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Transport and Growth Characteristics of Ultramicrobacteria (UMB) in Porous Media

by

Christopher Bradley Kowalski

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Transport and Growth Characteristics of Ultramicrobacteria (UMB) in Porous Media" submitted by Christopher Bradley Kowalski in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Present technologies for primary and secondary oil production generally produce less than 40 % of the underground oil reserves. There is, therefore, a high demand for the development of inexpensive, efficient tertiary recovery procedures. One such procedure is known as Microbial Enhanced Oil Recovery or MEOR.

A relatively new breakthrough in MEOR involves the use of small, dormant, starved bacteria known as ultramicrobacteria or UMB. These UMB penetrate underground reservoirs much easier than the large, stickier vegetative bacteria which may cause plugging around the wellbore.

This research studied the transport and growth of UMB in experiments performed using consolidated cores and sandpacks. Plugging occurred in every experiment but the plugging time was much shorter when using a rich nutrient as opposed to a relatively weaker foodsource.

A MEOR plugging simulator was also developed in which it was possible to study the effect of many biological parameters such as growth, substrate utilization and slime production.

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NOMENCLATURE

A	core cross - sectional area (cm^2)
K	core permeability (D)
K_s	parameter for Monod kinetics (g / L)
l	core length (cm)
N_{cell}	number of bacteria at any time (-)
N_{init}	initial number of bacteria (-)
P	bacterial product (slime) concentration (g / L)
ΔP	pressure drop across core (atm)
q	core flowrate (cm^3 / s)
$[S]$	substrate (nutrient) concentration (g / L)
S_o	parameter used in Carmen - Kozeny equation (D^{-2})
t	time (s)
t_d	doubling time for bacterial growth (h)
V_{pore}	volume of pore space in the core (cm^3)
V_{total}	total volume of the core (cm^3)

Greek Letters

α	parameter used in substrate utilization kinetics (-)
α_2	parameter used in product formation kinetics (-)

β	parameter used in substrate utilization kinetics (h^{-1})
β_2	parameter used in product formation kinetics (h^{-1})
μ	viscosity (cP)
μ_b	bacterial specific growth rate (h^{-1})
μ_{max}	parameter used in Monod kinetics (h^{-1})
ϕ	core porosity (-)

CHAPTER 1

INTRODUCTION

1.1 Definitions

Throughout the thesis, terminology specific to the fields of reservoir engineering and biology will be used. Many of these terms will be defined in the text as they are referred to. However, to avoid confusion, some of the more basic terms in both fields will be defined before the introduction begins.

Consolidated or Unconsolidated Porous Media - Porous media is a very broad term that describes a material through which flow may pass. Consolidated media is rock such as sandstone or carbonate in which the rock particles are cemented together. Unconsolidated media would be a sandpack or a soil sample containing loose particles without cementation. Particle size may be controlled in unconsolidated media to provide a very uniform and homogeneous system. Generally, fluid moves much easier through unconsolidated material.

Porosity - Porosity may be defined as the ratio of the open pore volume to the total volume in porous media as demonstrated in equation 1.1.

$$\phi = \frac{V_{pore}}{V_{Total}} \qquad (1.1)$$

Typical values range from 15 % for sandstone to 40 % for sandpacks.

Permeability - Permeability is a term used to describe the ability of porous media to transport fluid. It is described in units of darcies or millidarcies (D or mD) and is related to pressure drop, flowrate, viscosity and core dimensions. It can be calculated using Darcy's Law which will be presented later. Typical permeabilities for sandstone studied in this research range from 100 mD to 700 mD. Sandpacks are much more conducive to flow and permeabilities are generally over 1000 mD.

Darcy's Law - Darcy's Law is used to describe flow through porous media and relates pressure to flowrate as follows:

$$q = \frac{k\,A\,\Delta P}{\mu\,l} \qquad (1.2)$$

Where the units must be:

q	-	flow rate (cm^3/s)
k	-	permeability (darcies or D)
A	-	cross - sectional area (cm^2)
ΔP	-	pressure drop across core (atm)
μ	-	viscosity (centiPoise or cP)

l - length of core (cm)

For the experimental work in this study, most of the above values are constant. The porous media used is set in its geometrical dimensions so that area and length will not change. The tests are all run at room temperature so viscosity can be assumed to be constant and flowrate is decided upon before the test begins and maintained at the same rate throughout. This leaves only pressure drop and permeability as the changing parameters. They can be seen to be indirectly related so when pressure drop readings increase, permeability decreases.

Fingering Zone - In a reservoir, there are many complex channels through which flow may pass. In some cases, one of these channels may have a much higher permeability than the others and so flow will preferentially pass through this zone bypassing the remainder of the reservoir. This high permeability flow area is known as a fingering zone. If flow is initiated in the hope of sweeping oil through the media, only the oil in the fingering zone will be affected.

Bacterial Growth Phases (Lag, Exponential, Stationary, and Death) - Although bacterial species are incredibly diverse in their optimal growth conditions and nutrient requirements, a common growth profile exists. A sample of the typical batch

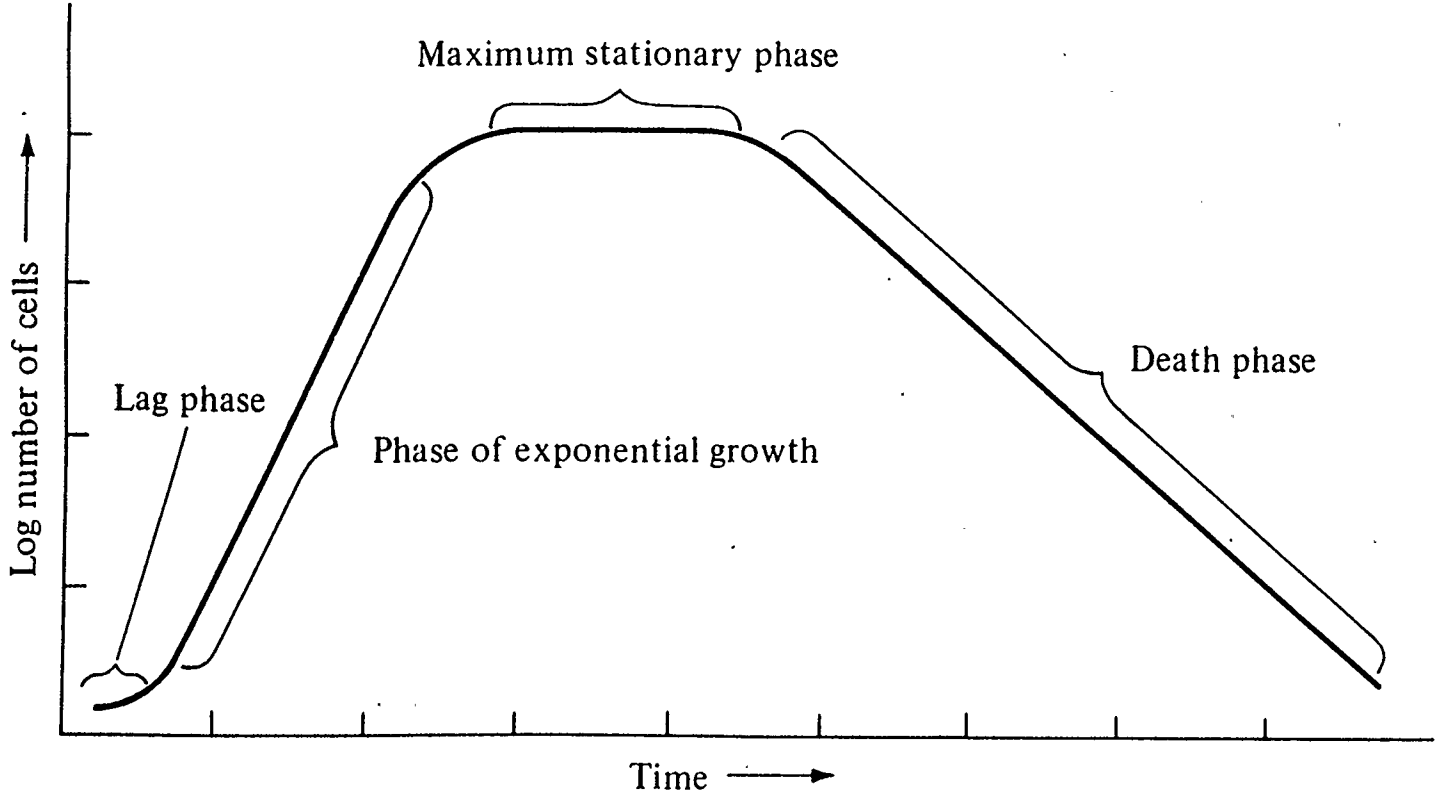

Figure 1.1 - Typical Profile for Batch Bacterial Growth (Bailey and Ollis, 1986)

growth profile can be seen in Figure 1.1. The bacteria are introduced to nutrient in a batch culture, usually a shaker flask, at the beginning of the profile.

The first phase is the lag phase in which the bacteria adapt to their environment and growth is very limited. This adaptation may be very short (one or two hours) or very long (days) depending on growth conditions. Once adaptation is complete, the bacteria move into the exponential phase and start to grow very rapidly. This phase usually lasts about eight to ten hours in a batch culture but again may vary depending on nutrient and bacterial concentrations. As the nutrient gets used up and the concentration of bacterial waste increases, the bacteria reach a peak count and their numbers remain steady. This plateau region is known as the stationary phase in which growth has slowed and become equal to the death rate. Bacterial count may remain stationary for a period of days before it starts to decrease. The death phase is then the final stage in which the bacteria, depleted of nutrient and subject to high concentrations of wastes, slowly die.

When dealing with bioexperimentation in this thesis, the important phases to consider are the lag phase and the exponential phase. The lag phase must be accounted for or else cell growth will be overestimated. The exponential phase is where most of the bioactivity takes place as cells grow and

produce metabolites of interest. After the exponential phase, the majority of the bioactivity has waned and so the stationary phase and death phase are of little interest in this study.

The specific growth rate (μ_b) is the growth parameter characteristic of the specific microorganism and substrate used and can be determined from Figure 1.1 by taking the slope of the exponential phase. The bacteria doubling time can then be calculated using the following expression:

$$t_d = \frac{\ln 2}{\mu_b} \qquad (1.3)$$

Doubling time is defined as the time required to double the bacteria population in a culture. It is a convenient and easily understood value that can be used to compare speed of growth in different situations.

1.2 General Oil Recovery Techniques

The first step to recovering oil from a reservoir is known as the primary recovery phase. As the driving force for this recovery is the underground reservoir pressure, the recovery process simply involves catching the oil as it is forced to

the surface. Unfortunately, primary recovery only works until the reservoir pressure is depleted and may only produce 10 % to 20 % of the total available oil. There is a considerable volume of oil remaining in the ground that must be produced by some other method.

Secondary oil recovery typically produces oil through waterflooding. Water is injected into one end of the reservoir and the oil it displaces is produced through wells at the other ends. In this case, the limiting factor is the oil mobility and the ability of the water to reach all of the pore spaces in which the oil is located. Generally, even after waterflooding, less than 40% of the original oil is produced (Ionescu, 1984). Further tertiary stages must be used in order to produce the remaining oil in the ground.

Many tertiary methods have been developed or are being developed in order to target this vast reserve of unproduced oil. For example, chemical injection of surfactant has been used to lower oil surface tension. Similarly, polymer injection can thicken injection fluid to increase waterflood sweep efficiency.

