colony forming units in the sandpack effluent was determined by diluting samples 10^3 to 10^6 in mineral salt medium without glucose and then plating on agar plates. Colonies were counted following two days of incubation at 42°C . Only plates containing between 20 and 400 colonies were used and the results reported represent the average counts from at least three plates.

Glucose concentrations in the effluent were determined using a Yellow Springs Instruments glucose analyzer.

5.2.3 Flow Experiments

Sandpacks 8 inches long and 1 inch in diameter were used as the model porous medium. The Ottawa sand used here is 99% pure quartz with trace quantities of iron oxide. The sand is sieved, washed repeatedly with distilled water and dried before being packed into the column. Three intermediate pressure taps were used to divide the sandpack into four sections with the following lengths : 1st section - 1 inch, 2nd section -2 inches; 3rd section - 4 inches; 4th section -1 inch (numbered from the upstream end of the porous medium). The pressure taps were connected to differential pressure transducers arranged in series for different pressure ranges (0 to 0.5 psi and 0.5-5 psi). The transducers were connected to a digital data recorder through a demodulator. The transducers are accurate to within 1% of the full scale reading. Figure 5.1 shows a schematic of the experimental setup. Fluid was pumped using constant rate HPLC pumps. The sandpacks were sterilized by flowing a mixture of DI water and ethanol (70%) for at least 8 hours to sterilize the medium.

In each experiment the sandpack was first vacuum saturated with the brine. The brine was flowed through the sandpack and initial permeabilities for all the four sections were measured. The cellular suspension was then injected into the sand column by displacing it in an accumulator with an inert oil that is being pumped through an HPLC pump. This minimizes any shear on the cells and prevents the generation of cell fragments and cell wall debris. The bacterial suspensions to be injected are prepared by diluting the culture with medium E to the desired concentration. Effluent samples are collected and the pressure drop across four sections of the column are monitored. These pressure drops are inversely proportional to the permeability for a constant flow rate. At the end of bacteria

injection, the flow is stopped and the cells are allowed to incubate at 49°C. An insulated heating tape connected to a temperature controller is used to maintain the column temperature at 40°C. Temperature variations of less than ± 0.2 °C are experienced over the course of several days. In situ growth of biomass is monitored by measuring the extent of glucose consumption at the end of the incubation period and by measuring viable cell counts. Since the primary focus of this work is on convective bacteria transport, results for incubation and growth studies are not presented. These results are available in Sarkar (1991).

5.3 EXPERIMENTAL RESULTS AND DISCUSSION

5.3.1 Effect of dispersant

To demonstrate the importance of electrostatic interactions between cells and between the cells and the porous medium, experiments were conducted with cell suspensions containing polyvalent anions (sodium pyrophosphate). Figures 5.2 and 5.3 show the dimensionless effluent bacteria concentration (effluent concentration divided by the injected concentration) and the permeability ratio (permeability at a given time divided by the initial permeability) of the upstream section of the porous medium as a function of the dimensionless time (t_D), which is defined as the cumulative volume of fluid injected divided by the pore volume of the sandpack. Since it is our objective to ensure the transport of high cell concentrations deep into the porous medium, minimizing coagulation of cells and increasing the electrostatic repulsion between the cells and the sand grains is highly desirable. Thus, 0.1% sodium-pyrophosphate was added as a dispersant. This polyvalent anion or peptizing agent acts as a dispersant for the bacteria by increasing the negative surface charge on the cell walls (Sharma et al., 1985). In the presence of the dispersant the breakthrough time (dimensionless time at which the effluent concentration is 50 % of the injected concentration) decreases and the maximum effluent concentration increases. The retained bacteria fractions (ratio of total retained to total injected cell mass at $t_D = 5$) are 0.37 and 0.80 for the two cases, respectively. In the presence of dispersant the permeability ratio of the upstream section is greater than 0.4 whereas in the absence of dispersant the ratio decreases to values below 0.1. This is probably a result of the formation of an internal filter cake in the upstream section preventing further transport through the medium. Hence, even though the injected bacteria concentration is higher in the experiment with dispersant, the bacteria are more easily transported.

through the medium. Hence, even though the injected bacteria concentration is higher in the experiment with dispersant, the bacteria are more easily transported.

The addition of a dispersant has several consequences. As shown previously (Sharma et al., 1985) it reduces the degree of coagulation and the effective size of bacterial aggregates. In addition it increases the repulsive forces between the depositing bacteria and the "collectors" (i.e. the grains of the solid medium) and reduces the retention by surface adhesion. Since addition of a dispersant has a significant effect on bacteria transportability it can be surmised that the effective bacteria size in suspension and the electrostatic interactions between the cells and the porous medium are both important in determining the extent of cell retention.

It is important to note that even though high bacterial counts are measured in the effluent, no significant permeability reduction occurred in sections 2, 3, and 4 of the sandpack. This clearly suggests that bacteria were primarily retained in the first section with very little retention downstream. This may have been caused by the absence of larger sized flocs and perhaps a streamlining of the flow trajectories. It has been shown that in the presence of a repulsive surface force barrier between the particles and collectors, a very low collection efficiency is obtained in the interior of a porous medium (Vitthal, 1991). If surface adhesion was the only retention mechanism, significant permeability reduction would have been observed in downstream sections where the flowing bacteria concentrations are as high as the injected concentration. Our data, therefore, suggest that in the upstream section of the sandpack, in the presence of repulsive energy barriers to particle deposition and coagulation, bacteria retention occurs either by straining or size exclusion of aggregates of cells. Small numbers of such aggregates were observed microscopically and seem to exist despite the large repulsive energy barrier for coagulation.

5.3.2 Effect of flow velocity

The effluent bacteria concentration and the permeability ratio of the upstream section as a function of the dimensionless time for various flow velocities are shown in Figures 5.4 and 5.5. In these experiments a dispersant was used in the bacterial suspensions and the injected bacteria concentrations were in the range of 10^8 cfu/ml. The bacteria first appeared in the effluent at $t_0 = 1.5$. This value is approximately the same for all the flow velocities tested. The time at which breakthrough occurred (the time for the effluent concentration to become 0.5 of the injected concentration)decreased and the maximum effluent concentration increases with increasing velocities (Figure 5.6). The permeability dropped very sharply during the initial period of injection and then further decreased gradually in a linear fashion.

The final permeability ratio of the inlet section as a function of flow velocity is shown in Figure 5.7. This figure also shows a decrease in the retained bacteria fraction with increasing flow velocity. The retained bacteria fraction is defined as the ratio of the cumulative number of bacteria retained in the sand column divided by the total number of injected bacteria. A velocity of approximately 25 ft/day at which the permeability impairment is minimum is observed. Under this condition the maximum effluent concentration is 0.70, the retained bacteria fraction is 0.70, and the breakthrough time is 3.5.

A higher retention of bacteria should result in lower permeabilities. Surprisingly, however, this was true at the lower but not the higher flow velocities examined. At flow velocities between 25 ft/day and 100 ft/day both the extent of retention and the permeability decreased. The reason for this effect is not clear. One possibility is that at high velocities cells block pore channels more efficiently and thereby cause large changes in permeability with a smaller retained fraction of bacteria.

Figure 5.8 shows the permeability histories for the second, third, and fourth sections. All these sections show permeability ratios to be above 0.75 irrespective of the flow velocity. Bacteria transported through the upstream section are less likely to be retained in the downstream sections. This result provides further evidence that individual bacteria are not retained by a surface adhesion mechanism. As pointed out earlier, the bacteria concentrations in suspension are high in the downstream sections, yet there was virtually no permeability reduction. This observation is probably due to the presence of small aggregates in the injected cell suspension which are preferentially retained in the first section.

5.3.3 Effect of injected bacteria concentration

The effluent bacteria concentration and the permeability ratio of the upstream section are shown in Figures 5.9 and 5.10 for two different bacterial concentrations. The results are grouped in two categories: with dispersant (solid curves) and no dispersant (dotted curves). In each category the injected concentrations differ by a factor of 10. For both cases, an increase in the injected concentration reduces the breakthrough time and increases the maximum effluent concentration significantly. It is difficult to compare the results between the two categories because the two flow velocities were different ('with dispersant'-10 ft/day, 'no dispersant'-100 ft/day). In the case of cell suspensions with no addend dispersant, coagulation is promoted at high cell concentrations and immediate reductions in the permeability of the first section are observed. Formation of such external

filter cakes was confirmed microscopically. Similar observations are made at lower velocities in the presence of the dispersant. The above observations also indicate that surface adsorption is not the primary retention mechanism in our experiments.

5.3.4 Effect of ionic strength

Figures 5.11 and 5.12 show the effluent bacteria concentrations and the permeability ratio for the upstream section when the salinity (ionic strength) of the injected suspension was increased from 0.5 % to 10.0% NaCl. (The high osmotic pressure in the 10% NaCl solution does not result in osmotic damage or lysis of *Bacillus licheniformis* which is halotolerant and is even capable of growth at this salt concentration (Goursaud 1989). The presence of 10% NaCl caused a dramatic decrease in permeability, indicating the formation of an internal filter cake. At the same time the effluent concentration ratio was reduced to zero. Higher ionic strengths result in decreased electrostatic iterations thus favoring the formation of cell aggregates and adhesion on pore surfaces. Both of these effects result in very little transport of cells.

5.3.5 Effect of temperature

Figure 5.13 shows the permeability ratio during bacteria injection at two different temperatures. These transport experiments were conducted at low flow velocities (2 ft/d), and as a result no significant transport of bacteria in the effluent could be observed. At low temperatures a decrease in the Brownian energy of the bacteria should increase coagulation resulting in the formation of bigger aggregates . The enhanced degree of coagulation is in turn responsible for the observed increase in retention at the face of the sand. There is, unfortunately, no experimental technique other than visual observation to quantify the size distribution of cells in a concentrated suspension. Techniques such as light scattering and Coulter counters require the suspension to be diluted and as such are not directly useful in this study.

5.3.6 Effect of the presence of two fluid phases

Figures 5.14 and 5.15 show the effluent concentrations and the permeability ratios of the upstream section during injection of a bacterial suspension in single- and two- phase flow cases. As described in the Materials and Methods section, to establish a residual oil phase, a brine saturated porous medium was used as a starting point. Decane was used to displace the brine to an apparent residual saturation. Brine was then used to displace the decane to obtain a residual oil saturation. This represents a depleted oil reservoir. In the

case of two phase flow the permeability ratio is based on brine effective permeability at residual oil saturation.

In two-phase flow, breakthrough occurs later and the maximum effluent concentration is lower. At $t_D = 5$, the retained bacteria fraction is 0.80 and 0.96 for the single- and two- phase cases, respectively. Although, a somewhat higher amount of bacteria is retained in the two- phase case, the permeability reduction is smaller. Because of the presence of a non-wetting residual phase, a relatively higher fraction of the bacterial suspension flows through the smaller pore throats and this may result in higher retention. This phenomenon may also be related to the adsorption of cells at the oil/water interface.

It should be noted that a comparison of the permeability ratios should be made with caution. In the case of two-fluid-phase flow, the dimensionless permeability ratio is calculated on the basis of the effective brine permeability (in the presence of a residual oil phase) but in the case single-phase flow the ratio is based on the single phase brine permeability.

5.3.7 Proposed Mechanisms of Bacteria Transport

Bacteria transport in a porous medium is a dynamic process involving the retention of bacteria and the consequent changes in the structure of the porous medium. Based on observations made during the course of this study and theoretical arguments (Vitthal and Sharma, 1992) we propose the following mechanisms for bacteria retention: (1) surface adhesion; (2) size exclusion; (3) pore bridging; (4) multi-particle hydrodynamic exclusion . Figure 5.16 schematically shows the postulated retention and permeability reduction mechanisms.

In the surface adhesion mechanism bacteria deposit smoothly on the surface of the medium. From the theory of particle attachment / detachment from surfaces (Sharma et al., 1991) the important adhesion parameters are: size and shape of bacteria, fluid velocity, viscosity, ionic strength, and dielectric constant; surface charge on bacteria and pore surfaces; the Hamaker constant and finally the fluid temperature. Surface adhesion is expected to be the dominant mechanism of retention when the pore sizes are much larger than the bacteria size. In general Brownian colloids (<1 μm) are more influenced by surface chemical interactions while larger particles are more influenced by hydrodynamic forces and gravity. Bacterial adhesion is affected significantly by polymeric binding of the cells to the surface (Ward and Berkeley, 1980). The polymeric binding is caused by extracellular polymers which consist of polysaccharides and/or glycoproteins whose

amount and composition vary with the stages of growth and environmental parameters such as nutrient composition, temperature, and culture pH. This material also plays an important role in the aggregation / coagulation of the bacteria in suspension. When the bacteria are retained by adhesion to pore surfaces the permeability is expected to decrease very slowly with time as is the case in deep bed filtration.

Pore throats with sizes smaller than the size of the bacteria are plugged by a size exclusion process. Regardless of the nature of the forces that mediate bacterial adhesion onto surfaces, permeability changes can arise due to physical clogging of pore throats by individual cells. Under these conditions the injected bacteria are retained as an external or internal filter cake with small depths of penetration. Studies in filtration suggest that the ratio of particle to pore size must be no more than approximately 1/5 if the formation of an external filter cake is to be minimized (Vitthal and Sharma, 1991). For typical bacteria sizes of approximately 2 μm, this translates into a minimum acceptable pore size of 10 μm. However, it should be noted that cells often form aggregates by reversibly or irreversibly binding together. The size of these aggregates may be substantially higher than the 2μm stated above.

The third proposed mechanism of bacteria retention is bridging. In the bridging process, bacteria deposit at pore throats and act as collectors for subsequent deposition. Gradually a bridge is formed which prevents further transport of bacteria. This is likely to be a dominant mechanism during transport of dilute bacteria suspensions at low flow rates. In this process the reduction in permeability should occur gradually. The stability of the bridges and hence the permeability impairment decreases with an increase in the flow velocity or the applied pressure gradient. If the surface interactions between depositing particles are attractive, i. e., deposition of one bacterium on another is favored, then bridging is likely to occur at the entrance to the porous medium and very few bacteria will propagate through the porous medium.

Finally, the hydrodynamic exclusion or "log-jam" effect is important in the transport of concentrated bacteria suspensions. In this case several bacteria may attempt to flow through a pore throat at the same time and effectively plug it by bridging across it. This effect has been discussed by Vitthal and Sharma (1992) and is also referred to as the "multi-particle hydrodynamic exclusion" mechanism. The trajectories of the incoming particles at a pore throat are interdependent due to hydrodynamic coupling. As the concentration of particles increases, the hydrodynamic retardation of the particles becomes sufficiently large so that they can not pass through the pore throat. This results in the spontaneous formation of a particle bridge across the pore throat. In situations where this

mechanism is dominant, flow reversals and fluctuations in flow velocity will enhance the transport of the bacteria (Stehmeier and Jack, 1987). In addition, increased applied pressure gradients will cause an increase in permeability as the deformable log-jams are broken and the bacteria are pushed deeper into the porous medium.

It is likely that in the transport of a bacterial suspension all these mechanisms are involved to varying degrees. The experimental results presented earlier can be discussed within the framework of the retention mechanisms outlined above. These mechanisms have been incorporated into a model for bacteria transport in a companion paper (Sarkar, 1992).

The effect of each of the experimental parameters on the proposed mechanisms is shown in Table 5.1. This qualitative description of the proposed mechanisms if useful because it allows us to understand the impact of each of the parameters (flow velocity, concentration etc.) on the retention of cells. From Table 5.1, it is evident that each mechanism is important under a certain set of conditions. It is instructive to break up the problem into two categories; (i) A repulsive surface force barrier exists between the bacteria and the grains (ii) the net surface forces are attractive. Under attractive surface force conditions (low surface charge density, high salinity etc.) the bacteria can be retained by all four proposed mechanisms; surface adhesion, bridging and size exclusion of aggregated bacteria. The retention is large and internal or external filter cakes quickly form that do not allow any subsequent transport of bacteria into the porous medium. In the case of repulsive surface force barriers between the bacteria and the collectors, (as is the case in most of our experiments) surface adhesion and bridging are unimportant. Size exclusion of cells and aggregates and hydrodynamic exclusion effects are dominant. Under these conditions, the effect of cell concentration and flow velocity will be large.