Another tertiary process involving the use of bacteria is known as Microbial Enhanced Oil Recovery or MEOR. Bacterial use is an attractive option due to their great versatility and

the fact that they can be used relatively cheaply. Bacteria can be found to live and actually thrive in nearly any reservoir situation including high temperatures (90 - 100 ^{o}C), high salinities, aerobic or anaerobic environments and a broad range of pH values.

Biochemical recovery is one large aspect of MEOR. Table 1.1 lists many of the applications in which microbes may be used. For example, many bacteria naturally secrete products that act as strong surfactants. Rather than go through an expensive chemical surfactant flood, these bacteria can be pumped through a reservoir with some inexpensive nutrient (substrate) and they will produce biosurfactant in situ to help mobilize additional oil. Other bacteria produce acids and gases as part of their natural metabolism. The biogas can act to repressurize a reservoir or dissolve in oil to reduce viscosity and increase mobility. Acids can dissolve carbonate rock, increasing porosity and permeability resulting in increased production.

Besides using biochemicals to enhance recovery, bacteria may be used as plugging agents. Plugging is desirable in some reservoirs in order to control the direction of waterflooding. Many slime - producing bacteria can provide strong, uniform plugs under certain reservoir conditions.

- Improvement of relative mobility of oil to water by biosurfactant and biopolymer production.

- Partial repressurization of reservoirs by methane and carbon dioxide production.

- Reduction of oil viscosity through dissolution of organic solvents in the oil phase.

- Increase in reservoir permeability and expansion of fissures and channels through etching of carbonaceous rocks by organic acid production.

- Cleaning of the well bore region by action of carbonic and organic acids or fermentation gas.

- Selectively plugging of highly permeable zones to increase areal sweep efficiency.

Table 1.1 - Bacterial Applications in MEOR

1.3 MEOR Strategies

One strategy for MEOR is based on the secondary recovery stage known as waterflooding. Figure 1.2 illustrates this procedure. Bacteria and nutrient are injected right at the beginning of the waterflood. Bacteria used here will be those that produce surfactant or polymer. Surfactant will serve to lower the surface tension of the contacted oil and so mobilize this oil towards a producing well. Biopolymer, on the other hand, will thicken the waterflood solution, thereby increasing sweep efficiency.

Another popular MEOR strategy for biochemical recovery is known as the huff - and - puff process. The huff portion involves injecting bacteria and nutrient into the reservoir down an injection well. This well is then shut - in for an incubation period in which the bacteria multiply and produce biochemicals. Biogas production is crucial because repressurization of the well is necessary in order for further oil production to take place. After the incubation period is complete, the puff of the process is initiated by reopening the well. The bacteria will have repressurized the well and, ideally, helped to mobilize additional oil as well. The former injection well now becomes a producer and enhanced production can begin. When the pressure finally dissipates,

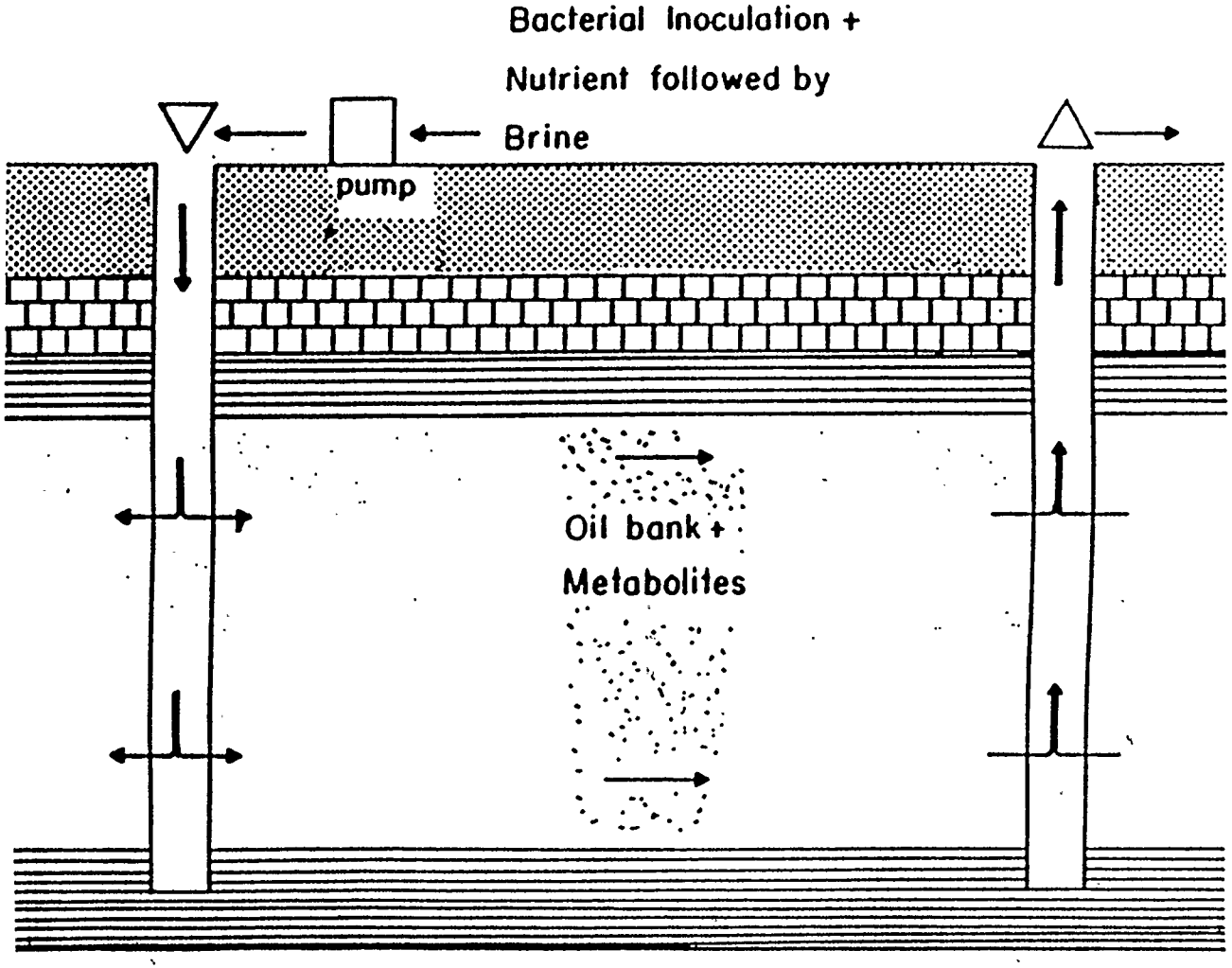

Figure 1.2 – Schematic for MEOR Waterflooding Technique (Yen, 1990)

the huff - and - puff procedure can be implemented again. In this case, only nutrient need be pumped into the well as the desired bacterial colonies already exist. This cycle of nutrient injection, incubation and production may be repeated many times before its effectiveness decreases (Jang et al, 1989).

Bacteria can also be used as plugging agents. Many reservoirs contain high permeability fingering zones that stretch from injection to production well as seen in Figure 1.3. As can be imagined, a waterflood will pass directly through this zone and this same water will be produced at the outlet end. The outlying oil in the reservoir will not be swept out. When bacteria are injected, they will permeate throughout the high permeability finger and then, upon growth, these bacteria will effectively seal the zone. They do this by producing exobiopolymer or slime and this sticky plugging substance produces a very effective barrier to flow. Waterflooding may now continue and, as the fluid bypasses the plugged finger, previously unreached oil will be swept towards producers.

1.4 MEOR Concerns

Unfortunately, no EOR scheme is perfect and MEOR does present

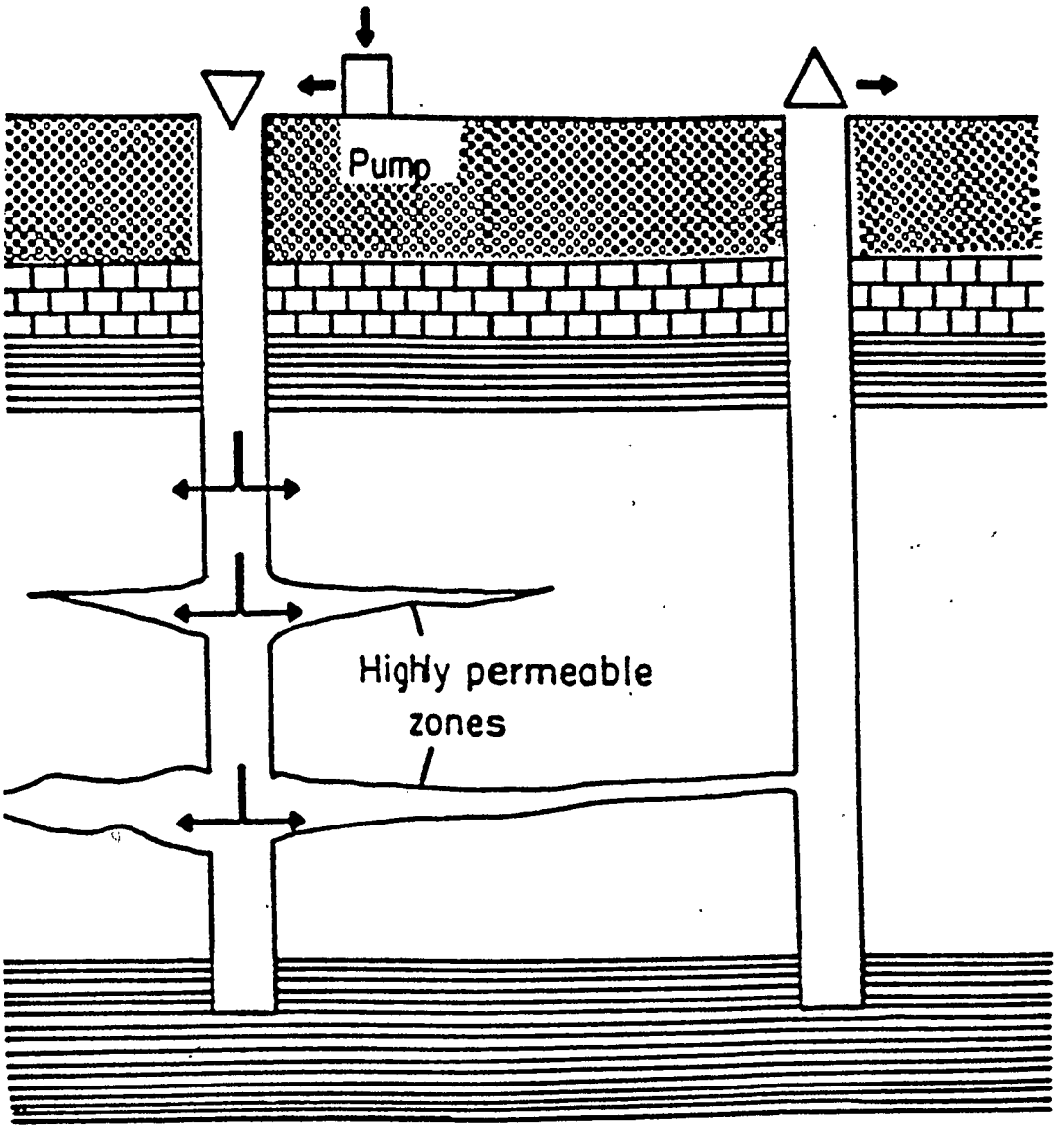

Figure 1.3 – Schematic of High Permeability Fingering Zones (Yen, 1990)

a number of possible problems that can be encountered. Bacteria are not simply particles but are living organisms and so there is always the possibility of inadvertently killing the inoculation before it can be used. Large, unexpected changes in pH, temperature, salinity or toxicity may not affect a chemical EOR procedure but would have drastic affects on a living culture.