It is observed in our experiments that high cell concentrations result in larger retention. Under repulsive surface force conditions, this could be due to more hydrodynamic interference between cells i.e. an increased likelihood of the formation of log - jams or due to the formation of a larger number of cell aggregates. In some experiments, high cell concentrations were observed in the effluent and no significant retention or permeability reduction was observed except in the first section. This suggests that the retention of cell aggregates by size exclusion is the primary retention mechanism in these experiments.

The velocity dependence of cell transport can be explained either by a cell aggregate retention by size exclusion or by a log - jam effect. Since the suspensions used in the above experiments contained relatively high bacteria concentrations, log-jams and cell

aggregates are likely to form even when repulsive electrostatic energy barriers exist for cell adhesion. At low velocities the fluid pressure gradients are not high enough to break cell aggregates plugging pores or to break any log-jams created. At higher velocities the pressure gradients are sufficient to break some of the bridges and log jams so formed.

In the presence of attractive surface forces between the cells and the collector, surface adhesion and bridging become viable mechanisms and a substantial amount of retention occurs in the inlet section. An internal or external filter cake is quickly formed. The filter cake retains all the injected bacteria so that no cells reach the downstream sections.

5.4 CONCLUSIONS

The following conclusions can be drawn from the experimental data presented.

(1) The transport of bacteria through sandpacks is controlled by the state of dispersion of the bacteria and the fluid flow velocity.

(2) Reducing coagulation by addition of a dispersant significantly reduces the breakthrough time and the permeability impairment. Addition of electrolyte, higher injected concentrations, or a decrease in temperature result in increased bacterial retention presumably because of the higher degree of coagulation. Increases in bacteria concentration result in higher retention due to an increase in the extent of multi-particle hydrodynamic exclusion.

(3) A higher flow velocity reduces the retained bacteria fraction and the breakthrough time and increase the maximum effluent concentration.

(4) In the presence of repulsive energy barriers for bacteria adhesion to collectors, multiparticle hydrodynamic exclusion and size exclusion of cell aggregates are the most likely operative mechanisms for bacteria retention in the upstream section of the core. Very little permeability reduction occurs in the downstream sections. This is observed in all the experiments at the various conditions studied.

(5) Under attractive surface forces, retention is almost complete with the immediate formation of an internal and / or external filter cake. Permeability is very rapidly reduced.

(6) The presence of a residual oleic phase increases bacteria retention, but decreases the extent of permeability reduction.

In summary the results of the study provide some insight into the mechanisms of bacteria retention in porous media and may be used to select optimal conditions for facilitating or retarding cell transport.

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Table 5.1 The Influence of Experimental Parameters on Cell Retention Mechanisms

Experimental Parameter Mechanisms	Dispersant Concentration	Flow Velocity	Ionic Strength	T	Cell Pore Size	Cell Concentration
Surface Adhesion	↓	↓	↑	↓	—	—
Size exclusion (cells and aggregates)	↓	↓	↑	↓	↓	↑
Bridging	↓	↓	↑	↓	↓	—
Log - jam	↓	↓	↑	↓	↓	↑

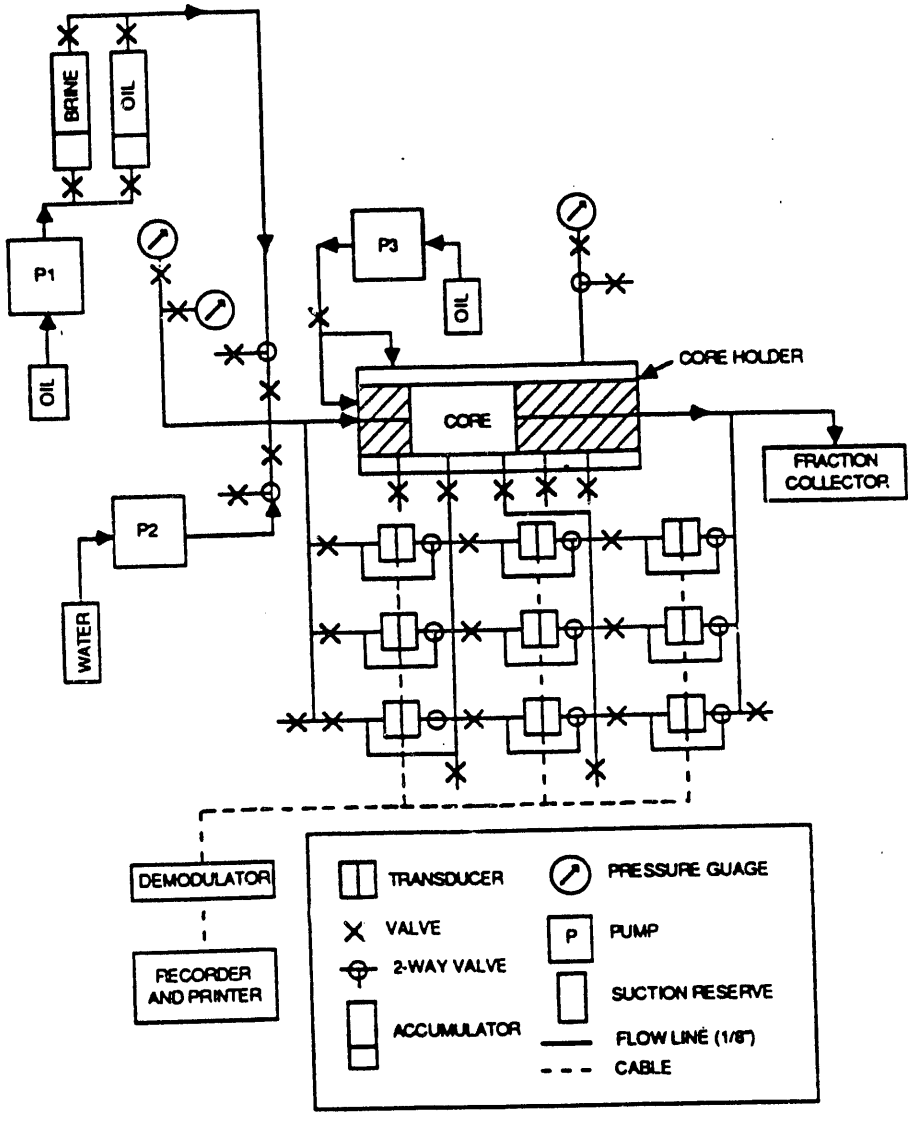


Figure 5.1 Schematic diagram of experimental apparatus.

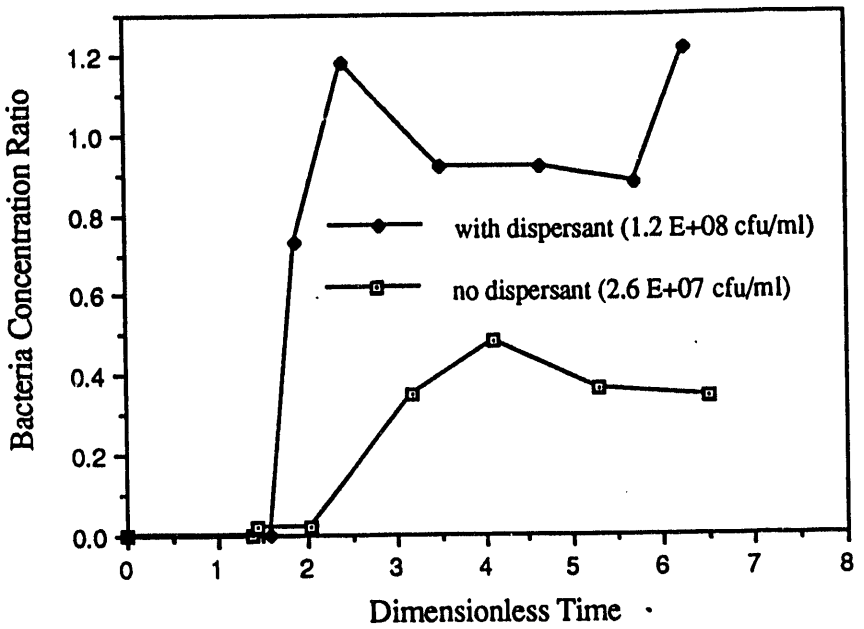


Figure 5.2. Effect of the presence of the dispersant on effluent bacteria concentration ratio history (velocity=100 ft/d).

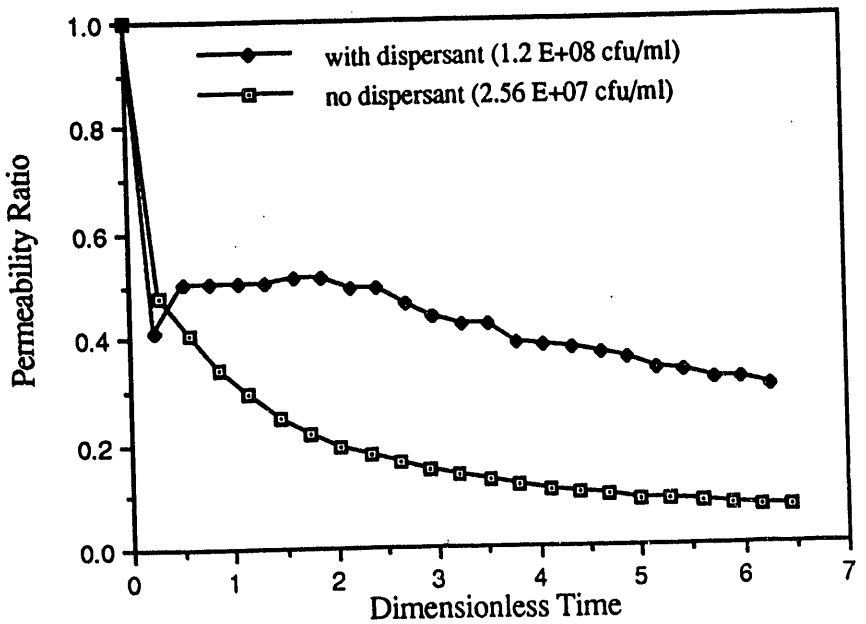
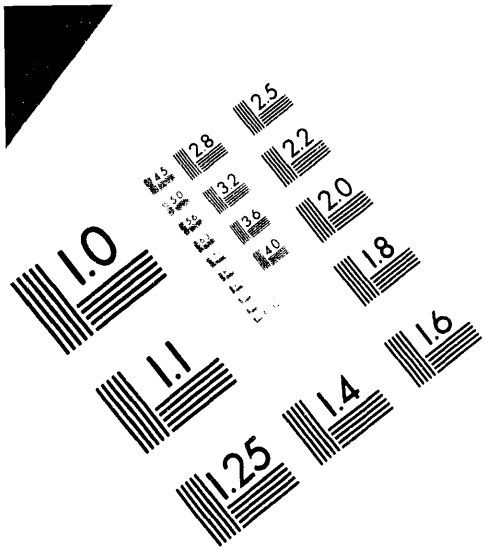


Figure 5.3. Effect of the presence of the dispersant on permeability ratio history of the upstream section (velocity=100 ft/d).



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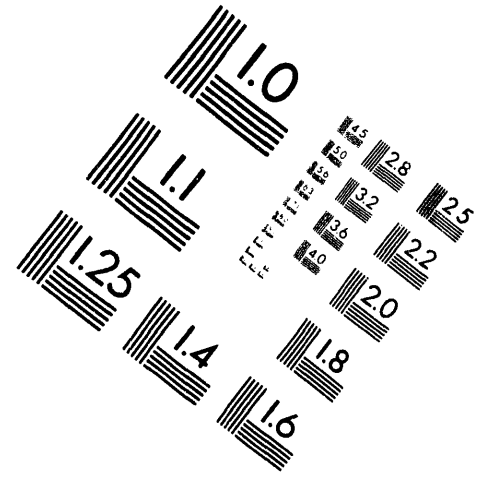

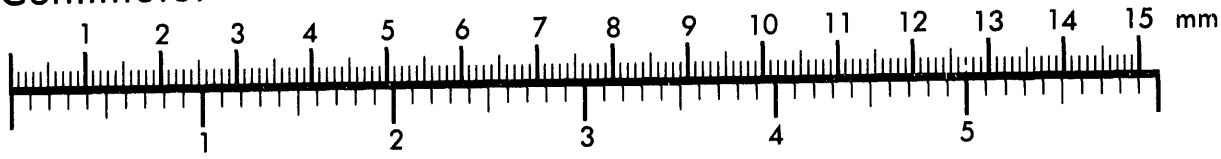

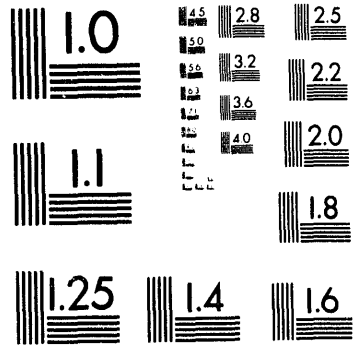

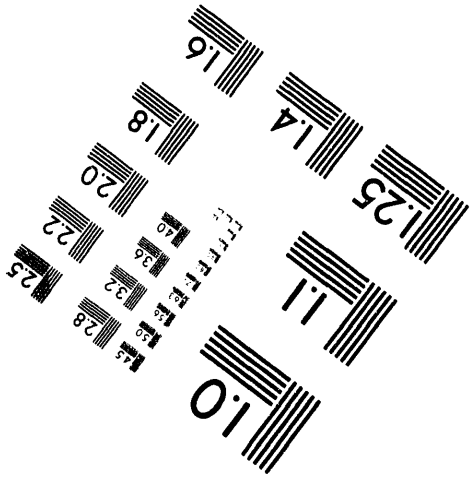

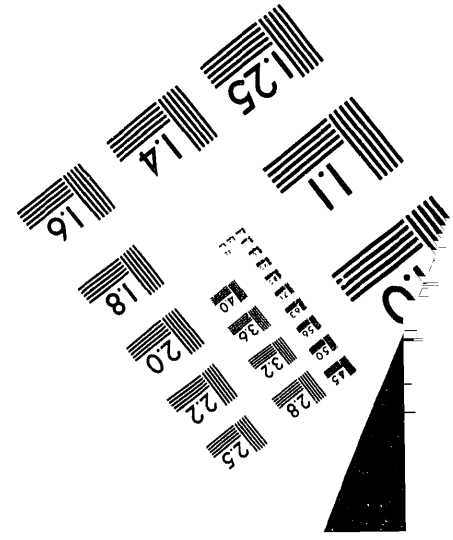

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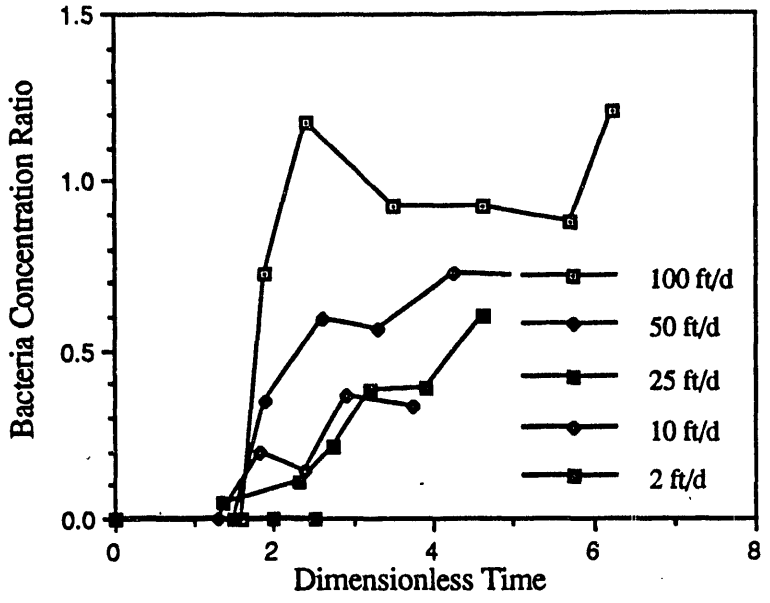

Figure 5.4. Effect of velocity on effluent bacteria concentration (injected conc.~10^8 cfu/ml).

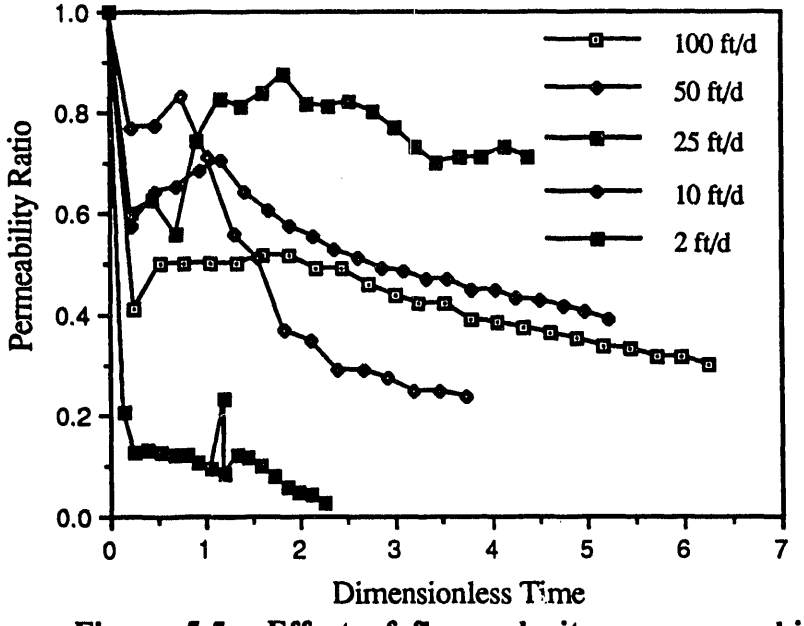

Figure 5.5. Effect of flow velocity on permeability ratio history of the upstream section (injected conc.~10^8 cfu/ml).

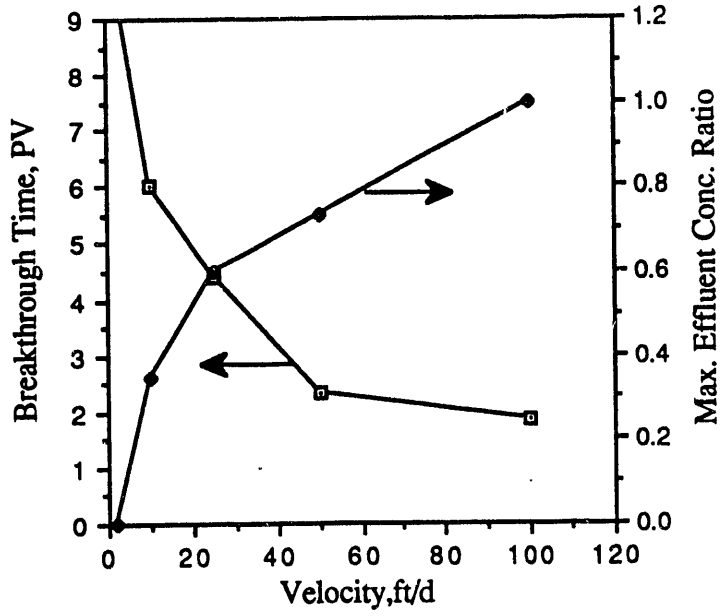

Figure 5.6. Effect of velocity on breakthrough time and maximum effluent bacteria concentration.

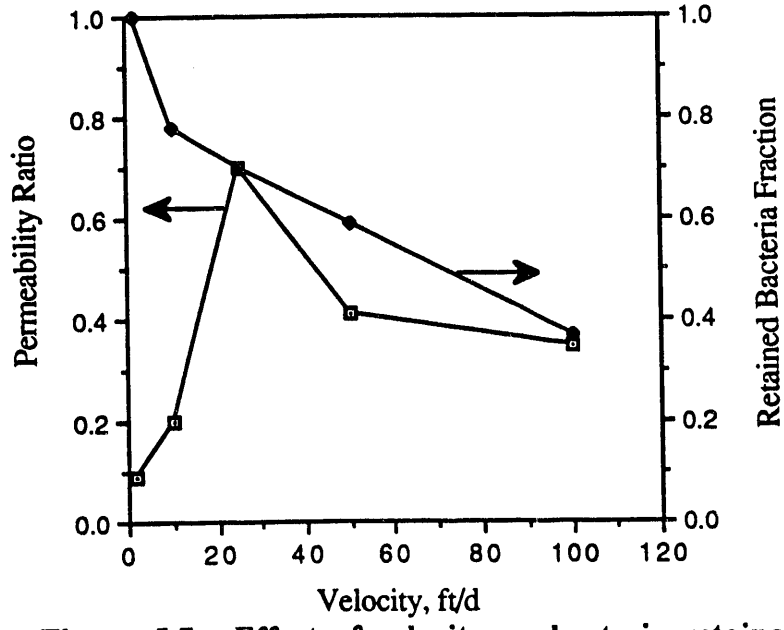

Figure 5.7. Effect of velocity on bacteria retained fraction and permeability ratio of the upstream section.

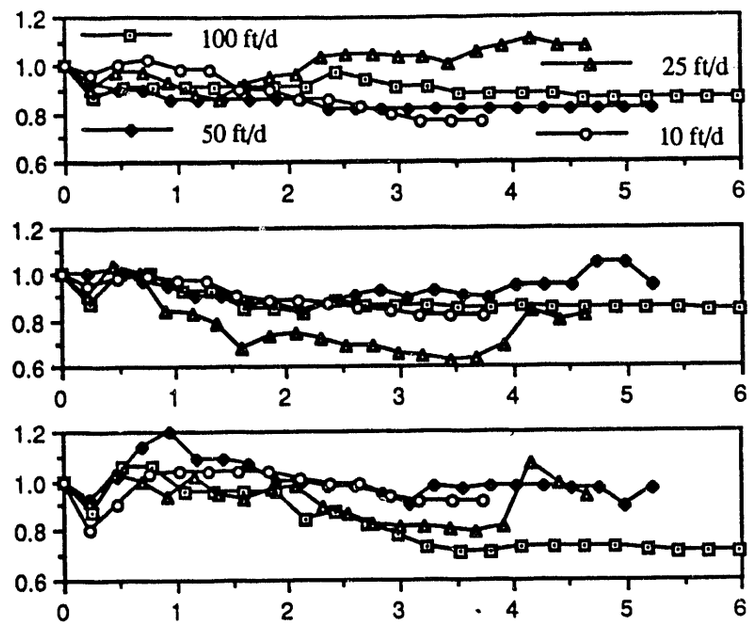

Figure 8. Effect of flow velocity on permeability ratio history of the 2nd, 3rd,and 4th sections of the medium (injected concentration ~10^8 cfu/ml)

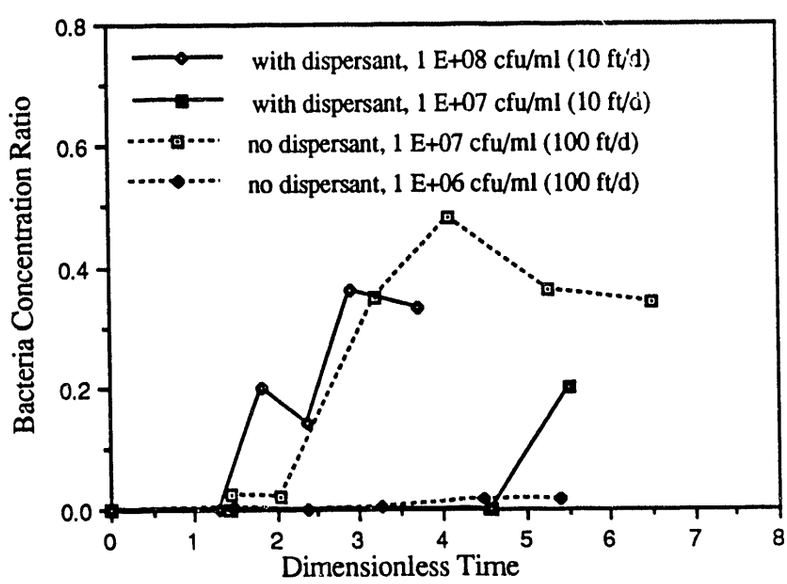

Figure 5.9. Effect of injected concentration on effluent bacteria concentration ratio history in the presence and absence of a dispersant.

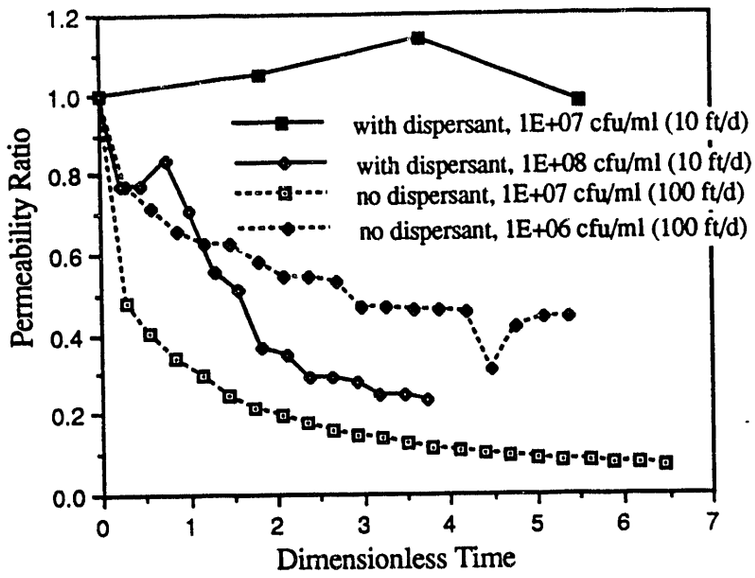

Figure 5.10. Effect of injected concentration on permeability ratio history in the presence or absence of a dispersant.

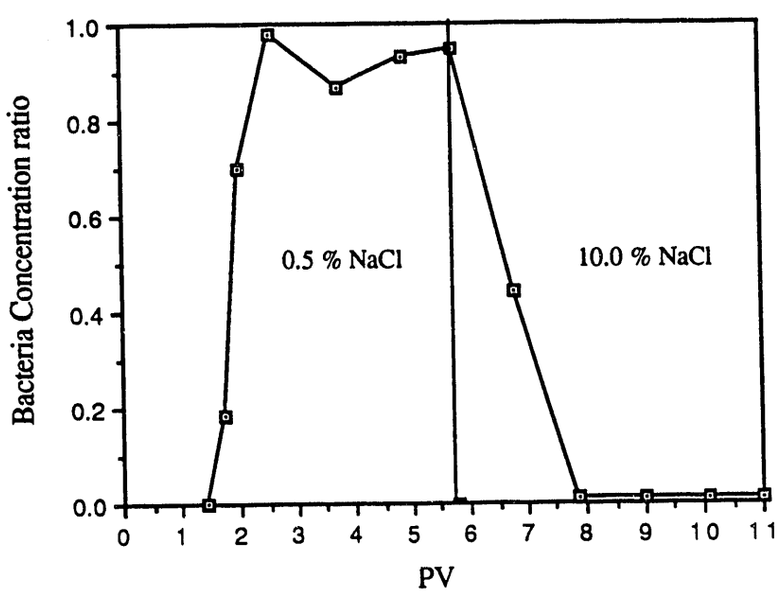

Figure 5.11. Effect of salinity in the injected suspension on effluent bacteria concentration history.

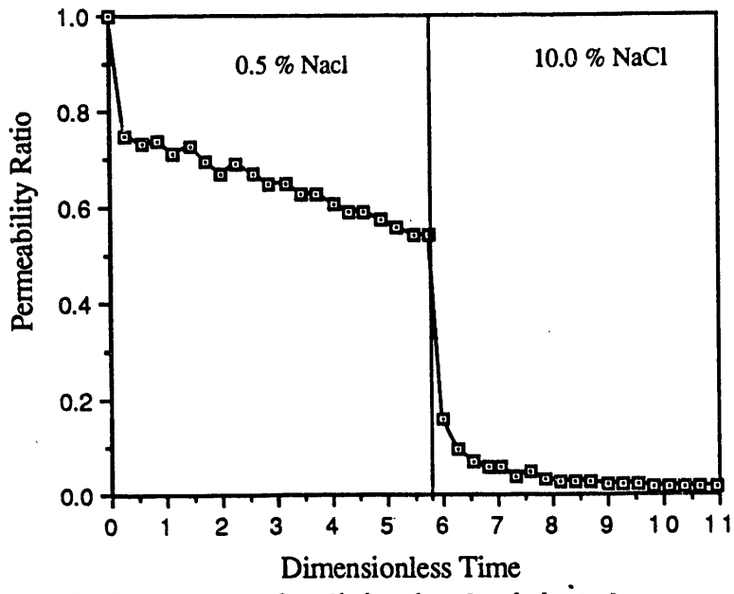

Figure 5.12. Effect of salinity in the injected suspension on permeability ratio history.

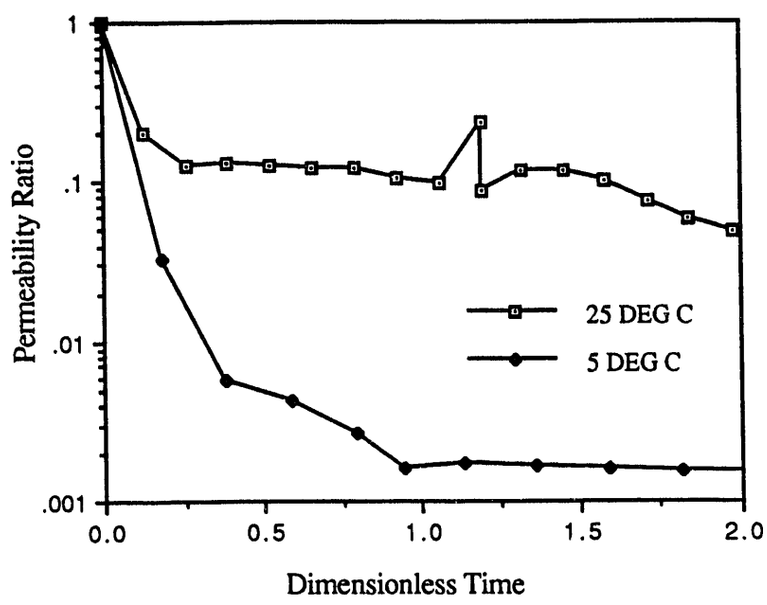

Figure 5.13. Effect of temperature of the injected suspension on permeability ratio history.