Nutrient (substrate) injection can sometimes cause problems. Maintaining the sterility of the nutrient is a high priority. It would defeat the purpose to inject a number of taylor - made bacteria into a reservoir and then follow it with a nutrient solution that contains even larger numbers of an unwanted competitive species. If the contaminant happened to be a slime producing bacterium or a larger sized organism, a skin plug would be formed in a tight reservoir. A skin plug is a short, undesirable plug that forms immediately around the injection point due to physical filtration. Skin plugging may greatly increase injection pressure and, subsequently, increase recovery expenses. Alternately, the contaminant may produce very little slime and be injected in high concentrations into a fingering zone in which plugging was the desired goal. In either case, these unwanted bacteria would most likely be those that survive in situ as they would be growing exponentially well before the target bacteria ever got a chance to be exposed to the foodsource.

Even if the nutrient sterility is maintained, another concern is that the indigeneous bacteria population already present underground may utilize the injected substrate. If the reservoir population is large enough, it may use all of the available nutrient for its own growth, thereby starving the target bacteria. For this reason, bacterial samples from a reservoir should be analysed and, if possible, one of these indigeneous species should be grown into larger numbers and used for the MEOR procedure. This also solves the microbial sensitivity problem as these bacteria would have already proven that they can survive under the conditions in which they would again be exposed to underground. Unfortunately, in many cases, indigenous bacterial samples contain organisms that sour wells. Since well souring can cause complete shut down of a reservoir, tests should be undertaken to ensure that these souring cells will not become the dominant culture.

1.5 Experimental History

The man considered to be the father of MEOR is Dr. Claude ZoBell. He first noticed an increase in oil mobility during unrelated experiments on *Desulfovibrio* hydrocarbon oxidation (ZoBell, 1947). This led to further bacterial research and the field of MEOR was initiated. As can be expected, early

experiments were very general, concerned only with whether or not sulfate reducing bacteria could aid oil production (Beck, 1947; Updegraff and Wren, 1954). As this research became more advanced, researchers started to focus on why MEOR was successful and what could be done to improve the process. It was noticed that many bacteria produce biosurfactants and so experiments tended to lean towards choosing these types of microbes (Jang et al., 1984). Also, reservoir properties had to be accounted for and so bacterial strains were chosen for their halotolerance and thermotolerance (Pfiffner et al., 1986). Presently, biochemical MEOR research has become very specific. Microbes are chosen for specific reservoir properties and results are published based on work conducted using specific oils and nutrients (Behlulgil et al., 1992).

Bioplugging has also drawn a lot of attention from researchers. Early work was actually done using dead bacteria in order to test the plugging effect on different rock types. In both constant pressure experiments (Hart et al., 1960) and constant flow experiments (Raleigh and Flock, 1965) it was demonstrated that although the bacteria went several inches through test cores, most plugged the inlet ends. However, other studies showed that bacteria could be found in core effluents using continuous injection (Jenneman et al., 1984). In these studies, plugging was minimal at first but occured rapidly after nutrient injection, resulting in permeability

reductions up to 80%. Another study proved that there is selective plugging of larger pores over smaller ones (Torbati et al., 1986). This, of course, is exactly what would be desired in the plugging of fingering zones; to leave the small pores open so that the oil may be pushed through the formation and subsequently produced.

Injection velocity is another factor that contributes to the success of MEOR processes. Slow velocities may deposit the majority of cells too close to the injection area. This will have the undesirable effect of causing the formation of a skin plug. Higher velocities ensure microbial flow throughout the formation (Marlow, 1991) and allow nutrients to move further through the zone before plugging starts. This would be beneficial as fingering zones have higher permeabilities and, therefore, permit relatively higher fluid flows than homogeneous zones.

There have been over 200 field tests performed over the years and summaries of these can be found in many sources (Yen, 1990; Donaldson et al., 1989). These tests have taken place all over the world but have mostly been focused in Eastern European countries and the United States. Results have varied but unfortunately, none of the tests have conclusively proven or disproven the effectiveness of any MEOR strategy. The main problem has been the apparent lack of planning and

optimization for the procedures. Many target fields have been fields that are no longer of interest to the producer so that workable oil reservoirs will not be put at risk. Also, conditions in many cases involve high temperature, high salinity and / or low permeability. Again, these characteristics will not be ideal for a bacterial recovery attempt. Results from well - planned field tests are necessary to properly determine the potential for MEOR in the future.

1.6 Novel Developments in MEOR Technology: Ultramicrobacteria (UMB)

Vegetative bacteria are relatively large (1-3 μm), sticky and grow very quickly. When these bacteria are injected into a reservoir they may form a skin plug because of their physical characteristics and, as a consequence, only a short area around the wellbore is affected. If the bacteria do manage to permeate throughout the finger, any nutrient injected will be used by the rapidly multiplying bacteria at the front of the finger. Very little growth will occur further into the zone because the entrance region becomes plugged and any nutrient that is swept further along is depleted. In either case, subsequent waterflooding simply shunts around the small

blockage and back into the high permeability zone. This skin plug may even obstruct the injection port entirely and some kind of treatment would be necessary just to use the well again (Cusack et al., 1987).

A fairly recent development has lent new life to the area of bioplugging. It was found that when many types of bacteria are starved, instead of simply dying, they shrink into a dormant state (Novitsky et al., 1976, 1977). These starved bacteria may be less than 0.3 μm in diameter and can easily be revived when subjected to the presence of a nutrient. They are known as ultramicrobacteria (UMB). A simple schematic seen in Figure 1.4 illustrates the differences between UMB and vegetative cells. The UMB look to be a sort of streamlined version of the healthy bacteria. They are smaller and, as they are dormant, they will not have the sticky exobiopolymer coating that many vegetative bacteria contain. A Scanning Electron Microscope (SEM) photograph in Figure 1.5 shows actual sizes and shapes for the species *Klebsiella pneumoniae*. This figure again illustrates the significant difference in size between starved and healthy bacteria.

UMB, being small and dormant, have the ability to permeate a long way through rock without causing any skin plugging. Also, when subjected to nutrient, they go through an extended lag phase while they become accustomed to growing again. This

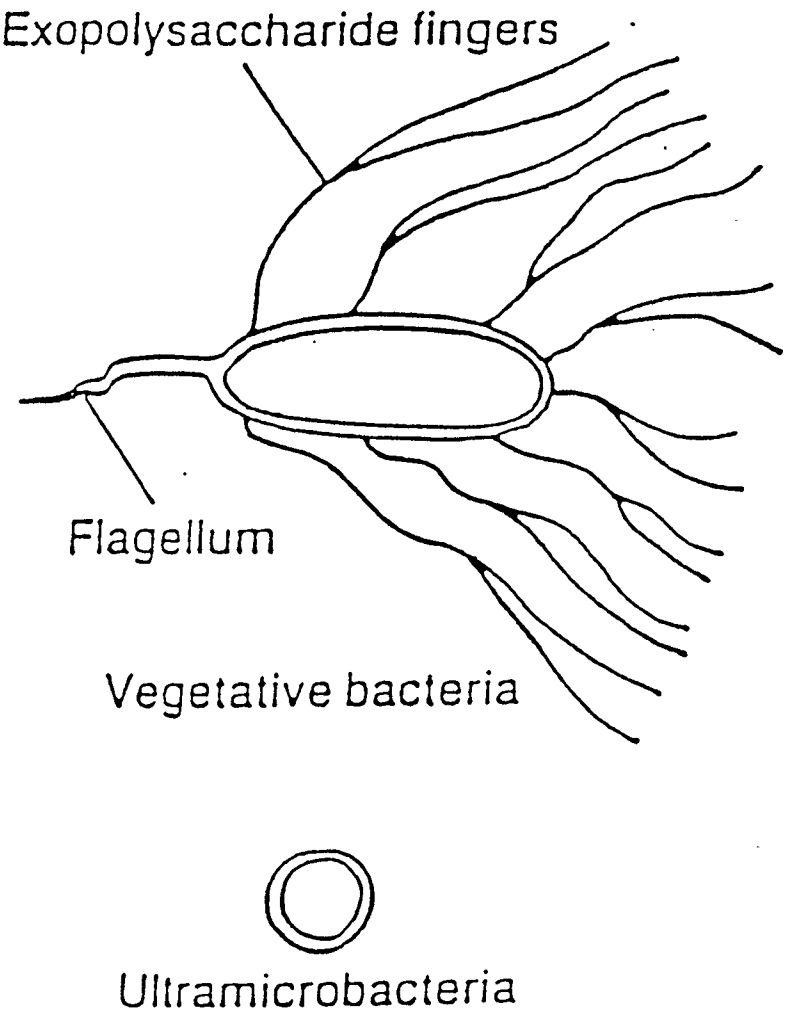

Figure 1.4 - General Difference Between Bacteria and Ultramicrobacteria (UMB) (Singh et al., 1988)

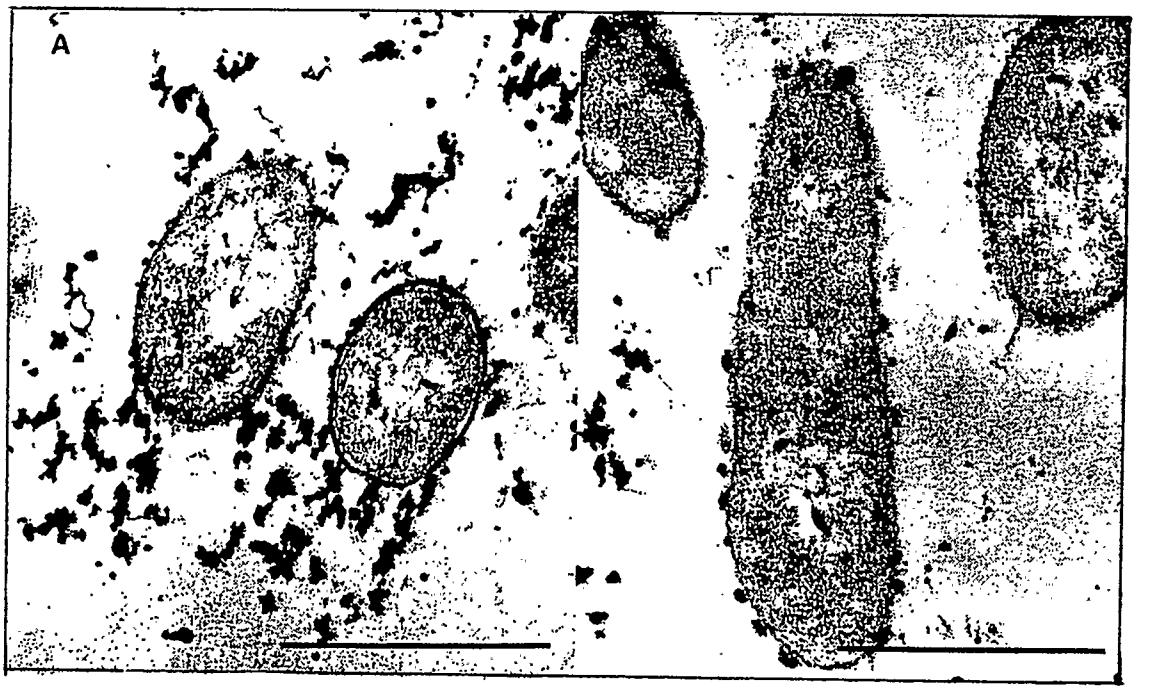

Figure 1.5 - Starved (A) and Full - Sized (B) Electron Microscope Photographs of *Klebsiella pneumoniae* (Bar = 1 μm) (Lappin - Scott et al., 1989)

phase will be dependent on the bacteria and the nutrient used but may last many hours or even days instead of just a few hours as is typical for healthy microbes. This allows much more time for nutrient to be pumped through the zone, forming a much longer plug.