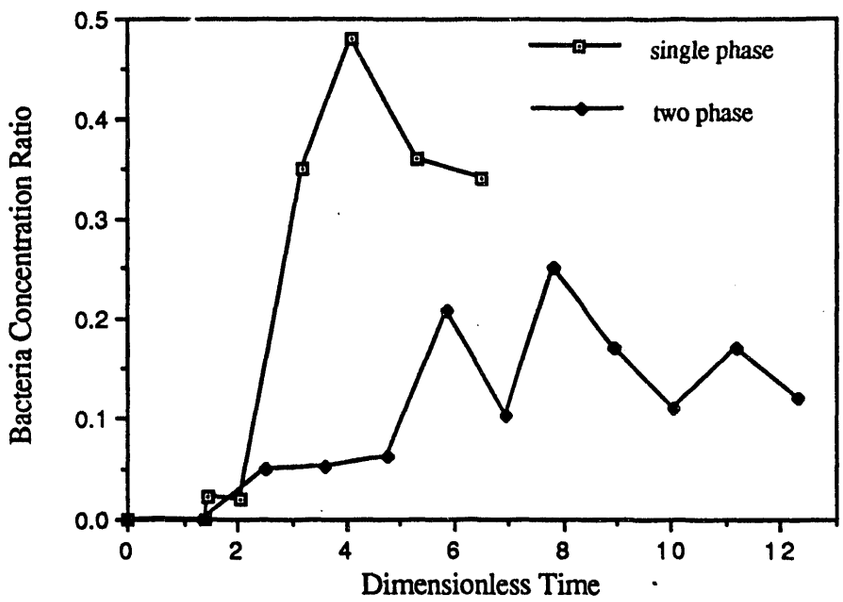

Figure 5.14. Effect of the presence of a residual oil phase on effluent bacteria concentration history.

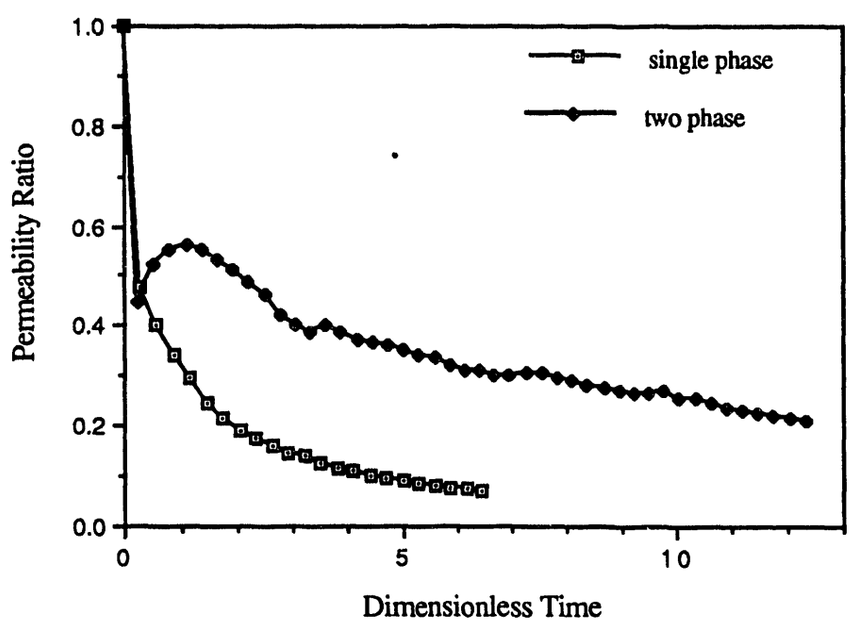

Figure 5.15. Effect of the presence of a residual oil phase on permeability ratio history.

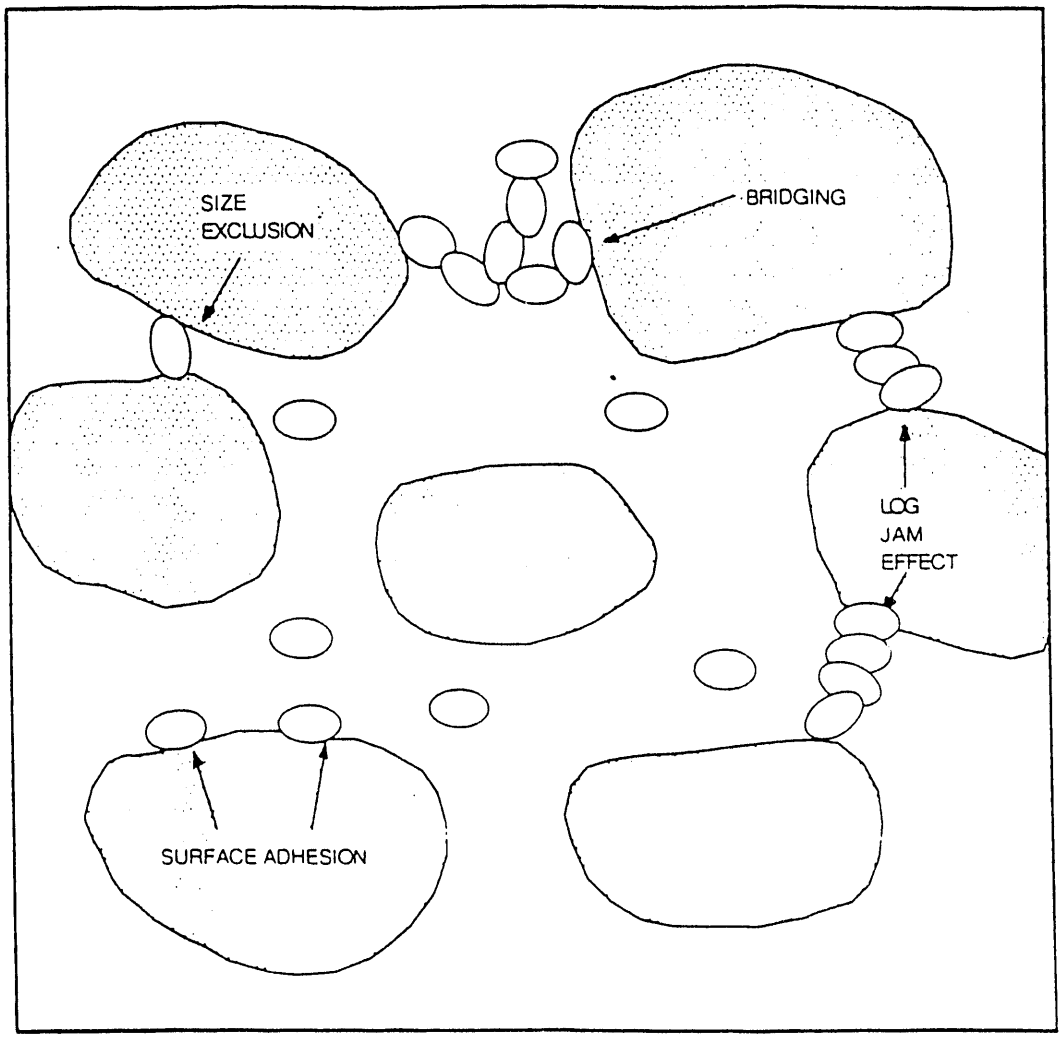

Figure 5.16 A schematic showing the four bacteria retention mechanisms proposed.

Chapter 6: Compositional Numerical Simulation of MEOR Processes

ABSTRACT

The retention and transport of bacteria in porous media has been modelled using a fractional flow approach. The various mechanisms of bacteria retention can be incorporated into the model through selection of an appropriate shape of the fractional flow curve. Reduction of permeability due to pore plugging by bacteria has been modelled using the effective medium theory. The rates of transport and growth of bacteria, the generation of metabolic products, and consumption of nutrients are strongly coupled. The governing conservation equations form a set of coupled, non-linear partial differential equations that are solved numerically. The model can be used to predict the in - situ transport and growth of bacteria, nutrient consumption, and metabolite production. It will be particularly useful in simulating laboratory experiments and in scaling bacterial processes to the field. The reasonably good agreement between the model and experimental data indicates that the physical processes incorporated in our model are adequate. It also points to the need to determine the transport and growth parameters from in-situ measurements.

6.1 INTRODUCTION

In Microbial Enhanced Oil Recovery (MEOR) processes bacteria and nutrient are injected into a porous medium containing residual oil. The microbes consume nutrient and produce biomass and various metabolic products such as gases, surfactant, acids, alcohols, and polymers. Some of these metabolic products or the biomass, help to mobilize residual oil. It is important to understand the potential mechanisms of MEOR processes before a mathematical model can be developed. The transport of bacteria, and other components such as nutrients, and metabolic products are important issues. Numerical simulation of this highly complex and coupled process is necessary to predict oil recovery and to investigate sensitivity towards different process variations and physico-chemical parameters. Using the oil recovery rates an economic feasibility study can be conducted.

Transport and growth of bacteria in porous media are essential to the implementation of bacterial/biochemical processes such as microbial enhanced oil recovery (MEOR) and bioremediation of contaminants in groundwater. The functioning of specific mechanisms in oil recovery or bioremediation are obviously related to the in - situ transport

and growth processes. The objective of this work is to develop a model for the transport and growth of bacteria in porous media that may be used for the analysis of laboratory results and for scaling these results to the field.

Experiments presented in the chapter have shown that the flow velocity, injected concentration, presence of residual phases, and the state of dispersion of the cell suspension (which is controlled by the concentration of dispersant, ionic strength, and temperature) are the major factors affecting the transport of bacteria in porous media. The ratio of the cell size to average pore throat size, shape of bacteria, and the presence of extracellular polymers on the bacteria surface are other important factors (Kalish et al., 1964 and Shaw et al., 1985). The retention of bacteria and permeability reduction was observed to be significantly higher in the first few centimeters (~2.5 cm) of the porous medium as compared to the downstream sections. The transport of pathogenic bacteria through soil may involve other factors such as the type and amount of clay present. Size exclusion, surface adhesion, bridging, and hydrodynamic exclusion are considered the major mechanisms for the retention of bacteria and reduction of permeability (Sarkar et al., 1992). In - situ cell growth depends on both kinetic expressions inferred from fermentation studies as well as cell metabolism in a nutrient limited environment. A model for transport and growth of bacteria, nutrients and metabolic products in porous media must include the above mechanisms to appropriately represent this complicated process of bacteria capture, flow and growth.

Transport of bacteria has been modelled as a rate process, using expressions for rates of clogging and declogging or rates deposition and release that do not account for the various mechanisms listed above (Corapcioglu et al., 1985; Jang et al., 1982).

In a review of problems with using existing transport models to describe microbial transport in porous media, Brown (1986) pointed out the need for treating microorganisms as colloids rather than as molecular species in solution. The lack of sufficient data to validate the existing models is also mentioned. Appropriate bioremediation/biorestoration mechanisms or oil recovery mechanisms (Sarkar et al., 1990) need to be included in the model for simulating a particular bacterial process.

The model, described here, has been used for simulating laboratory experiments on the transport and growth of bacteria in sandpacks. Experiments showing the effect of velocity, injected concentration, and presence of two phases on transport of bacteria have been simulated and the results on effluent bacteria concentration histories and permeability histories are compared with experimental results. Reasonable agreement is obtained by adjusting the transport and effective radius parameters. Growth of bacteria in the sandpack was simulated and the results on effluent glucose concentration histories and permeability

profiles after static growth are successfully matched with the experimental results by adjusting the growth parameters.

6.2 OIL RECOVERY MECHANISMS IN MEOR

A better understanding of the mechanisms of oil recovery is extremely useful for selection of appropriate bacterial strains, design of optimal operational procedures and for mathematically modelling the process to predict laboratory and field results. The net oil recovery (E_r)is expressed as the product of the volumetric sweep efficiency (E_v) and the displacement efficiency (E_d),

$$E_r = E_d * E_v$$

In a heterogeneous reservoir, the by-passed residual oil may be recovered by improving the volumetric sweep efficiency by decreasing the mobility ratio, but to recover true residual oil (oil contacted by displacing agent but not displaced) the capillary number must be increased.

Several mechanisms, utilizing these metabolic products, have been proposed for MEOR processes. In this section a order-of-magnitude analysis of some of these mechanisms is presented.

6.2.1 Effect of gases

Different gases produced during a microbial growth process are CO_2 , CH_4 , H_2 and N_2 . These gases may increase reservoir pressure, improve solution gas or gas cap drive mechanism, miscibly displace oil, or swell the oil phase and reduce the viscosity of the oleic phase.

From our gas build up experiments in a Hungate tube it was found that 6.0 SCF/BBL PV (46 mM) of CO_2 was produced (Sarkar, 1990).

$$\frac{CO_2 \text{ concentration}}{\text{Glucose consumed}} = 1.53 * 10^{-3} \text{ gm mole } CO_2 \text{ / gm glucose}$$

$$\frac{CO_2 \text{ concentration}}{\text{cell produced}} = 3.891 * 10^{-3} \text{ gm mole} CO_2 \text{ /gm cell mass}$$

For a 1 % glucose solution the theoretical limit for gas production is 300 mM or 39 SCF/BBL. But, at 100° F temperature and 1000 psi pressure the gas solubility is 150 SCF CO2 /BBL water (Lake, 1989, Fig.7.23). The huge amount of nutrient that will be required for a significant increase in CO_2 production will make the process uneconomical. From the plot of viscosity ratio of the gas oil mixture to oil as a function of saturation pressure (Lake 1989, Fig.7-21) viscosity reduction can be evaluated to be negligible. From the plot of oil swelling factor as a function of mole fraction of CO_2 in oil (Lake, 1989, Fig.7.22) it can be concluded that this concentration is too low to cause any oil

swelling. In laboratory studies conducted with no back-pressure, the produced gas may create a trapped gas saturation that reduces the residual oil saturation (Kyte et. al, 1956, Fig. 4,5). But, this explanation can not be extended to the field.

Under anaerobic conditions, typical high-CO_2-producing microorganisms (for example *Zymomonas mobilis*) form about 1 mole of CO_2 for every 12.5 grams of cells (wet weight) formed (Gottschalk, 1983). A simple calculation reveals that a large amount of cell mass (approximately 6 % biomass wet weight per unit weight of oil) would have to be generated in order to produce the amount of CO_2 needed for satisfactory oil recovery. Among other things, the generation of such high concentrations of biomass is very likely to cause serious plugging problems. Similarly, Grist (1983) conducted a simple economic analysis and concluded that *in-situ* production of biogas is not economical because of the large amount of nutrients that must be injected.

It has been reported by several authors that the gas produced during microbial growth process helps in the drive mechanism for oil recovery. From a overall material balance (Dake, 1978) two individual drive mechanisms (a) solution Gas Drive and (b) gas cap drive may be analyzed separately. It can be clearly demonstrated that the volume of gas that can be generated by bacteria is insufficient to contribute to either of these mechanisms. It is conceivable, however, that small quantities of gas may buildup localized pressure and substantial gas saturation in the near wellbore region (1 to 2 ft.). But, it is unclear how this will improve oil recovery.

6.2.2 Effect of acids

Various acids produced during microbial growth include acetic acid, formic acid, lactic acid etc. These acids may cause rock dissolution and improve the permeability of the reservoir. From our experimental study in batch fermentation the maximum concentration of acetic acid is 5 g/l (83 mM). The theoretical limit of acid production is 9 g/l (3*50 mM). Very low pH or high acid concentrations can not be obtained because it will inhibit cell growth.

The acid component can react only with limestone and dolomite and not with clay or silica. Acetic acid has a low dissociation constant and 50 % acid remains unconverted (unreacted). At 0.5% acid concentration the dissolving power of acid (volume of rock dissolved/volume of acid reacted) is 0.0016 for limestone and 0.0014 for dolomite (Williams, 1979, Fig. 8.1). The reaction products of calcium acetate and magnesium acetate are soluble in spent acid. From the plot of permeability ratio (final to initial permeability) as a function of time, a properly designed mud acid gives a permeability ratio improvement of 4. So, the permeability improvement from acetic acid alone (without HF

acid) will be much less and from the definition of capillary number this low improvement can not cause any order of magnitude increase in capillary number. Grist (1983) also concluded that the production of organic acids is probably not a primary mechanism for enhancing the flow of oil. Since, the permeability (k) improvement is very small microbial acid stimulation is very unlikely to improve productivity.