Dr. Costerton and co - workers at the University of Calgary realized the potential for UMB in MEOR applications and have focused on research in this area (Macleod et al., 1988). In one study, fourteen cores were injected with UMB in large volumes and then resuscitated with nutrient (Lappin - Scott et al., 1988). In all of the cores injected with nutrient, permeability dropped below 5% of the original value. Also, SEM photographs of the cores upon completion of the experiments showed penetration and slime production from inlet to outlet in most of the cores. Two cores showed skin plugging and little growth in the last half of the core. These cores had been treated with a much richer foodsource than the others. This leads to the conclusion that media selection may be very important in designing the UMB plugging procedures.

In another study, a three - dimensional physical simulator was built and run with sandpack only (homogeneous) and then with sand packed around a sandstone core (heterogeneous) (Cusack et al., 1990). Both packs were injected with UMB and then

followed by nutrient resuscitation. The homogeneous simulation showed a uniform plug that formed throughout the pack with no skin plugging. The heterogeneous simulation also resulted in complete uniform plugging of the sandpack but the sandstone core was not plugged. Cells were present in the sandstone but did not grow as the nutrient flowed through the path of least resistance which was through the surrounding sandpack. This last experiment provides the most promise for the future. It proves that high permeability zones will be preferentially plugged with little or no damage to the outlying areas.

1.7 Bioremediation as a UMB Application

Interest in utilizing UMB for transport through porous media has not been limited to MEOR. Environmental contamination has become a very pressing problem all over the world. As industrial technology improves, toxic chemicals become very prominent as either feedstocks or waste products. Transport of these chemicals can be very dangerous when hazardous spills are a possibility. Large enough spills may greatly endanger entire ecosystems if not treated properly and quickly. Bioremediation has long been used to treat hazardous waste sites through choosing bacteria that will digest toxic

chemicals and convert them to less dangerous compounds. In situ bioremediation would be an ideal application for UMB. Presently, sites are cleaned by removing the contaminated soil and treating it in bioreactors. As UMB so readily permeate porous material, they could be injected into the soil and allowed to remediate the site in situ. Alternatively, plugging UMB could be injected around and/or underneath the site to provide a wall of biomass that would contain the contamination. Containment is crucial in many situations because if the toxic material reaches the water table it can be spread over great distances and becomes extremely difficult to track for treatment.

1.8 Modelling Attempts

As can be imagined, modelling of MEOR would be very advantageous to the design of an efficient and optimized procedure. Unfortunately, although there has been a fair amount of MEOR research, modelling attempts are not nearly as numerous. Fines entrainment and deposition has been modelled before (Gruesbeck and Collins, 1982; Imdakm and Sahimi, 1991) and can be linked in many ways to the transport of bacteria through porous media. The difficulties arise when the biological factors are considered. As the models deal with

living organisms, factors such as growth rate, product formation and nutrient transport and utilization must all be considered.

More specifically, bacterial adsorption has been modelled (Cheneviere et al., 1989) but agreement with experimental data is less than ideal. Plugging models may also be established to predict plugging behaviour (Islam and Gianetto, 1993; Kowalski et al., 1990). Again, experimental data is not available or easily comparable to these results. Another factor to consider is that bacteria in small reservoir spaces may behave differently than in batch cultures because they will form biofilms on the rock. In this case, it may become necessary to study the in situ biofilm interactions (Taylor and Jaffe, 1990).

In addition, models have been established that take into account other types of biochemical processes such as reservoir souring (Ametov and Entov, 1982), oil recovery through biosurfactant production (Sarkar et al., 1989) and bioremediation in soil (Dhawan et al., 1993). The shortcoming to all of these models can be found in the equations necessary to explain reservoir and bacterial behavior. There are many parameters that cannot be calculated and must be estimated or backfit from experimental data. These factors may lend credibility to results that do not properly model the system.

1.9 Outline of Study and Specific Objectives

This study will attempt to provide a more fundamental understanding of the process of MEOR. Experimental data will be presented for tests dealing with the plugging of porous media using UMB with nutrient resuscitation. The porous media used began as consolidated sandstone but inconsistent rock properties caused many problems in data interpretation. Experiments were changed to incorporate unconsolidated sandpacks for the remainder of the study. Variables such as nutrient selection, UMB concentration and UMB injection volume were all studied to examine the effects on the plugging process. Also, effluent bacterial counts were monitored in order to maintain an idea of how the growth was proceeding. After each sandpack experiment, the packs were cut and samples were taken along the length in order to determine plugging depth through magnitude of cell numbers.

The purpose of this work is to provide a better understanding of the processes behind microbial plugging in porous media. Each experimental run will be performed in a very methodical manner so that analysis of the results will provide insight as to how the plugging proceeds. UMB flow through porous media may first be examined and then the effect of UMB resuscitation and growth can be studied.

Finally, the important equations dealing with MEOR are discussed and used in a computer simulation to predict plugging profiles. This simulation is very useful as a tool for sensitivity analysis as there are many different biological parameters that will influence the success of a MEOR procedure.

CHAPTER 2

MATERIALS AND METHODS

This chapter will explain the procedures used to prepare for experimentation, the experimentation itself and any post - experimentation analysis performed. Nutrients and other solutions will also be described in terms of their ingredients and their preparation.

2.1 Preparation of Ultramicrobacteria (UMB)

The chosen bacterial strain for UMB preparation was *Klebsiella oxytoca*, obtained from the biology department at the University of Calgary. This strain was used because of the following characteristics:

1. Facultative Anaerobe - This bacteria will grow aerobically in the prescence of oxygen but has the capability to grow when oxygen - deprived as well.
2. Slime Production - *K. oxytoca* can be considered a moderate slime producer and so is useful for plugging experiments.
3. Growth Conditions - *K. oxytoca* grows very well on many

different nutrient sources and needs no special treatment. As this species is non-pathogenic, experimental handling is safe and relatively simple. Also, room temperature is adequate for fast, healthy growth.

4. UMB Capability - The University of Calgary Biology Department has previously used *K. oxytoca* for UMB production and found it to produce suitable starved bacteria (Cusack, 1992). UMB can be expected to be somewhat similar to those of *K. pneumoniae* seen in Figure 1.5 as they are of the same bacterial species.

Starving *K. oxytoca* takes some time in order to ensure a healthy culture with a minimum of contamination. The vegatative cells are first inoculated into a 100 ml half strength Brain Heart Infusion (BHI) broth and allowed to grow overnight. The nutrients and media used are described in detail in the next section of this chapter. A 2 mL sample from this flsk is then inoculated into another sterile 100 mL flask of media again for overnight growth. This transfer is done three times to ensure a healthy exponential growth phase is achieved and a viable cell count of 10^9 colony forming units (CFU) / mL is reached. At the time of the final transfer, two large 2 L volumes of sterile media are inoculated in order to have a large number of cells for starvation.

The next step is to separate the cells from their nutrient source

to start their starvation. This is achieved through repeated centrifuging. Six sterile centrifuge jars are filled about half full with bacteria and media from the 2 L flasks and weighed to balance the fluid amounts for smooth centrifuging. The cells are separated at 8000 rpm for 20 minutes. The supernatant is then removed and the remaining pellets are washed by resuspending them in a phosphate buffer saline (PBS) solution. The centrifuging and washing process is repeated three times and then the "clean" cells are collected and transfered to two large 2 L flasks of sterile PBS with stirbars. These flasks may then be placed in a sonicator for 40 - 60 seconds in order to break up any remaining clumps.

Finally, the flasks are placed on stir plates and left to allow the bacteria to slowly starve in a medium-free environment and shrink to form UMB. The UMB will form in 1-2 months and will remain in this form until they are introduced to another nutrient source. The final concentration of UMB in the flasks was about 10^9 CFU/mL.

2.2 Preparation of Solutions

Two different media were used in the plugging experiments. One was a half strength Brain Heart Infusion (BHI) nutrient, which is considered very rich but is not chemically defined. The other nutrient is known as Sodium Citrate Media (SCM) and is considered

weaker than BHI but its chemical composition is well known. Another solution that was prominent in the study was used for the starvation of UMB and for dilution tubes used in post - experimental analysis. This solution is known as Potassium Buffer Saline (PBS) and, while not harmful to the bacteria, will not promote growth of any kind. The recipes for BHI, SCM and PBS can be found in Table 2.1.

BHI and SCM are generally sterilized in a number of different quantities depending on the volume needed. The important thing is to sterilize them immediately after preparation and keep them sterile to prevent biomass from contaminating. PBS may also be sterilized in different volumes but usually, it is pipetted into test tubes in 9 mL and 9.9 mL quantities to be used for dilutions. PBS may be kept unsterile for long periods of time as it contains no growth substrate but should be sterilized before use as a precaution against minor contamination.

Also prepared before experimentation are BHI agar plates. The recipe in Table 2.1 is followed and then each 300 mL flask is sterilized. Immediately after sterilization, while the solution is still warm, the flask is poured into 15 petri dishes with approximately 20 mL going into each. These dishes are left to cool and when they do, a solid gel is formed along the surface of the plate.

Brain Heart Infusion Substrate (BHI)	**1/2 strength**
BBL BHI Media (Becton Dickinson) (Cockeysville, MD, USA)	18.5 g
Distilled Water	1 Liter
Brain Heart Infusion Agar	**1/2 strength**
Bacto - BHI Agar (Difco - Detroit)	26 g
Bacto - Agar (Difco - Detroit)	7.5 g
Distilled Water	1 Liter
Potassium Buffer Saline Solution (PBS)	
Sodium Chloride	8.7 g
Potassium Phosphate Dibasic	1.23 g
Potassium Phosphate Monobasic	0.4 g
Distilled Water	1 Liter
set to pH = 7.0	
Sodium Citrate Media (SCM)	
Sodium Citrate	6.45 g
Ammonium Sulfate	0.198 g
Potassium Phosphate Dibasic	5.23 g
Potassium Phosphate Monobasic	2.72 g
Magnesium Sulfate	0.12 g
Ferric Chloride	0.008 g
Distilled Water	1 Liter
set to pH = 7.0	

Table 2.1 - Solution Recipes

The uses for all of the aforementioned substances will be described as they are needed in the remainder of this Chapter.

2.3 Preparation of Cores

Consolidated and unconsolidated cores were prepared in order to perform UMB flood experiments followed by nutrient resuscitation. The gradual plugging of these cores could then be studied and conclusions drawn on the effectiveness of the plugging technique.

During the core flooding experiments, there were a number of problems encountered which will be discussed later. Due to the attempts to rectify these problems, three different core preparation methods were used, one for consolidated rock preparations and two for sandpack experiments.

Sandstone consolidated cores were obtained from the stores of both the University of Calgary Biology Department and the Imperial Oil Research Center. These cores were drilled to either 1.5 inch or 2 inch diameters using a diamond bit core driller. Each core was also cut to 4 inch lengths using a diamond bit rotary saw. Metal endplates were constructed that were about 2 inches long to fit on either side of the core. The core with its endplates was set up on a slow lathe and coated with epoxy and then wrapped with fiberglass

tape. About ten layers of epoxy and fiberglass was found to be more than sufficient to handle the pressures experienced during floods. The epoxy attains its final properties in 72 hours but the cores can be handled after an overnight drying. Sterilization of the cores was then achieved by submitting each core to ethylene oxide gas for 4 hours.

Sandpacks were first prepared by packing quartz sand into lead sleeves and then inserting these into specially designed core holders. The core holders acted to seal both ends and provide space around the outside of the sleeve for overburden pressure. These packs were 2 inches in diameter and 6 inches long and were sterilized by placing them in a 150 ^{o}C oven overnight while simultaneously applying a vacuum to the packs. Equipment problems forced these sandpacks holders to be abandoned after 5 runs.

The second sandpack system also involved packing quartz sand into lead sleeves but these sleeves had to be fitted with 2 filters (400 mesh and 40 mesh) and a thin (0.5 inch) metal endplate on each side. The entire system was then wrapped with teflon tape except for the o-ring around the endplates. Shrinkable tubing was then fitted around the core and heat shrunk to seal the system at the o-rings. These packs ended up being 1.5 inches in diameter and 6 inches long. Sandpack sterilization was achieved through autoclaving the packs at 121 ^{o}C and 15 psi for 45 minutes. The whole pack was then placed in a core holder and overburden pressure

was applied to help maintain the seal and prevent core leakage and bypassing.

The quartz sand is Agsco #3 / 0 Quartz - Inspected and contains properties and particle size distribution as seen in Table 2.2. Prior experimentation has demonstrated that this sand gives a permeability of 1 to 4 D with pore throats of approximately 15 to 20 μm. Actual permeabilities and porosities will be discussed in the experimental section.

2.4 Experimental Procedure

The experimental setup is illustrated in Figure 2.1. The holding containers and all of the tubing were sterilized by autoclaving at 121 ^{o}C and 15 psi for 45 minutes. The pump used was a Ruska pump due to the low flow rates possible and the minimal pulsing that occurs due to the screw type pumping mechanism. Bayol oil was used as the pumping fluid and pumped to an accumulator where the nutrient was held. The nutrient was then forced out of the accumulator and passed through a 7 micron filter before entering the core. Pressure transducers were located at the core inlet and outlet to determine the flooding pressure drop. There was also a pressure gauge located at the inlet to the core. The gauge was used to give a general idea of the pressure range so that the

Representative Chemical Analysis

Total Silicon Oxide	95.5 - 96.6 %
Other Oxides	< 2.5 %
Chromium	< 0.0002 %
Phosphorous	< 0.01 %
Carbonate	< 0.1 %
Water (trapped)	< 0.5 %

Physical Properties

Specific Gravity	2.65
Shape	Angular
pH	6.8 - 7.2
Hardness	7 (Moh's Scale)

Screen Analysis For
#3 / 0 Quartz - Inspected

U. S. Sieve #	Sieve Opening (mm)	Percent of Sand in Tray (%)
100	0.149	trace
140	0.105	8.9
170	0.88	12.3
200	0.74	24.3
270	0.53	24.0
325	0.44	17.0
Pan	(-)	13.4
		99.9 %

Table 2.2 - Properties of Sand Used in Sandpacks

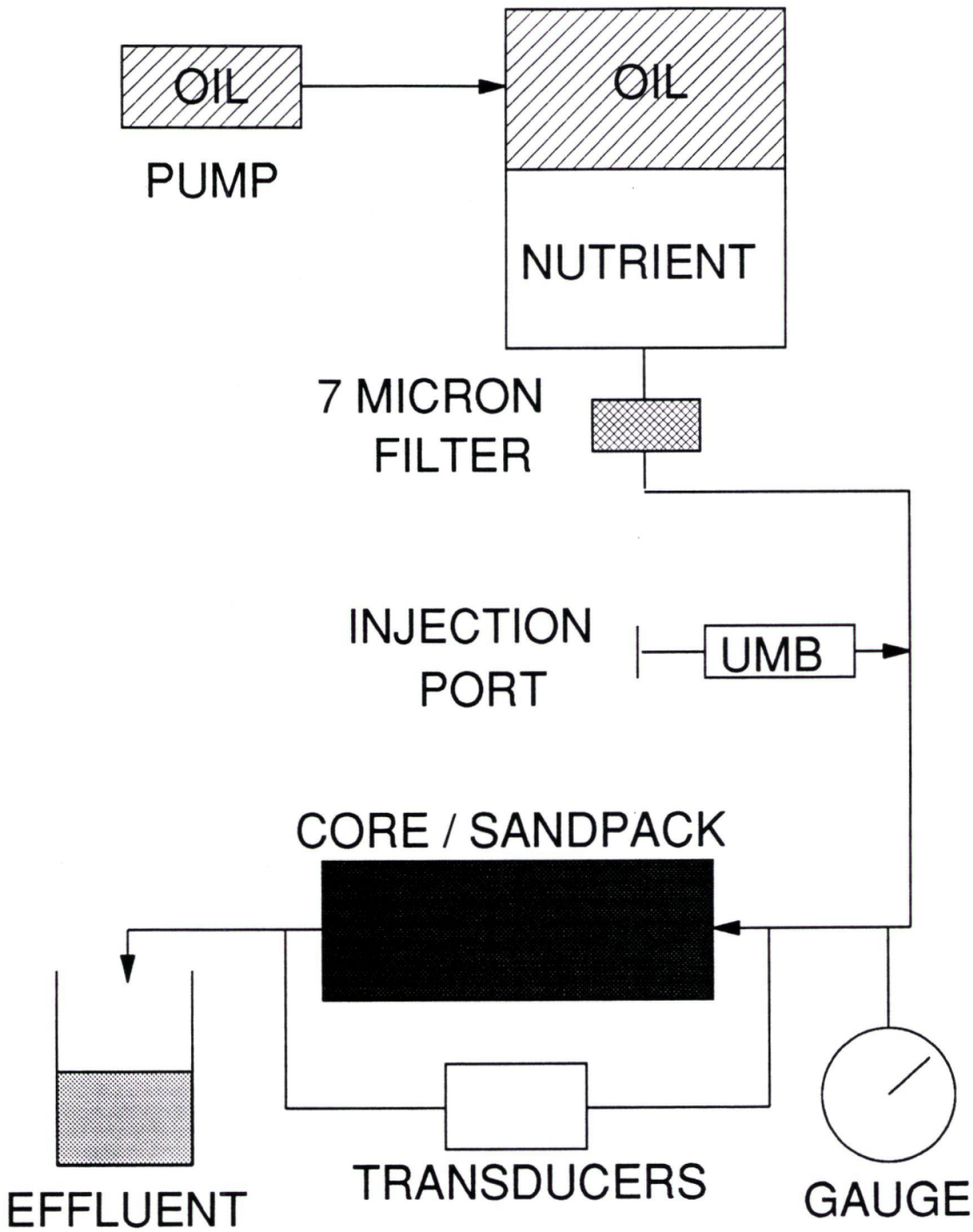

Figure 2.1 - Schematic of Experimental Setup

proper pressure transducer could be opened to the system. This served to protect the more sensitive transducer diaphragms from being overburdened. The inlet line was used to inject the UMB solution manually using a syringe. Effluent was collected at the core outlet in 250 mL sterile flasks.

Once UMB, porous media and the necessary solutions were ready, the actual plugging tests began. The experimental procedure was started by determining the core porosity. The core was evacuated through the outlet for 1 - 2 hours while the inlet piping of the system was filled with nutrient. With the core evacuated and the outlet valve closed, nutrient was pumped into the core until the pressure could be seen to be rising appreciably. Once this pressure stabilized, the inlet line was opened and the backflowing fluid collected in a graduated cylinder. Once the flow stopped, the measured collected volume was subtracted from the volume of liquid pumped into the core and the porosity could be calculated.

Core permeability also had to be determined. Nutrient was pumped through the core at two or three constant flow rates and the corresponding pressure drops were recorded. These values were then used in Darcy's Law to calculate core permeabilities. The flow was maintained for about two pore volumes for the sandpacks to wash out any mobilized fines and stabilize the pressure drop.

After the core had been characterized, the experiment was run

immediately with the injection of UMB followed by nutrient. The core was then shut in for 24 hours to allow the UMB to resuscitate and become viable, slime producing bacteria. After this first shut in period, nutrient was injected every twelve hours to check the permeability decrease as well as to resupply the bacteria with fresh media. The experiment carried on until the permeability had stabilized, usually for 60 - 80 hours. In some experiments a backflow run was done at the end to test the plug stability.

Each effluent sample collected was weighed and then stored at 5 ^{o}C until analysis was performed.

When the consolidated cores were used, the plugged cores had the endplates sawed off, re - machined, and reused while the cores were sterilized and disposed of. Core analysis was not done due to the limited availabilty of the diamond saws necessary to cut sandstone.

In the case of the sandpacks, each one was dismantled and sand samples were taken from four equally spaced lengths along the pack (inlet, 2 inches, 4 inches, and outlet). These sand samples were placed in sterile flasks containg PBS, weighed, and then also stored at 5 ^{o}C until analysis could be completed.

In some cases, the experimental procedure was slightly changed in order to examine some different phenomena and these changes will be discussed as they are presented in the results section.

2.5 Post – Experimental Analysis

The effluent solutions and sand samples were analysed at the end of the experiments to measure bacteria concentrations by performing bacterial counts. The samples were stored at 5 ^{o}C when not being used in order to prevent the bacteria from growing and thus giving erroneous results.

The bacterial solution, PBS dilution tubes and BHI plates were all placed on a counter next to an open flame from a bunsen burner. The open flame is necessary to maintain the sterile environment. When each flask or tube has its top removed, the edge or lip of the container is placed in the flame. This will burn away any outside contamination that may have developed through handling even though the inside was protected.

A vortex mixer was used to mix the effluent and then 0.1 mL was pipetted out of the flask and into a tube containing 9.9 mL of PBS. This served to dilute the solution by a factor of 10^2. Similarly, to dilute the solution further, the tube can be vortex mixed and 0.1 mL removed to be placed in another 9.9 mL PBS tube to give a total dilution of 10^4. If a dilution factor of only 10^1 is desired, 1 mL can be taken from the source solution and placed in a 9 mL PBS tube.

Once the desired dilutions were obtained, plating took place. 0.1 mL was pipetted from the dilution sample and dropped onto a petri dish containing BHI agar. This drop was then spread using a bent glass rod until it was dry. The rod was sterilized before each use by ethanol immersion and then the ethanol was burnt off by passing the rod through the open flame. The rod was then touched to the inside of the petri dish before spreading in order to cool it enough so that bacteria were not killed upon contact.

These plates were incubated at room temperature (23 ^{o}C) for one to five days depending on the viability of the organisms. Once cultures were visible, they were counted and since each culture began from one single bacteria, the original cell number can be determined. Counts should be between 30 and 300 for maximum accuracy and if the plates are too full or too empty, a more suitable dilution will have to be plated. It should be noted that since only 0.1 mL is plated, the final dilution will be increased by another factor of 10 to obtain the amount of colony forming units (CFU) / mL. Also, plating was done in duplicate or triplicate to gain accuracy and an average value was used for the actual count.

CHAPTER 3

RESULTS AND DISCUSSION

In this chapter, results from laboratory experimentation will be presented. The results will be discussed with regards to their particular pertinence to MEOR plugging procedures. In many cases, possible explanations will also be given for observed behavior that is not easily explainable.