6.2.3 Effect of alcohol

Microbially produced alcohols may act as a solvent to cause oil swelling, reduce oil viscosity, lower IFT in combination with a biosurfactant, act as a cosurfactant to reduce salinity sensitivity of the biosurfactant, or displace oil in a miscible manner.

From our anaerobic experiments the maximum concentration of butanediol during anaerobic fermentation is 3 g/l (33 mM). The theoretical limit for butanediol production is 7 g/l. Butanediol is highly soluble in both water and oil. Since, the amount of alcohol produced is very little, it can not cause any swelling of oil or reduction of oil viscosity, but it may be a potential agent for near wellbore stimulation.

The water solubility of surfactants commonly used in conventional surfactant floods increases with an increase in the concentration of low molecular cosurfactant (n-butanol) and decreases with an increase in high molecular weight cosurfactant. Cosurfactants generally increase optimal IFT, but it has been reported that addition of n-pentanol to a biosurfactant produced by an aerobic species H-13 significantly lowered IFT from 10^{-2} to 10^{-5} mN/m (Brown et al., 1986). This indicates that a particular surfactant and alcohol system must be studied to determine the final IFT of the system against an oil.

6.2.4 Effect of biopolymers

The sweep efficiency of the displacement process may be improved by an increase in the viscosity of the displacing phase or by the biomass selectively plugging high permeability zones.

It is known that good mobility control is achieved when the displacing fluid has a viscosity between 10 to 50 cp at a shear rate of 1 sec^{-1}. It has been reported (Knapp et al., 1987) that a biopolymer, which can be grown anaerobically at 50°C temperature and at a salinity of 8 %, has the potential of increasing culture viscosity to 4 cp at a shear rate of 150 sec^{-1}. Also some of the strains along with extracellular biopolymers are good plugging agents and can reduce permeability by 70 % or more. The selective reduction of reservoir permeability and increase in the viscosity of the displacing fluid can improve displacement efficiency and mobilize residual oil from a heterogeneous reservoir.

6.2.5 Effect of biosurfactants

Biosurfactants produced during exponential or stationary phase of bacterial growth may reduce interfacial tension (IFT) between oil and water, and increase capillary number to cause a decrease in the residual oil saturation. Isolation and characterization of the biosurfactants produced by *Bacillus licheniformis* JF-2 is a subject of current research. The lowest IFT of the fermentation broth has been found to be 0.03 mN/M. Assuming a surfactant molecule containing 12 carbon atoms and having a molecular weight of 300, the theoretical limit for surfactant production is 25 mN/M (7.5 g/l or 7500 ppm). For a good quality surfactant 1/25 th of that amount may be sufficient for lowering the IFT of the medium. Since only small quantities of surfactant are required to achieve a high capillary number, this is the most promising agent for MEOR.

It has been estimated (Lake, 1989) that the interfacial tension must be lowered to 10^{-2} to 10^{-3} mN/m (corresponding N_{ca} of 10^{-3} to 10^{-4}) before significant oil recovery is achieved. Biosurfactants, produced anaerobically, which are capable of reducing the IFT to such a low value have been reported (Brown et al., 1986). Production of the surfactant at the location where it is most needed should minimize losses due to adsorption on the rock.

6.2.6 Effect of Wettability Alteration

Microbes growing on rock surfaces may cause wettability alteration, which may in turn mobilize oil if the change is toward more water wetness. It has been shown (Kianipey et al., 1986), using a flow visualization cell, that the medium became more water wet and oil became emulsified (4 to 80 μm size) as a result of biosurfactants, gas and other metabolites. In case of JF-2 (mainly a biosurfactant producer) wettability alteration was more prominent in the case of an initially oil wet medium and the oil recovery was slightly higher compared to a water wet medium. In case of *Clostridium* (mainly a CO_2 producer), although the wettability change was of the same magnitude but the oil recovery actually went down (16 % to 12 %). From these results one might conclude that the IFT reduction is more important and the effect of wettability alteration is as yet unclear. The exact mechanism of wettability alteration is an area of further research.

6.3 MODEL FORMULATION

The basic equations governing the transport of oil, water, bacteria, nutrients, and metabolites in MEOR processes are species mass conservation equations. In our formulation (n_c -1) component mass balance equations and one overall mass conservation

equation are used. In addition to this, specific models for bacteria transport, growth and permeability reduction need to be developed. Constitutive relations for rock properties, as well as initial and boundary conditions have to be specified to complete the mathematical description.

Some assumptions need to be made in order to make the analysis tractable:

(i) The presence of only two incompressible fluid phases is considered.

(ii) Precipitation/dissolution, cation exchange, or other chemical reactions are not considered.

(iii) Bacteria, nutrient, and metabolites do not partition into the oleic phase.

(iv) Pressure, volume, and temperature changes from chemical reactions, bacteria growth, and mixing are negligibly small.

6.3.1 Material Conservation Equations

The differential form of the material balance equations for isothermal, multicomponent, multiphase transport of chemical/biochemical species in permeable media can be written as (Lake, 1989)

$$\frac{\partial W_k}{\partial t} = - \nabla . N_k + R_k \qquad (1)$$

where W_k , N_k and R_k are the overall concentration, net flux, and source terms respectively of a component k.

The overall concentration of component k (W_k) may be expressed as

$$W_k = \varnothing (1 - \sum_{k=1}^{n_C} \hat{C}_k) \sum_{l=1}^{n_P} (\rho_k C_{kl} S_l) + \varnothing \rho_k \hat{C}_k \qquad (2)$$

where n_C is the total number of components and n_P is the total number of phases. C_{kl} refers to the volumetric concentration of component k in phase l, and $\hat{C}_k$ refers to the volumetric concentration of component k adsorbed on the rock surfaces based on the original pore volume. $\varnothing$ is the porosity of the medium, S_l is the saturation of phase l, and ρ_k denotes the density of component k.

The flux term (N_k) consists of convective, dispersive, and coupled dispersive fluxes. It may be expressed as

$$N_k = \sum_{l=1}^{n_P} \{ (u_l \ \rho_k C_{kl}) - \phi S_l (D_{kl} \cdot \nabla(\rho_k C_{kl}) + D'_{kl} \cdot \nabla(\rho_{k'} C_{k'l}))\} \qquad (3)$$

where D_{kl} and D'_{kl} are hydrodynamic and coupled hydrodynamic dispersion coefficients respectively. Subscript k' refers to the indices for the component with which component k is coupled. For example, in MEOR processes there are diffusive fluxes in addition to the molecular diffusive flux. Bacteria have a chemotactic diffusive flux driven by a nutrient concentration gradient (from a lower nutrient concentration towards a higher nutrient concentration) and a tumbling diffusive flux driven by the bacteria concentration itself (from a higher concentration of bacteria towards a lower concentration of bacteria).

The source terms are a combination of all rate terms for a particular component, e. g., injection, production, growth, and lysis (of bacteria) and may be expressed as

$$R_k = \sum_{l=1}^{n_P} \phi \ S_l (R_{k\,l} \ \rho_k C_{k\,l} + R'_{k\,l} \rho_{k'} C_{k'\,l}) + \frac{Q_k \ \rho_k}{V_b} \qquad (4)$$

where Q_k indicates rate of injection or production and V_b is the bulk volume. R_{kl}, R'_{kl} are the specific rate constant and the coupled specific rate constant of component k in phase one. First-order rate expressions have been used here for reaction rate terms. For example, in the case of nutrient, the coupled rate coefficient indicates that the rate of consumption is dependent upon the concentration of live bacteria and the concentration of inhibitory metabolite and in the case of metabolites, the rate of generation depends on the concentration of bacteria and the nutrient. These specific rate terms are discussed in detail later.

If the component density (ρ_k) is assumed to be constant then the final form of the material balance equation obtained is,

$$\frac{\partial}{\partial x} \sum_{l=1}^{n_P} u_l = \sum_{l=1}^{n_P} \phi S_l \{ \sum_{k=1}^{n_c} (R_{kl} C_{kl} + R'_{kl} \frac{\rho_{k'}}{\rho_k} C_{k'l} \} + \sum_{k=1}^{n_c} \frac{Q_k}{V_b} \qquad (5)$$

The continuity equation describing the conservation of total mass can be obtained by summing the above material conservation equations over all components. Combining the continuity equation, Darcy's law and the capillary pressure relation, and by neglecting gravity, the final form of the pressure equation in terms of the aqueous phase pressure (P1), can be obtained as (Sarkar, 1992).

$$-\frac{\partial}{\partial x}\left\{ K\lambda_{r1}\frac{\partial}{\partial x}(P_1)\right\} = \sum_{l=2}^{n_p}\frac{\partial}{\partial x}\left\{K\lambda_{rl}\frac{\partial}{\partial x}(P_{c\,l1})\right\} + \sum_{k=1}^{n_c}\frac{Q_k}{V_b}$$

$$+ \sum_{l=1}^{n_p} \phi S_l\left(\sum_{k=1}^{n_c}(R_{kl}C_{kl}+R'_{kl}\frac{\rho_{k'}}{\rho_k}C_{k'l}\right) \qquad (6)$$

Details of this derivation are provided by Sarkar (1992). Here K is the single phase permeability and λ_{rl} is the relative mobility of phase l. Other terms are defined in the nomenclature.

6.3.2 Derivation of Specific Rate Terms

The kinetics of bacteria growth, consumption of nutrient and generation of metabolites needs to be specified in the material conservation equations. For bacteria, the specific rate constant (Rbl), considering growth and lysis processes, can be written as (Bailey and Ollis, 1986)

$$R_{kl} = R_{bl} = \mu - K_d, \qquad (7)$$

where the specific growth rate (μ) can be approximated by the Contois model as (Goursaud, 1989)

$$\mu = \frac{\mu_{max}}{1+B\frac{C_{bl}}{C_n}} \qquad (8)$$

where C_{bl} and C_n are concentrations of bacteria and nutrient, respectively. μ_{max} and B are empirical constants that need to be determined from fermentation studies. For the species used in our transport experiments, *Bacillus licheniformis* JF-2, extensive studies on fermentor kinetics were conducted in our lab (Goursaud, 1989).

This specific growth rate is modified by inhibitory metabolites such as alcohols or acids, which become important towards the end of the growth process when the concentration of inhibitors become significant (Aiba, 1968).

$$\mu = \frac{\mu_{max}}{1+B\frac{C_{bl}}{C_n}} * \frac{k_i}{k_i+C_i} \qquad (9)$$

where k_i is an experimentally determined constant and C_i is the concentration of inhibitory metabolite. The specific lysis rate for most bacteria under limited nutrient conditions may be considered a constant, K_d=0.01 hr^{-1} (Corapcioglu, 1985). In the case of limiting nutrient (in our case, glucose), the specific rate constant (R'_n) for nutrient consumption can be written as

$$R'_{kl} = R'_n = A_n\,\mu + B_n \qquad (10)$$

In popular terminology,

$$A_n = \frac{1}{Y_B} \quad \text{and} \quad B_n = m, \tag{11}$$

where Y_B and m are the yield and maintenance coefficients of bacteria respectively.

The generation of metabolic products has been traditionally modeled using the Leudeking-Piret equation (Bailey and Ollis, 1986; Georgiou and Shuler, 1986) and the specific rate constant for metabolite production can be written in general as

$$R'_{kl} = A_k \mu + B_k \tag{12}$$

A_k and B_k are the yield (generation) parameters of a metabolic product with respect to growth and maintenance of the bacterial population respectively.

The above expressions provide a framework within which the growth kinetics of any specific strain growth measured in the laboratory can be incorporated for use with our model. All kinetic parameters were measured experimentally through fermentations conducted in our laboratories (Goursaud, 1989).

6.3.3. Transport and Retention of Bacteria

The modeling of bacteria retention by the porous medium is crucial to the simulation of bacteria transport processes. There are several approaches that have been attempted in the past: (i) Empirical filtration coefficient models; (ii) Trajectory analysis (Paytakes, et al., 1973) and (iii) Network models (Sharma and Yortsos, 1987). In general, it would be desirable to simulate this process as a transient one where the retention of bacteria results in a change in the pore structure and local flow field resulting in a continuously changing (in time and space coordinates) local filtration coefficient. This is, however, tedious and in most cases impractical especially when coupled with the problems of cell growth and metabolism.

The approach chosen here is unique. A fractional flow function is defined that relates the local flowing cell concentration to the total (flowing + entrapped) cell concentration. Local equilibrium is assumed. This implies that the rate of cell capture is much larger than the rate of convection. This is a reasonable assumption (Sharma and Yortsos, 1987) and it provides us with a tremendous savings in mathematical and computational complexity in describing cell transport and capture. The shape of the fractional flow curve will in general depend on the mechanisms of bacteria retention. As an example, a particular form of the fractional flow function can be derived for cases in which size exclusion is the only retention mechanism i.e. for the flow of dilute suspensions when the particle size is comparable to the pore throat size. For this case of retention by size

exclusion, a straight line fraction flow function intersecting the x axis at C^* (Figure 6.1) is appropriate. This shape of curve implies that all bacteria are retained at total concentrations less than C^*. This is so because pores smaller than the bacteria size are still available to retain them. All cells in excess of C^* flow because no more small pores are available to entrap them. This is, of course, an overly simplistic example model for cell retention. In general, cells will partially plug pores that are both smaller and larger than the cell size and they will continue to do so until bridging causes an internal or external filter cake to form. In such situations, the fractional flow function will deviate from the above behavior (Figure 6.1). The more the extent of surface adhesion and bridging the lower the flowing concentrations will be. Eventually, the total bacteria concentration will be so high as to cause the flowing concentration to go to zero (Figure 6.1) i.e. complete trapping occurs. Similar non - linear effects are observed in traffic flow theory (Herman, 1966) where the flux of automobiles increases and then decreases as the traffic density increases. A log - jam is said to occur. The log - jam or multiparticle hydrodynamic exclusion effect proposed by Vitthal and Sharma (1991) can be seen to occur in Stokesian dynamics simulations when several particles approach a pore opening simultaneously.

A simple fractional flow curve was chosen to simulate some of the experiments and illustrate the use of the method. The equation used is,

$$C_{fD} = \frac{A\, C_{TD}}{1 + B\, C_{TD}} \tag{13}$$

where

$$C_{fD} = \frac{C_f}{C_T}$$

$$C_{TD} = \frac{C_T - C^*}{C_T}$$

The trapped bacteria concentration ($\hat{C}_k$) is

$$\hat{C}_k = C_T - C_f$$

C_{fD} and C_{TD} are dimensionless, flowing, and total concentrations of bacteria, C_f is the flowing bacteria concentration and C_T is the total bacteria concentration. The retention parameters A, B and C* need to be determined experimentally.

It is important to note that the use of a fractional flow function is extremely useful and convenient. It allows us to get away from the problem of writing conservation equations for trapped and flowing bacteria in a manner analogous to Sharma and Yortsos (1987). Such equations are extremely complex and difficult to solve and would make the problem of bacteria transport and growth intractable.

The transport of bacteria is very sensitive to the size of the bacteria in suspension. The shape of the fractional flow curve will depend on the 'effective' bacteria size in suspension. There are two approaches that can be adopted for obtaining the effective coagulated size of the cells. The state of coagulation of the suspension can be obtained by computing the potential energy of interaction between the individual bacterium and hence the collision efficiencies. This is an involved computation. A simpler more empirical approach is used instead where the bacteria floc size is empirically assumed to depend on the suspended bacteria concentration and the flow velocity. The effective radius of bacteria in the flowing suspension (r_s) is represented as a function size of a single cell (r_{si}), using an empirical relation of the form,

$$r_{sD} = F * \frac{C_D{}^e}{V^f} \tag{14}$$

where

$$r_{sD} = r_s/r_{si} \tag{15}$$

$$C_D = \log(C_F/C_{CC}) \tag{16}$$

where C_{CC} is the critical coagulation concentration, V is the fluid velocity, e, f, and F are an empirical constant, and e and f are the effective radius parameters. The critical coagulation concentration is expected to be a function of ionic strength, temperature, and bacteria type. The empiricism introduced through the relations above is far from ideal and a more exact relationship based on the potential energy of coagulation needs to be developed in the future.