3.1 Bacterial Growth Tests

One of the most important factors in experiments dealing with bacteria is the rate of growth. Temperature, pH, media selection, and bacterial species are just a few of the variables that influence this growth. Temperature for all of the experiments remained constant at room temperature (T = 23 ^{o}C) and pH was approximately neutral throughout. The bacteria chosen was *K. oxytoca* for the reasons discussed earlier and it was grown on two different media. The first nutrient used was a half-BHI nutrient which is considered very rich and so the bacteria reach exponential phase quickly and level off in the stationary phase at a high count. The second medium was SCM and, while it contains the necessary growth components,

microbes grow slower and obtain lower final counts than with the richer BHI.

The viable cell growth counts and profiles can be seen in Figure 3.1. These bacteria have been grown in their respective media for two transfers before the profiles were recorded. This ensures that the curves are not marred by unhealthy or dormant cells. Flasks containing nutrient are inoculated with a viable bacteria sample and placed in a shaker where they remain for the duration of the test. Samples are taken every two hours for 10 hours and then much later samples are taken ($t > 24$ hours) to determine the stationary cell counts. As can be seen in the curve, the BHI profiles are typical growth curves. There is a small lag phase starting the test in which the bacteria become accustomed to their new environment and then rapid exponential growth occurs. After about 15 hours the curve levels off at a high count of 10^9 CFU / mL. The doubling time can be calculated as described in Chapter 1 and it is found to be approximately 1 h. That is, the bacterial numbers will double every hour they are exposed to BHI while growing in the exponential phase.

The SCM curve does not show a definite lag phase. Rather, the lag and exponential phases blend together to give a much more gradual growth history. The slope of the exponential phase

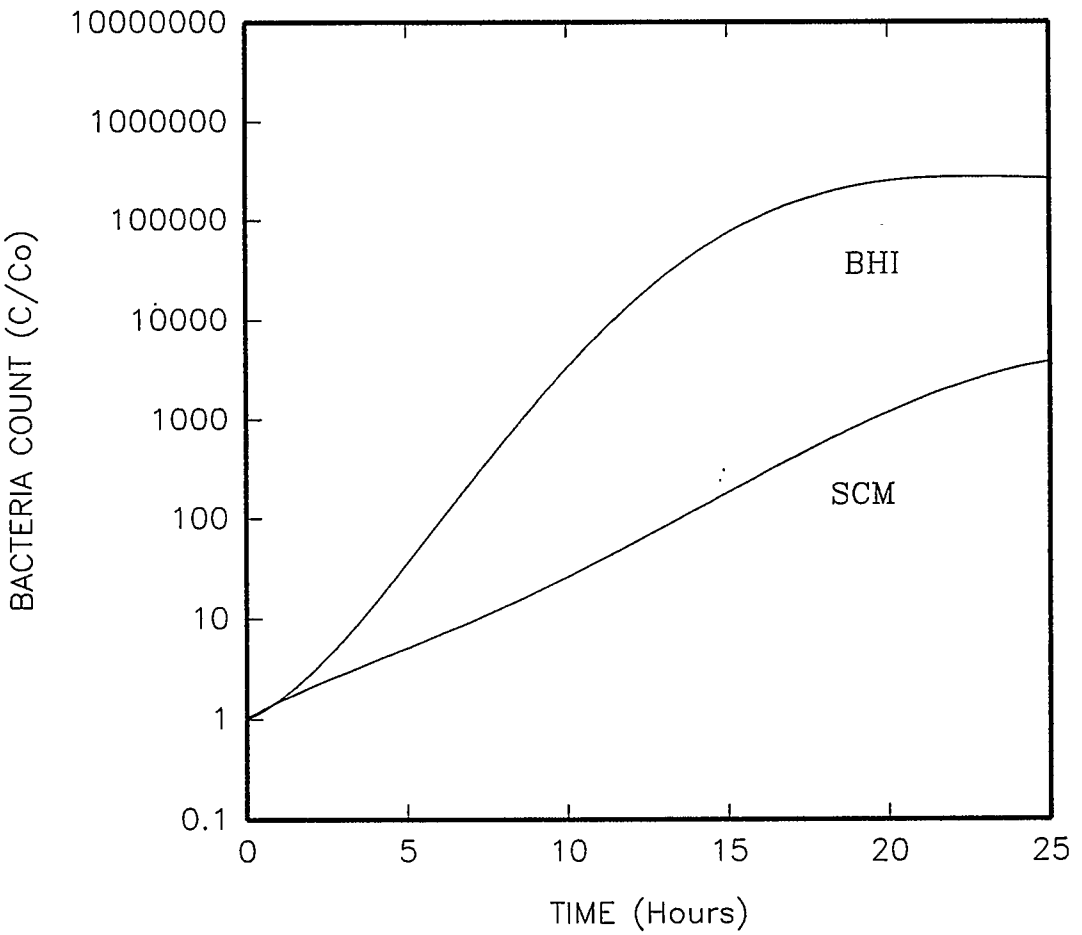

Figure 3.1 - Growth Experiment Results Using BHI and SCM Media

can be seen to be much smaller than that for BHI, indicating a slower cell doubling time. In fact, the doubling time calculated to be 1.9 hours is nearly double that of BHI. The SCM is only half as potent a growing medium. Also, the bacteria grown in SCM reach a maximum count of only 10^8 CFU / mL, an order of magnitude lower than those grown in BHI.

These differing trends will become very important when dealing with MEOR and will be discussed later in this chapter. There are advantages and disadvantages to both nutrient sources and ideally, media could be tailored to incorporate all of the beneficial traits while eliminating the problem sources.

3.2 Consolidated Cores

Originally, the research was to be focused on floods through a number of consolidated cores with varied permeabilities. Unfortunately, some major problems occured with the cores. Two rock samples were obtained from the stores of the Department of Biology at the University of Calgary. These samples were marked 300 mD and 400 mD and each was cut into three smaller cores. After wrapping and sterilizing, permeability tests were run and the results were puzzling. Theoretically, the pressure drop across the core should have

stabilized quickly and remained constant while the flow was constant. Instead, the pressure rose throughout the test and even after a full pore volume of flow, the pressure would not settle.

Despite these problems, MEOR experiments were run with these cores and the results are analysed later in this chapter but in the meantime, new core samples were obtained from the Imperial Oil Research Lab. These cores were originally ordered from the Cleveland Quarry and were marked 100 mD, 250 mD, and 500 mD. These samples were also cut into three smaller cores and prepared for MEOR testing. Upon permeability testing, the same phenomenon was observed with the pressure increasing even though the flow was constant.

Clay swelling was first hypothesized to be the problem. A 12 % Calcium Chloride solution was used on a previously unwetted core to try to eliminate or at least reduce the swelling problem. This treatment did nothing to steady the pressure drop and so another solution had to be found. Finally, chips of the rock were taken and analysed under a scanning electron microscope (SEM). These samples showed no problems at low magnifications and actually showed some very prominent pores and pore spaces. However, at higher magnifications, a major clay problem was observed as can be seen in Figure 3.2. The clays were not of the swelling type, which can be treated, but

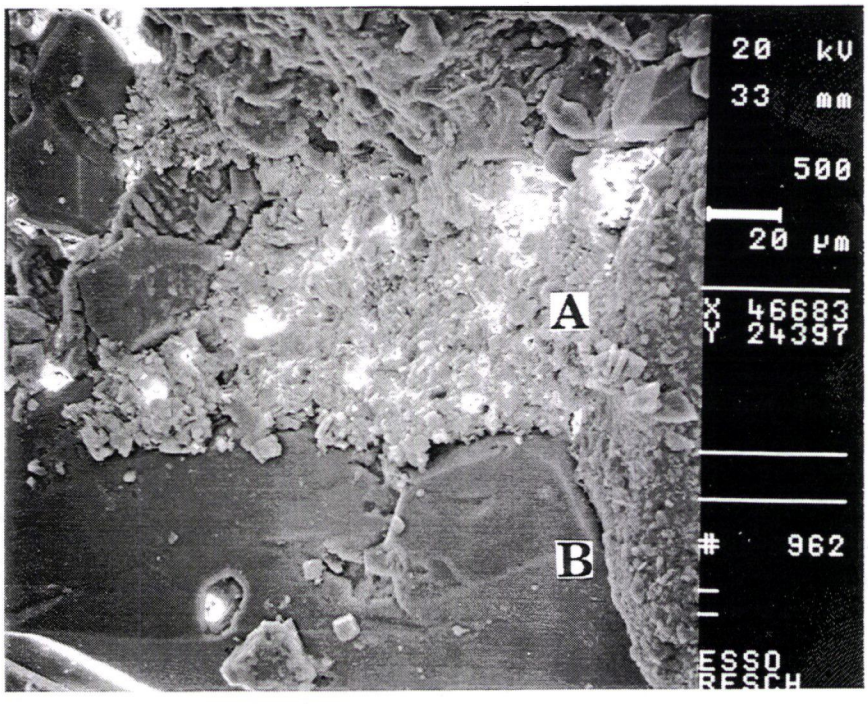

Figure 3.2 - SEM Photograph Illustrating the Clay Fines (A) on the Rock Surface (B)

identified as fines migration clays which are very difficult to treat (Kwan et al., 1989). It was concluded that the cores were probably tested for permeability using air flow which would have a substantially smaller effect on the fines than liquid flooding and so lead to erroneous labelling.

Figure 3.3 shows a control experiment on a core marked 200 mD. This core was flooded with BHI nutrient but without UMB injection. It can be seen that with every new flow test, the core retained its last permeability reading and continued increasing from there. This is indicative of the clay fines forming filter plugs through the core. It should also be noted that this core exhibited a large change in behavior when it was backflushed at the end of the test. Instead of an increase in the final pressure reading as can be seen between 48 hours and 60 hours, there was a decrease. This again supports the fact that fines migration is the problem because backflowing would serve to somewhat reverse any filter that the fines had formed. This clay fines problem ensured that the permeabilities were nowhere near their marked values, instead being in the range of 10 mD to 20 mD. In fact, in one case, a 400 mD core had a higher pressure drop than a 300 mD core run at the same time under the same operating conditions. Any reference to these permeabilities later in this thesis will be made as an attempt to distinguish between cores and not to describe the samples themselves. The values will be

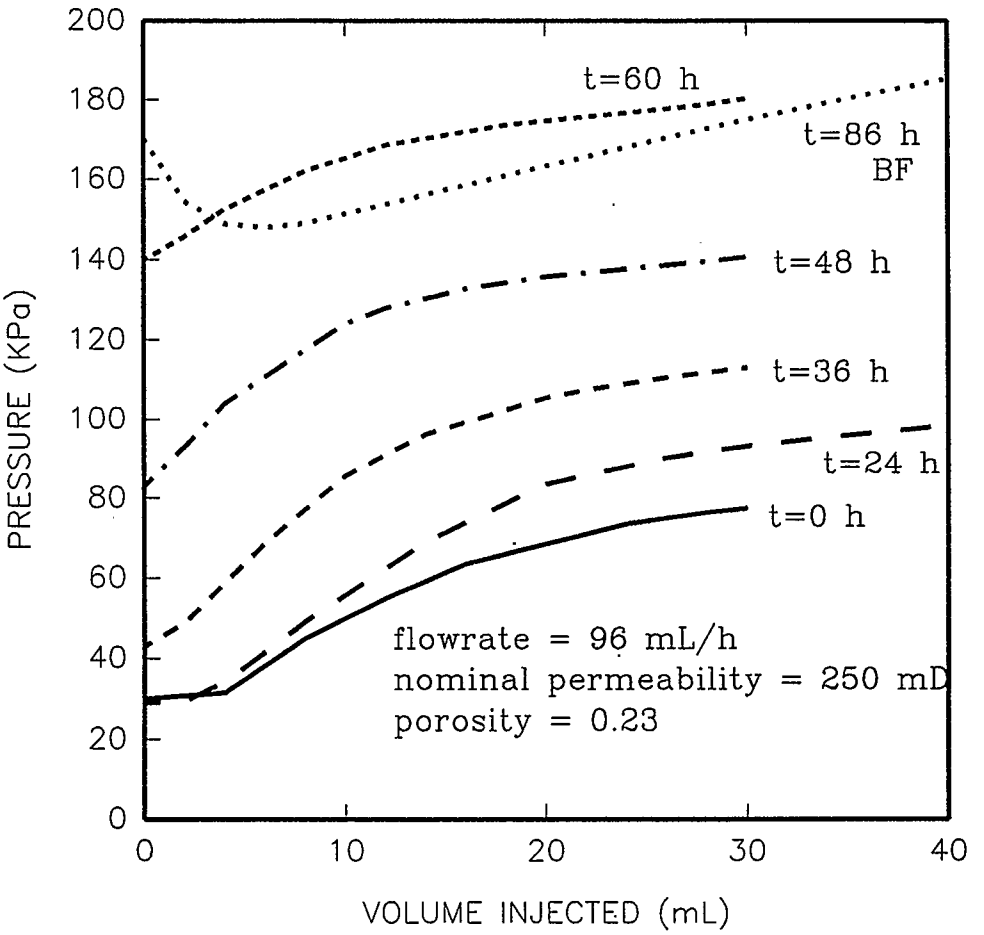

Figure 3.3 - Pressure Profiles for a Control Core (No Bacterial Inoculation) Containing Clay Fines

considered nominal only.

Figures 3.4 and 3.5 show pressure profiles for core A (300 mD) and core B (400 mD) respectively. These experiments were run as discussed in the procedure section with UMB injection followed by BHI media flooding and each core was treated identically. The only difference between the two cores was their actual starting permeabilities which can be calculated from the pressure drop at t = 0 h.

The beginning of each profile, while erratic, is insignificant to the experiment as the starting pressure varied for each flood. It is the last half of the profiles that show the core response to the flooding. The pressure drop in both cores increased for each time step until t=48 h. After this flood, the pressure increases very little for core A and not at all for core B. This is where the bacterial growth has come into play. The bacteria plug up many of the core channels so that the flow only passes through certain areas. If the flow had an alternate route, this core would most likely be plugged entirely with no flow passing through. In these experiments however, flow is forced through the core and so the fluid must find a channel from inlet to outlet. As this same channel is used for every flood after the bacterial plugging stage, any clay fines not already held in place by slime get washed through or into other slime - filled places to become

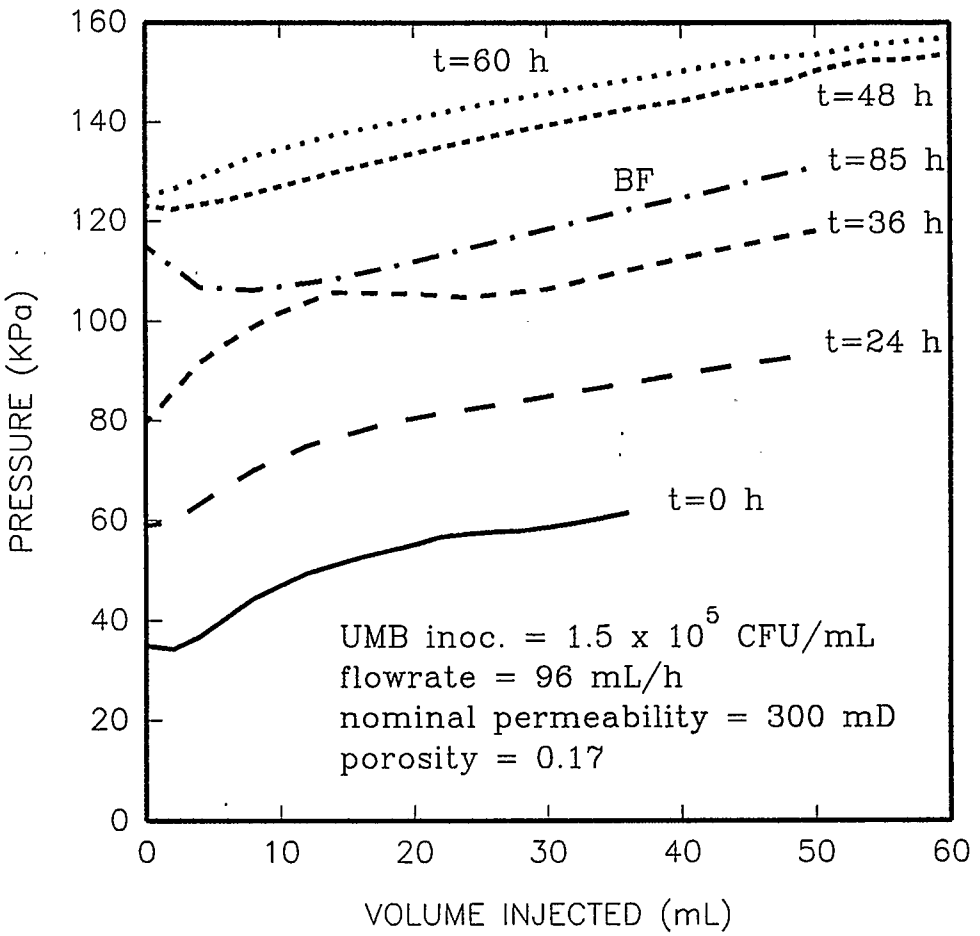

Figure 3.4 - Pressure Profiles for Core A (Clay Fines)

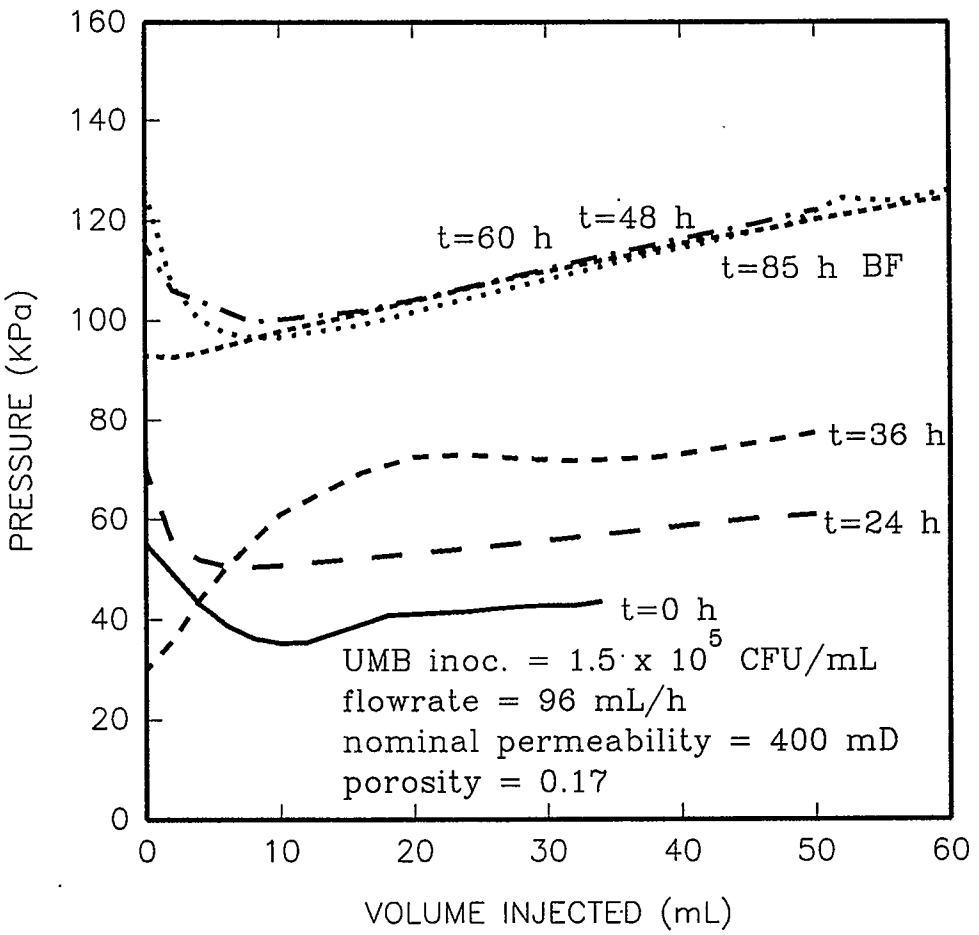

Figure 3.5 - Pressure Profiles for Core B (Clay Fines)

stabilized. Core B shows a very stable plug as the backflow test at t = 85 h still shows the same pressure profile. Core A does not seem to have the same stability. The pressure still increased, albeit slightly, after t = 48 h and the backflow test decreased the pressure substantially. This seems to imply that the smaller permeability core does not allow for UMB and nutrient migration as uniformly as in Core B. Core B may have larger pore throats and allow access to more parts of the core so that the plug is solid and not intermittent and therefore vulnerable to backflow.

With all of the problems encountered with the rock samples obtained, an error in the experimental setup was also suspected. This was discounted when one rock sample marked 700 mD was tested and found to behave exactly as it should for its marked permeability. This rock was included in the samples from the Biology Department but must have originally come from a different source as SEM tests showed that this rock did not have the same overwhelming clay problem as the others. Unfortunately, the sample was only big enough to form one core for experimentation.

Figure 3.6 shows the pressure profile for the test with core C (700 mD). The data is presented differently than in the previous figures because the pressure drop during each flood was much more stable. There was no need to show the pressure

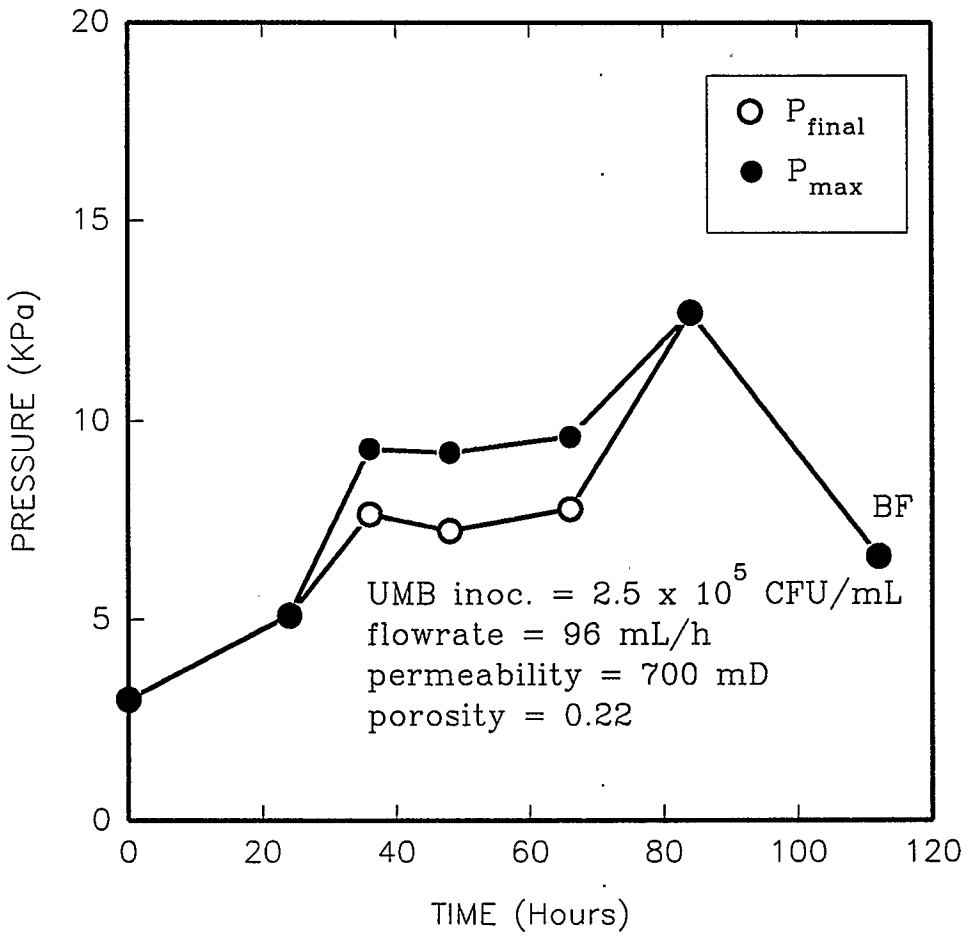

Figure 3.6 - Pressure Profile for Core C (No Clay Fines)

change over the duration of each flood because there was little or no change.