6.3.4 Permeability Reduction Model

The permeable medium is represented as a three-dimensional network of pore throats and pore bodies (Sharma and Yortsos, 1987). As bacteria plug off flow channels, the conductance distribution, i.e. the pore size distribution of the network, changes. The effective medium theory (EMT) can be used to estimate the permeability reduction. EMT provides an approximate method for calculating the effective hydraulic conductance (g_m) if the conductance distribution [f(g)] i.e. the pore throat size distribution in a network is known.

$$\int_0^\infty \frac{g - g_m}{g + \alpha g_m} f(g)\, dg = 0$$

where $\alpha = \frac{z}{2} - 1$

g is the individual bond (pore throat) conductance and z is the coordination number of the network, i.e. the number of pore throats that join at each interior pore body. The conductance (g) may be expressed as a function of pore throat radius (r) as

$$g \alpha r^n \qquad 3 \leq n \leq 4 \tag{17}$$

The above equations can be combined to obtain (Sarkar, 1992)

$$g_m = \frac{\int_0^\infty \frac{r^n f(r)}{r^n + \alpha g_m} dr}{\int_0^\infty \frac{f(r)}{r^n + \alpha g_m} dr} \tag{18}$$

where f(r) is the size distribution of open pores.

To illustrate the model a Rayleigh pore size distribution was chosen.

$$f(r) = 2\alpha\, r\, e^{-\alpha r^2} \qquad r \geq 0, \tag{19}$$

where α is a parameter in the distribution and the mean (μ) is represented as

$$\mu = \frac{1}{2}\sqrt{\frac{\Pi}{\alpha}} \tag{20}$$

The ratio of the permeability of the damaged porous medium (k_D) to the initial medium (k_i) can be expressed as a function of the ratio of the corresponding mean conductances.

$$\text{Permeability ratio} = \frac{k_D}{k_i} = \frac{g_{mD}}{g_{mi}} \tag{21}$$

where g_{mi} and g_{mD} are the mean conductances for an undamaged and a damaged medium respectively.

6.3.5 Constitutive Relations and Phase Behavior

Modelling the petrophysical properties of the rock and the fluid phase behavior is a very important aspect of this model. In addition to the constitutive relations constraints are placed on the saturations, phase concentrations and total concentrations (to ensure that they sum to one). The approach taken here is similar to that taken in the reservoir simulator UTCHEM (Bhuyan, 1989) and the interested reader is referred to this publication for further details.

We have taken a simple approach to phase behavior by assuming that the oleic phase consists of only the oil component, and that the bacteria, the nutrient, and the produced metabolites remain in the aqueous phase. This is a good assumption. The phase saturation may be expressed as

$$S_o = C_o \quad ; \qquad S_w = 1 - S_o \tag{22}$$

The phase concentrations of aqueous phase components may be expressed as

$$C_{kw} = \frac{C_k}{S_w} \tag{23}$$

6.3.6 Initial and Boundary Conditions

It is necessary to specify initial values for (n_c -1) overall component concentrations for species conservation equations and an aqueous phase pressure value for the pressure equation. The boundary conditions for no flow, inflow, and outflow boundaries have been treated as described earlier by Datta Gupta (1985) and Lake et al. (1984).

The set of equations developed above completely describes the multicomponent, multiphase, flow of bacteria and the associated components in porous media.

6.3.7 Numerical Solution Procedure

The mathematical model developed above is solved numerically using a finite difference technique. The finite difference formulation of the species conservation equation and overall material balance equation (pressure equation), treatment of initial and boundary conditions and handling of different rate terms are discussed in detail by Sarkar, 1992 and Pope et. al., 1984. The model problem has been solved for a one dimensional medium using a block centered type of grid. An IMPES (Implicit pressure and explicit saturation) technique is used to solve one pressure equation and (n_c-1) species conservation equations. A bandsolver has been used to solve the tri-diagonal pressure matrix.

The model can be used for simulating injection of different sizes and compositions of slugs in the presence of one or more phases. Profiles (concentration as a function of distance) and histories (concentration as a function of time) can be obtained for any component or phase.

6.4 RESULTS AND DISCUSSION

Up to 10 components in two phases have been considered in our simulations. The model can be easily adapted for additional components and phases. A sensitivity study of the model parameters was conducted. It was evident that the shape of the fractional flow curve was critical in determining the location and concentration of cells in - situ. The next most important factor was found to be the kinetic parameters. As discussed later, the measurement of kinetic parameters in - situ may be critical in adequately modelling bacterial processes in porous media. A sample run is presented in the next section.

Sample Run

Numerical results are obtained by running the simulator for the following conditions.

(i) Initial condition. Core (1-D system) at waterflood residual oil saturation.

(ii) Operations. The following operations are simulated.

(a) Injection of bacterial slug (2 PV)

(b) Injection of nutrient (2 PV)

(c) Static incubation (63 hrs)

(d) Brine postflush (2 PV)

In this experiment sufficient PVs of bacteria and nutrient slugs were injected such that concentration of these components were uniform throughout the length of the medium. The results are shown in Figures 6.2 through 6.6. The first three figures show the results for the static incubation period, and next 2 figures show the results for the brine postflush period.

Fig. 6.2 shows growth of bacteria and consumption of nutrient as a function of time during static incubation inside the porous medium, in the presence of oil and water phases. From this result one can predict when the nutrient will be completely consumed. The bacteria concentration increases exponentially in the beginning, but as the inhibitor concentration builds up the rate of growth decreases.

Fig. 6.3 shows the change in metabolite concentration as the incubation continues. The final metabolite concentration of acids and alcohols match the results from fermentation studies (Goursaud, 1990).

Fig. 6.4 shows changes in IFT as the surfactant concentration increases during incubation. When the surfactant concentration reaches the CMC the IFT does not change. It is possible to predict from this result the time when incubation can be stopped and displacement may be started to recover oil.

Fig. 6.5 shows oil recovery during the brine postflush. It also shows the change in capillary number in the effluent block as the flood continues. As the capillary number decreases oil recovery continues to decline and finally stops below a certain critical capillary number value. The number of PVs needed to obtain the maximum oil recovery can be predicted. Fig. 6.5 shows oleic and aqueous phase production rates as a function of PV injected.

6.4.1 Comparison with Experiments

To evaluate the ability of the model to simulate experimental results, a few laboratory data sets were chosen for which at least some of the model parameters had been measured. Experimental results reported by Sarkar et al. (1991), Sarkar (1992) and those reported by Stehmeier and Jack (1987), on the transport and growth of bacteria in sandpacks, were used. The simulated and experimental results on effluent bacteria concentration histories and permeability ratio histories of the upstream section are compared while simulating the transport experiments. The results on effluent glucose concentration histories and permeability ratio profiles after static growth are compared in simulating the growth experiments. To obtain a match between the results only the transport and growth parameters were adjusted. The parameter values chosen for the simulations are shown in Table 6.2.

6.4.2 Simulation of Convective Bacteria Transport

To compare our simulations with experiments, two experiments that represent the most efficient transport experiments conducted were selected. These experiments were conducted at flow velocities of 100 and 25 ft/day. In the '100 ft/day' experiment the bacteria retained fraction and the breakthrough time were minimum, and the effluent concentration approached the injected concentration by 3 PV. The '25 ft/day' experiment is interesting in the sense that the permeability reduction at the upstream end was minimum, although the bacteria retained fraction and the breakthrough time were higher than those in the '100 ft/day' experiment.

The effluent bacteria concentration histories are compared in Figure 6.7. For the '25 ft/day' experiment the agreement is good. In the '100 ft/day' experiment the agreement after some initial portion of the bacteria flood is good, although the breakthrough is not accurately predicted. The experimental data points show a very sharp increase in effluent concentration after the initial breakthrough at 1.5 PV. Moreover, they show some fluctuations in concentration values. The simulated results show a gradual increase in the effluent concentration.

The permeability ratio histories of the upstream section are compared in Figure 6.9. The overall match for both the cases is reasonably good. The experimental data points show a sudden decrease and then an increase in permeability during the initial stages of the bacteria flood. After the initial portion of the flood the permeability ratio decreases gradually. The simulated results show a gradual decrease in the initial stages and no change later on. It is conjectured that the decrease and increase in the permeability experimentally observed may be due to the breaking of particle bridges as the pressure gradient increases. This is not modeled in the simulator. Any pressure fluctuations observed will, therefore, be smoothed over in the numerical simulation.

Figure 6.10 shows comparisons of the simulated and experimental effluent bacteria concentration ratio histories for two experiments (in which the injected concentrations were varied by an order of magnitude (~10^7 to 10^8 cfu/ml). The simulated and experimental permeability ratio histories for the upstream section of these experiments are compared in Figure 6.11. For the low concentration experiment the simulated result shows no permeability reduction at all. The fluctuations observed in the experimental values may result from the formation and breaking of bridges at the pore throats. For the high concentration experiment the agreement is reasonable.

Figure 6.12 shows a comparison of the simulated and experimental effluent bacteria concentration ratio histories for the two-phase experiment in which a residual mineral oil phase was present during the bacteria flood. The agreement is good during the initial stages of the bacteria flood. Later as an internal cake begins to form at the upstream end the experimental effluent concentration is smaller than that predicted by the model.

The simulated and experimental permeability ratio histories of the upstream section for the experiment are compared in Figure 6.13. The overall agreement during the initial stages of the flood is reasonably good, but because of internal cake formation the permeability ratio decreases continuously while the simulated results show a constant permeability ratio.

Addition of electrolytes, dispersants, and changes in temperature affect the state of dispersion of the bacteria, which in turn determine the effective size of the bacteria. These three parameters will also affect the surface adhesion mechanism of retention. The effects of these parameters are not modelled explicitly but their influences are implied in the retention and effective radius parameters.

6.4.3 Simulation of Stehmeier and Jack's Experiments

Stehmeier and Jack (1987) conducted several single-phase bacteria transport experiments using sandpacks (~3 ft long). Run #20 was chosen because it was one of the

most efficient transport experiments they conducted. Run #22 was conducted under similar experimental conditions except for a smaller flow velocity. The flow velocities (87 and 35 ft/day) and the injected concentrations (~10^8 cfu/ml) for these two experiments were similar to the two experiments conducted in our lab. The values of the other parameters were very different, but as discussed earlier the flow velocity and the injected bacteria concentration are two of the most important parameters in bacteria transport experiments. The values for the different variables were obtained from the experimental conditions specified in their report (Stehmeier and Jack, 1987). The permeability reduction results are not available. Figure 6.13 shows a comparison between experimental and numerical results for bacteria effluent concentration histories for these experiments. The agreement is reasonably good in both the cases.

6.4.4 Simulation of Transport and Growth Experiments

The growth of bacteria in a porous medium is simulated for the '100 ft/day' experiment. In this experiment, cells were injected in the sandpack at a high rate and allowed to grow in - situ for a period of several days. After incubation, the cells and the glucose were displaced with brine and the effluent concentrations as well as the permeabilities of each section were monitored. This sequence of injections was numerically simulated. The simulation results from the transport part of the experiment are described in the previous section. Figure 6.14 shows a comparison of the simulated and experimental effluent glucose concentration ratio histories. A comparison of the simulated and experimental permeability ratio profiles after static growth is shown in Figure 15. The agreement is good in both the cases. The two ends show higher permeability reduction because the initial permeabilities at these locations were less than those for the middle sections.

It is important to note that to obtain good agreement with the experiments the kinetic growth parameters A_n and B_n were chosen to be significantly smaller than the values measured using fermentors. The same effect was repeatedly seen with simulation of other experiments as well. The difference can only be due to large differences in in-situ and ex-situ growth kinetics. This suggests that as far as possible in-situ growth and kinetic measurements need to be used when simulating growth and transport of cells in porous media. Additional experiments on different species of bacteria need to be conducted to see if similar effects are observed with other cells.

6.5 CONCLUSIONS

A fractional flow approach to modelling the transport of bacteria is used together with component conservation equations to obtain a model for the coupled growth and transport of bacteria, nutrients and metabolic products in porous media.

Reasonable agreement between our model and the experimental data indicates that the physical processes incorporated in our model are adequate while the overall trend in permeability reduction is simulated, the transient fluctuations in the permeability are not. This study also points to the need to determine the transport and growth parameters from in-situ experimental data.

Nomenclature

a	Minimum fraction of pore throats open to flow
A, B	Retention parameters in fraction flow equation. Eq. 13
A_n, B_n	Parameters in the specific rate constant eqn. 10
A_k, B_k	Parameters in the specific rate constant for metabolite production, Eq. 12
C^*	Threshold concentration below which all the particles will be trapped
C_{cc}	Critical coagulation concentration
C_f	Flowing particle concentration
$\hat{C}_k$	Trapped and adsorbed particle concentration
C_{kl}	Concentration of a component k in phase l
C_T	Total particle concentration
D'_{kl}	Coupled dispersion coefficient for a component k in phase l
D_{kl}	Dispersion coefficient for a component k in phase l
e, f	Effective radius parameters for concentration and velocity effects
$\tilde{f}(g)$	Conductance distribution for conductive pores
$f(r)$	Pore throat radius distribution function
$f_D(r)$	Damaged pore throat radius distribution function
F_r	Empirical constant in effective radius equation
g	Individual bond (pore throat) conductance
g_m	Effective/mean hydraulic conductance in a network
g_{mD}	Mean conductances for a damaged medium
g_{mi}	Mean conductances for an undamaged medium
k	Absolute permeability in the flow direction.
K_d	Specific death rate constant.
k_D	Permeability of the damaged medium
k_i	Permeability of the undamaged medium

k_{rl}	Relative permeability of phase l.
m	maintenance coefficient
N	Original number of pore throats per unit bulk volume
n	Exponent in equation 17
N_k	Net flux of component k(mass/(area.time)).
N_{kl}	Net flux of component k in phase l.
n_p	Number of phases.
P1	Pressure in aqueous phase.
$P_{cll'}$	Capillary pressure between phase l and l'.
P_l	Pressure in phase l.
Q_k	Rate of injection (+ve sign) or production (-ve sign) of component k.
R'_{kl}	Coupled specific rate constant for component k in phase l
R_{kl}	Specific rate constant for component k in phase l.
r	Pore throat radius
r_s	Effective radius of particles from bacterial growth process
r_{si}	Original bacteria size
S_l	Saturation of a phase l
t	Time.
V_b	Bulk Volume of sandpack
W_k	Overall mass concentration(mass/bulk volume)
y	Fraction of nonconductive pores
z	Coordination number of the network

Subscripts

i,j,k	Correspond to x, y, z of the coordinate system
k	Index for a component.
k'	Index for the component that is coupled with k
l	Index for a phase

Greek Symbols

∇	Gradient operator
μ_l	Viscosity of phase l
ρ_s	Number-averaged density of solid particles
ø	Porosity of the medium
μ_{max}	Maximum specific growth rate of the bacteria.

ρ_l Density of phase l.

μ_l Viscosity of phase l.

λ_{rl} Relative mobility of phase l. ($= K_{rl}/\mu l$)

λ_{rT} Total relative mobility.

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APPENDIX 6A

The initial undamaged mean conductance (g_{mi}) is written as

$$g_{mi} = \frac{\int_0^\infty \frac{r^n f(r)}{r^n + \alpha g_m} dr}{\int_0^\infty \frac{f(r)}{r^n + \alpha g_m} dr} \tag{A.1}$$

In the damaged permeable medium, the conductance distribution function [f(g)] consists of a fraction (y) of nonconductive pores and a fraction (1-y) of conductive pores.

$$f(g) = y\,\delta(g) + (1-y)\tilde{f}(g) \tag{A.2}$$

where $\tilde{f}(g)$ is the conductance distribution for conductive pores and is expressed as

$$\tilde{f}(g) = f_D(r)\left|\frac{dr}{dg}\right| \tag{A.3}$$

where $\delta(g) = 0$ for $g \neq 0$,

where $\delta(g)$ is the Dirac delta function and $f_D(r)$ is the damaged pore throat size distribution function. By definition, the area under pore throat distribution curve is the unit area. The damaged pore throat size distribution can be related to the initial size distribution as follows:

$$f_D(r) = \frac{1 - \sum_k \hat{C}_k}{1-y} f(r) \qquad r < rs \tag{A.4a}$$

$$f_D(r) = \frac{1}{1-y} f(r) \qquad r > rs \tag{A.4b}$$

Here, r_s is the bacteria size and a is the minimum fraction of pore throats open to flow.

$$a = \int_{r_s}^\infty f(r)\, dr \tag{A.5}$$

$\hat{C}_k$ is the trapped particle concentration and C^* is a threshold concentration below which all the particles will be trapped.

Let N be the original number of pore throats per unit bulk volume. It is assumed that a single cell completely plugs a pore smaller in size. If the total number of particles per

unit bulk volume is more than N^*, the particles in excess will flow. So, for particle flow, N^* forms a threshold concentration, which can be written in units of volume fraction as

$$C^* = \frac{(1-a)\, N^* \, \frac{4}{3} \pi \, r_s^3}{\varnothing} \tag{A.6}$$

The damaged mean conductance can be written as

$$g_{mD} = \frac{(1-y) \displaystyle\int_0^\infty \frac{r^n f_D(r)}{r^n + \alpha g_{mD}} \, dr}{\dfrac{y}{\alpha g_m} + (1-y) \displaystyle\int_0^\infty \frac{f_D(r)}{r^n + \alpha g_{mD}} \, dr} \tag{A.7}$$

Table 6.1. Dispersion coefficients and specific rate constants for MEOR components.

Component		Name	Dispersion Coefficient		Specific Rate Constant	
k	k'		D_{kl}	D'_{kl}	R_{kl}	R'_{kl}
1	*	Water	0.0	0.0	0.0	0.0
2	*	Oil	0.0	0.0	0.0	0.0
3	4	Nutrient	D_{3l}	0.0	0.0	$-(A_N*\mu+B_N)$
4	3	Bacteria	$(D_B+D_T+D_F)_{4l}$	$-D_{C4l}$	$(\mu-k_d)$	0.0
5	4	Polymer	D_{5l}	0.0	0.0	$A_P*\mu+B_P$
6	4	Surfactant	D_{6l}	0.0	0.0	$A_S*\mu+B_S$
7	4	Inhibitor	D_{7l}	0.0	0.0	$A_I*\mu+B_I$
8	4	Spore	D_{8l}	0.0	0.0	0.0
9	4	Alcohols	D_{9l}	0.0	0.0	$A_{CS}*\mu+B_{CS}$
10	4	Acid	D_{10l}	0.0	0.0	$A_A*\mu+B_A$

k, k'	Index for a component and coupled component
D_{kl}, D'_{kl}	Dispersion and coupled dispersion coeff. of component k and k' in phase l
R_{kl}, R'_{kl}	Specific and coupled specific rate constant of component k and k' in phase l
μ, k_d	Specific growth rate and death constant for bacteria
A, B	Metabolite generation/growth parameters
Subscripts: N, P, S, I, CS & A	Nutrient, Polymer, Surfactant, Inhibitor, Cosurfactant and Acid.

Table 6.2. List of transport and growth parameters and their values used in simulating laboratory experiments.

Expt. No.	A	B	e	f	Comment
3	3.0	10.0	1.08	0.285	Fig. Nos. 1, 2, 8, 9
5	0.4	1.0	1.50	0.0	Fig. Nos. 1, 2
6	21.0	80.0	1.08	0.69	Fig. Nos. 3, 4
8	10.0	80.0	1.08	0.16	Fig. Nos. 3, 4
12	1.0	10.0	1.08	0.25	Fig. Nos. 5, 6
Stehmeier and Jack, 1987					
20	5.5	75.0			
22	0.94	30.0			

Other parameters:

C_{cc}=0.164E-3; n=0.12E-05; F_r=5.0.

Note: Notations are described in the nomenclature

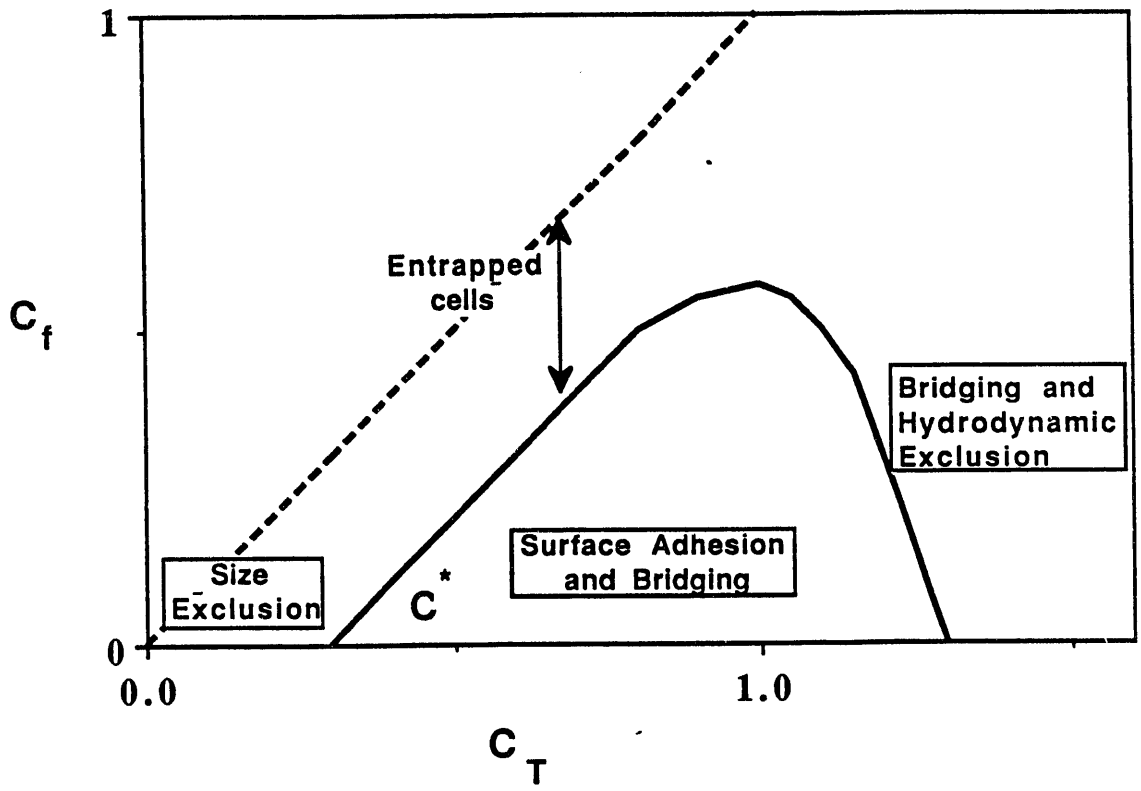

Fig. 6.1 Schematic of a fractional flow curve for the flow of colloidal suspensions in porous media.

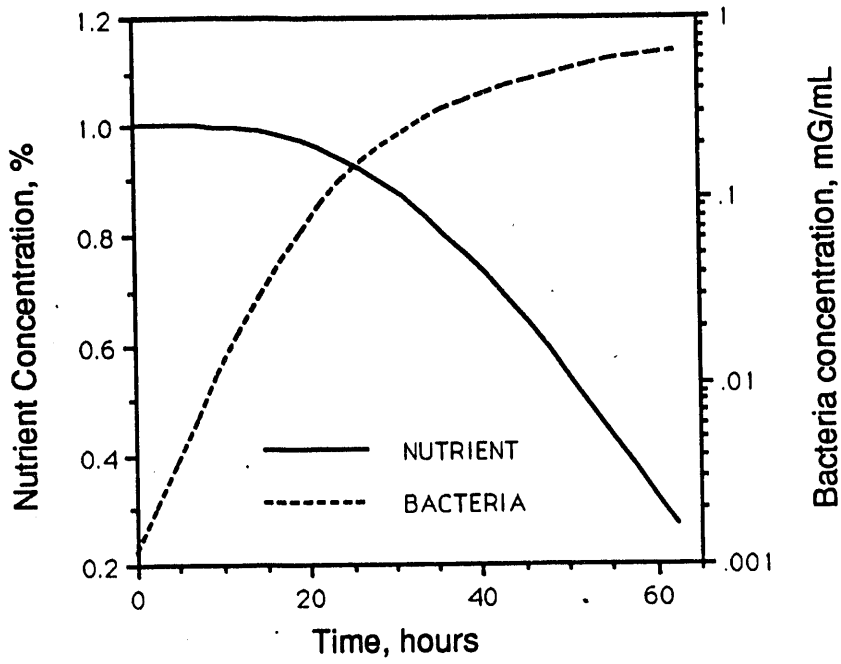

Fig. 6.2 Growth in porous medium during incubation.

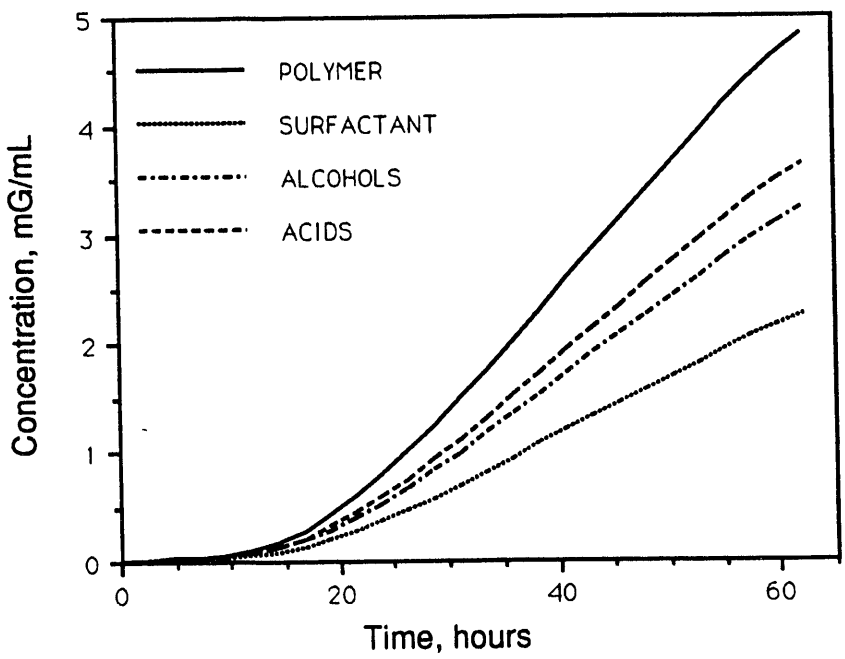

Fig. 6.3 Metabolite concentration during incubation.

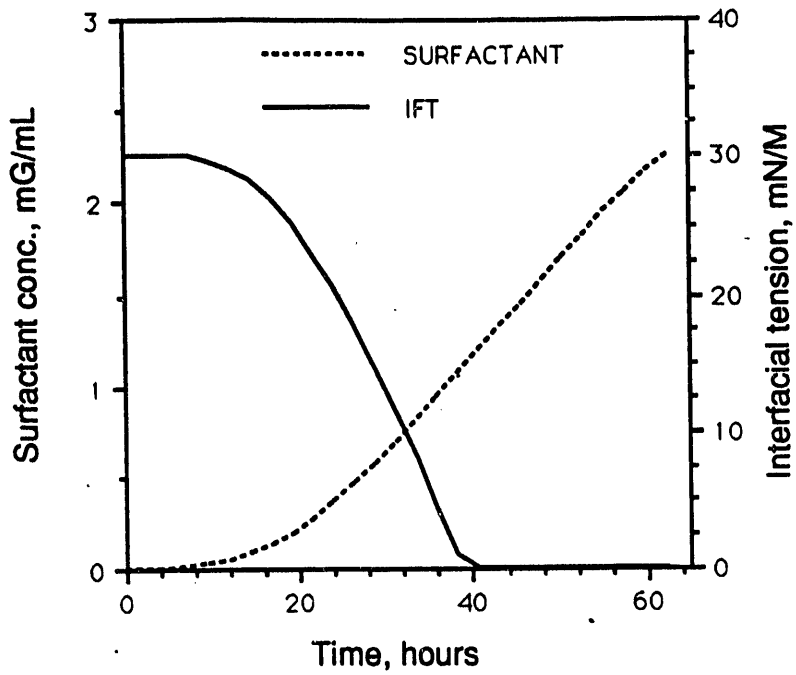

Fig. 6.4 Reduction of interfacial tension during incubation

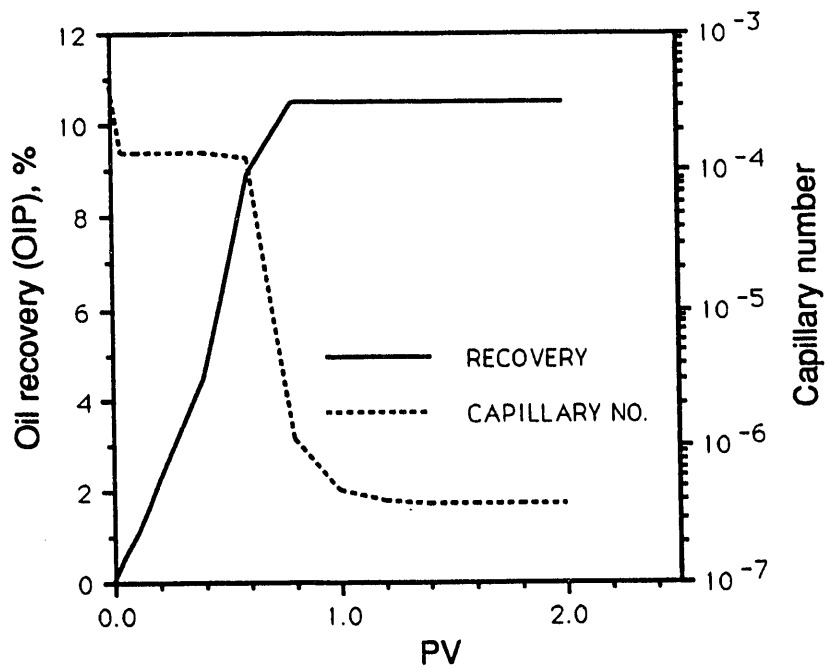

Fig. 6.5 Oil recovery during brine postflush.

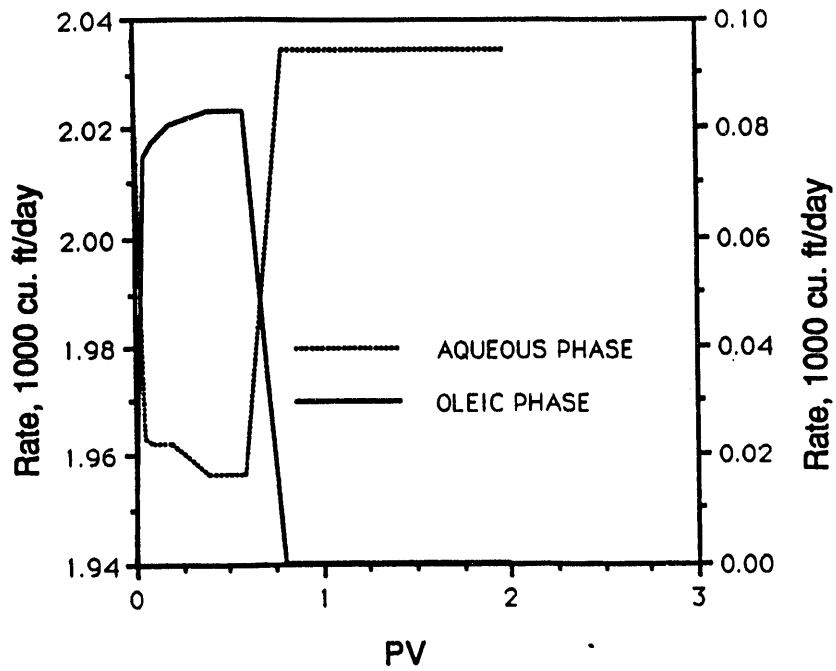

Fig. 6.6 Oleic and aqueous phase production rates.

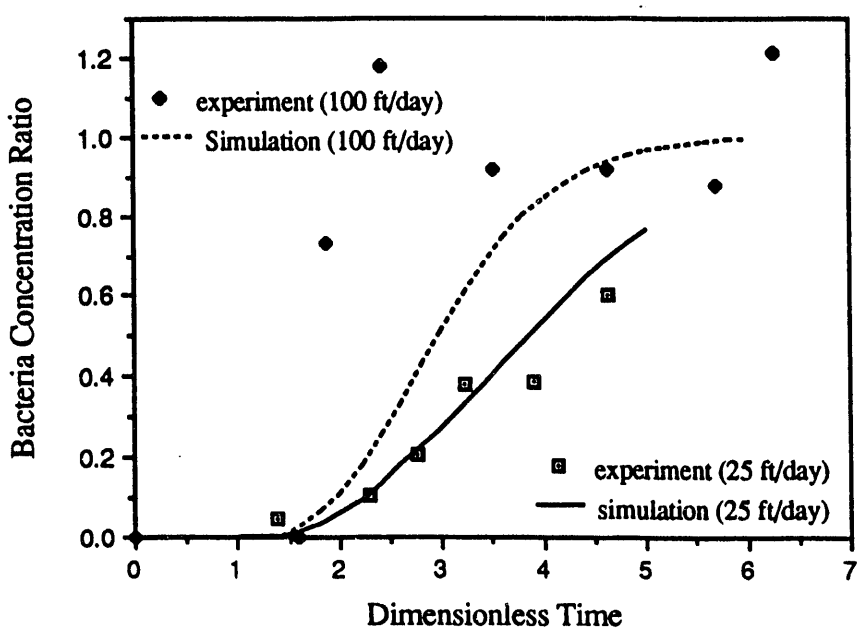

Figure 6.7. Comparison of the simulated and experimental effluent bacteria concentration histories at different velocities.

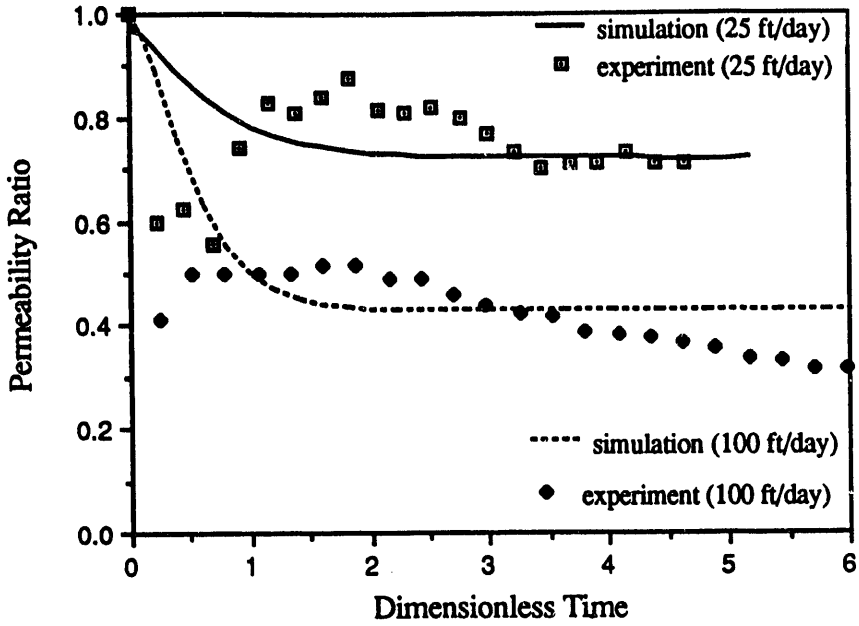

Figure 6.8. Comparison of simulated and experimental permeability ratio histories for the upstream section at different velocities.

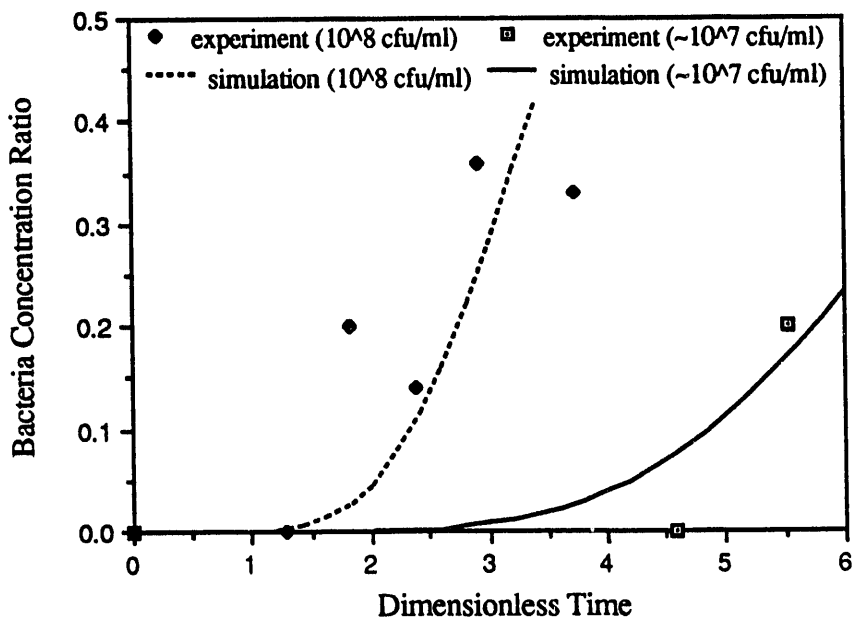

Figure 6.9 Comparison of the simulated and experimental effluent bacteria concentration histories at different injected concentrations.

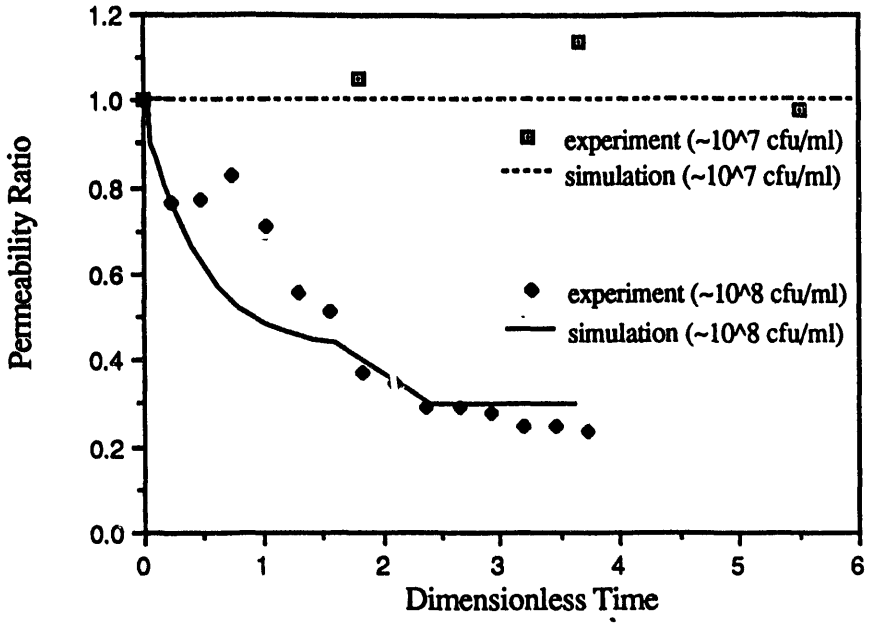

Figure 6.10 Comparison of the simulated and experimental permeability ratio histories for the upstream section at different injected concentrations.

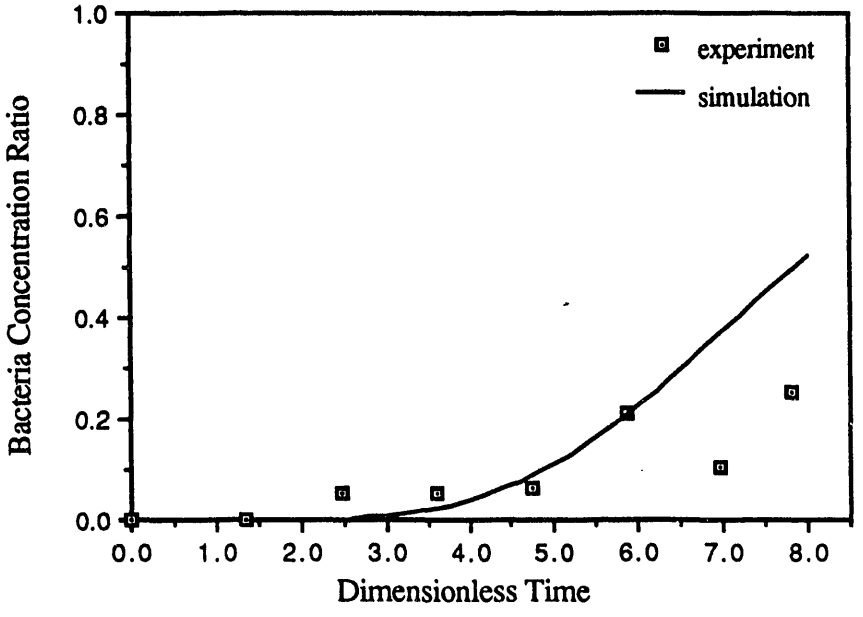

Figure 6.11 Comparison of the simulated and experimental effluent bacteria concentration histories in the presence of two phases.

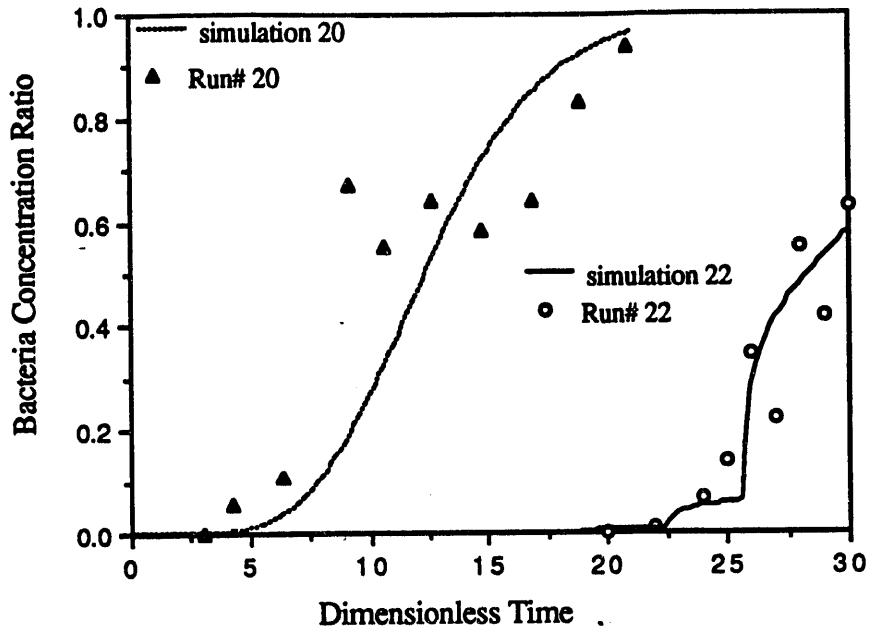

Figure 6.12 Comparison of simulated and experimental effluent bacteria concentration histories (after Stehmeier and Jack: Run Nos. 20 and 22).

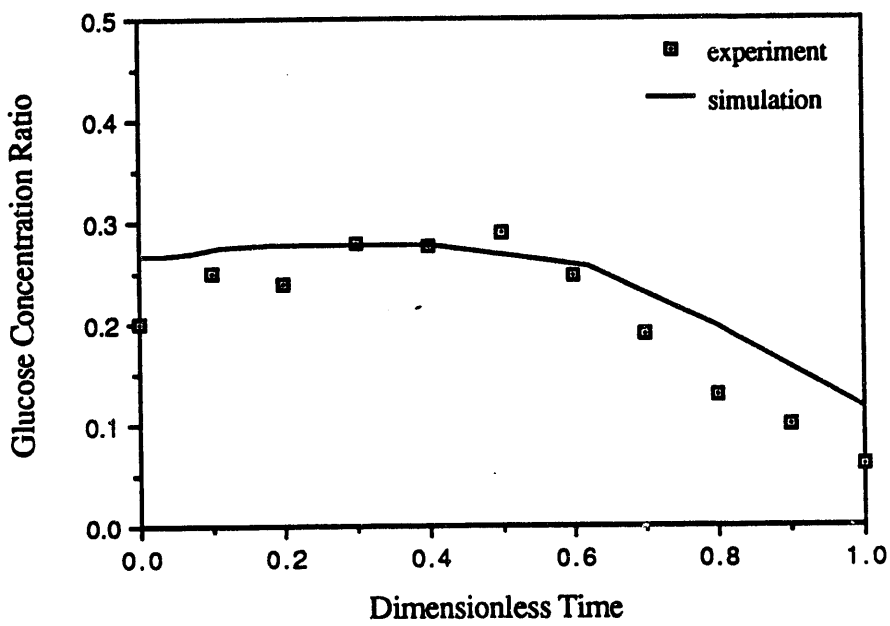

Figure 6.13 Comparison of the simulated and experimental effluent glucose concentration histories after static growth.

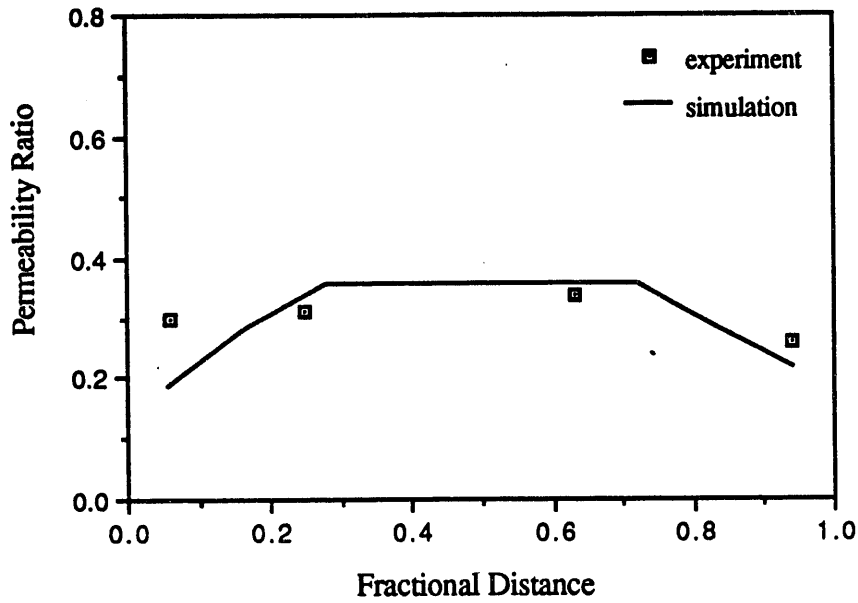

Figure 6.14 Comparison of the simulated and experimental permeability ratio profiles after static growth.

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