As bacterial growth and plugging became prominent, the pressure drop was slightly erratic at the beginning of each flood until stability was reached. The lower profile in the figure shows the final pressure reading at each time. The upper profile shows the maximum pressure drop encountered through the test, usually during the early erratic phase. The maximum pressure drop encountered during this behavior gives an indication of the extent of plugging before a path is cleared for the fluid to flow through. At early times, growth is minimal and so the maximum and the final pressures are the same. Once growth takes over, after about 24 h, the core can be imagined to have biomass throughout. However, the cells and slime would not necessarily be well packed or established so the pressure jumps at the beginning but decreases as the biomass is pushed into non-flow pore space and through into the effluent. At t=80 h the biomass has reached stability in the non-flow areas and has started to impeach on the flow channel that the fluid is attempting to use. Also, the number of bacteria in the core is very large and so a filter cake starts to form throughout the core, causing the pressure to increase throughout the test until the flow is stopped. This is why the maximum and final pressure drops are once again the same. The backflow test serves to remove some of the excess

biomass forming filters and also to change the channel of flow and remove more bacteria. The permeability still remains about half of the original even when backflow is applied.

Figure 3.7 shows the bacterial effluent counts collected at each time test for the three cores. Cores A and B allowed very little of the initial UMB injected to travel through into the effluent. The bacteria concentration was decreased by nearly three orders of magnitude by being passed through the rock. This indicates that nearly all the UMB remained in the core after injection and subsequent nutrient flooding although the clay fines may have aided this retention. It also shows that UMB did not just gather at the entrance and form a skin plug as at least some made their way through. Core C had opposite results. The effluent concentration was smaller but of the same order of magnitude as the inlet concentration. This indicates that for the higher permeability, UMB have no problem permeating completely through the core while still leaving some bacteria behind.

After the initial shut in period of 24 hours, it can be seen that growth has been initiated in all of the cores. Cores A and B show an increase in microbes but the stable count is not reached until after another shut in period of 12 hours. Core C shows that its stable level can be achieved immediately after the first 24 hour period. This may have to do with the

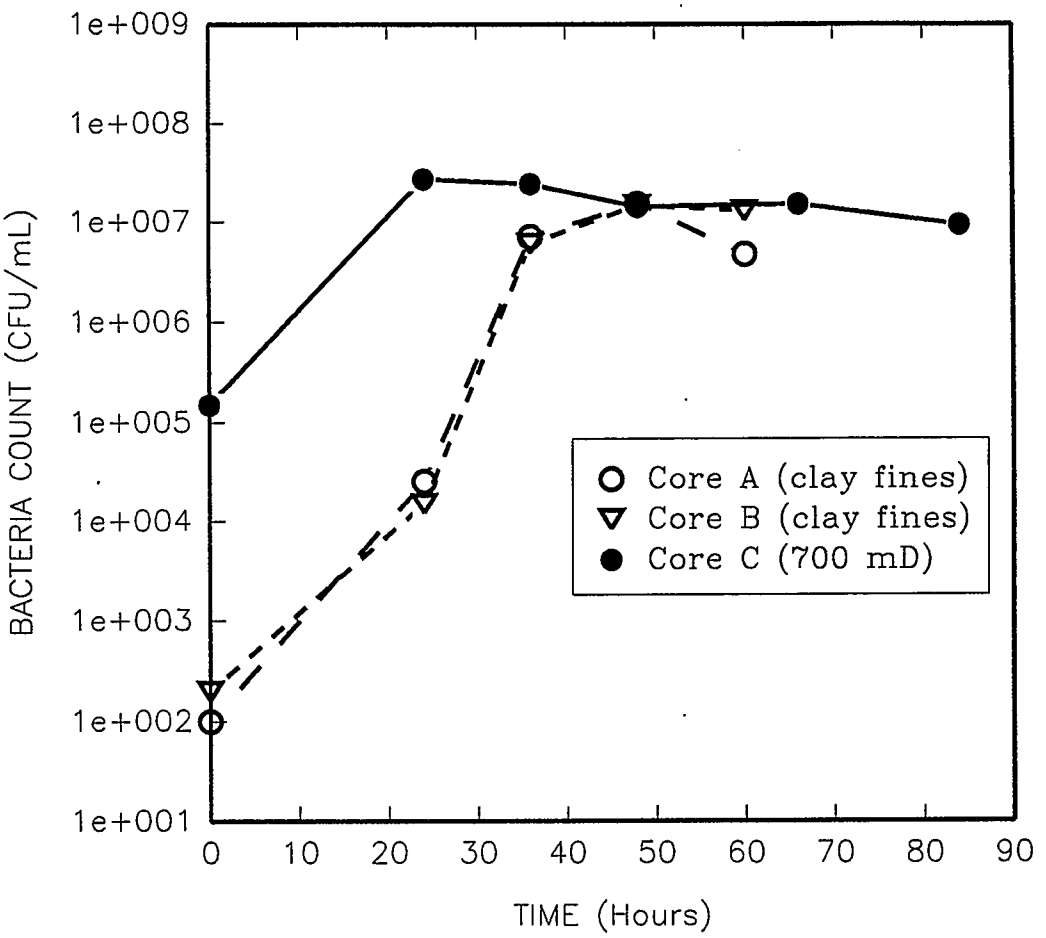

Figure 3.7 – Effluent Bacterial Concentrations for Cores A, B, and C

fact that it is easier for the nutrient to reach the UMB through the clean pore space with little clay to hinder transport. It could also be that cores A and B do not allow the bacteria themselves through because they get stuck up in the spaces blocked by clay fines. The stable level may only be reached when the effect of these clay particles has diminished.

In all three cases, the backflow experiments also had their bacterial numbers monitored. All of them were of the order of 10^8 CFU / mL, ten times higher than the other counts. This is as expected because the forward flow path will probably still be used by the backflow to wash through the regular counts but any filters formed will also be partially washed out. Another factor is that the biolayers have grown to withstand forward flow and do not have the same strength or anchorage when backflow is applied.

3.3 Sandpacks with BHI

After the unexpected problems encountered with consolidated cores, a decision was made to use sandpacks for further research. Although these packs were of higher permeabilities, they were easier to handle in both the sterilizing phase and

during post - experimental analysis. Also, sandpacks are not the best representative for reservoir rock but act very similar to a soil ecosystem through which bioremediation procedures would take place.

Figure 3.8 shows the pressure profile for Pack A. Again, the two lines represent the final pack pressure drop and the maximum value reached during a particular flood. The largest discrepancy can be seen at 48 hours where the maximum pressure indicates a permeability decrease to less than 25 % of the original value. As seen with the consolidated cores, after 48 hours the pressure drop stabilizes and the biomass appears to have completed its plugging action.

Figure 3.9 shows similar profiles for pack B but there are some noticable differences. The difference between the two packs is in the UMB injection scheme. Pack A had two pore volumes of UMB injected at a concentration of 10^5 CFU / mL. Pack B had only one pore volume of UMB injected but the concentration was 10^7 CFU / mL. Both cores started with nearly the same permeability and nutrient flooding (BHI) was also maintained constant. The differences in profiles should be attributed to higher UMB concentration versus higher volume of UMB fluid injected.

The first major difference occurs after the first shut - in

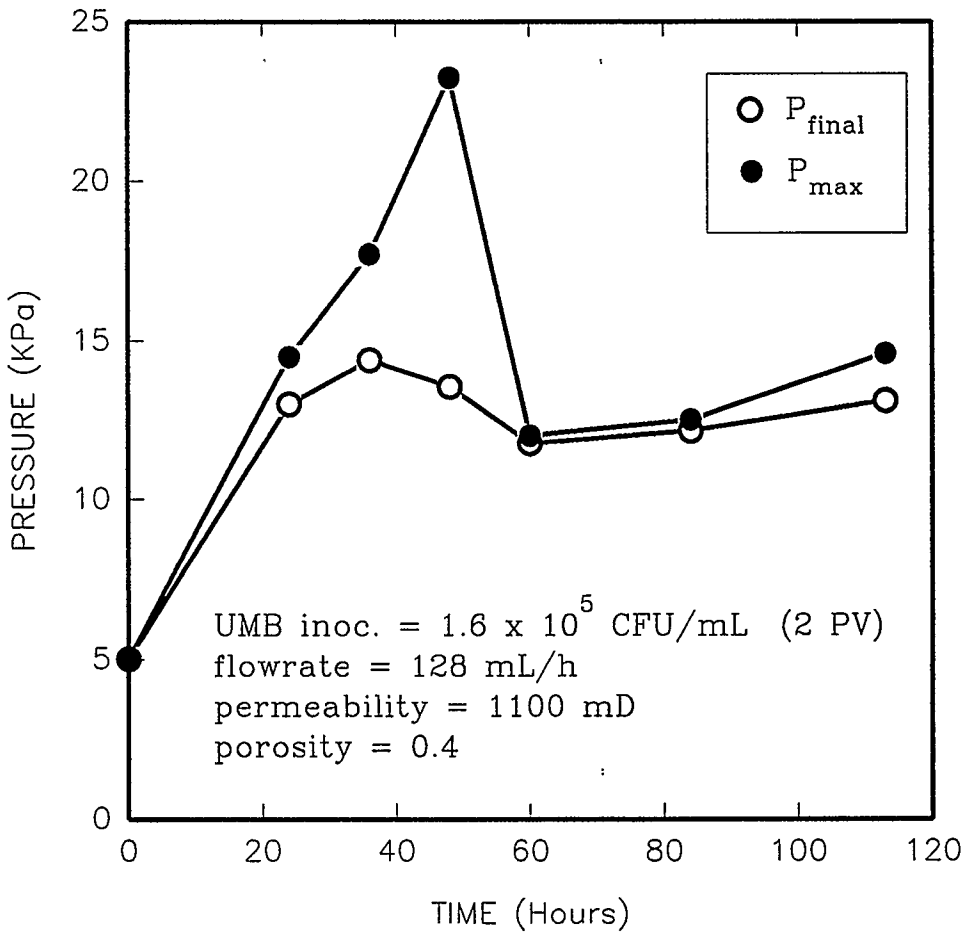

Figure 3.8 - Pressure Profile for Pack A

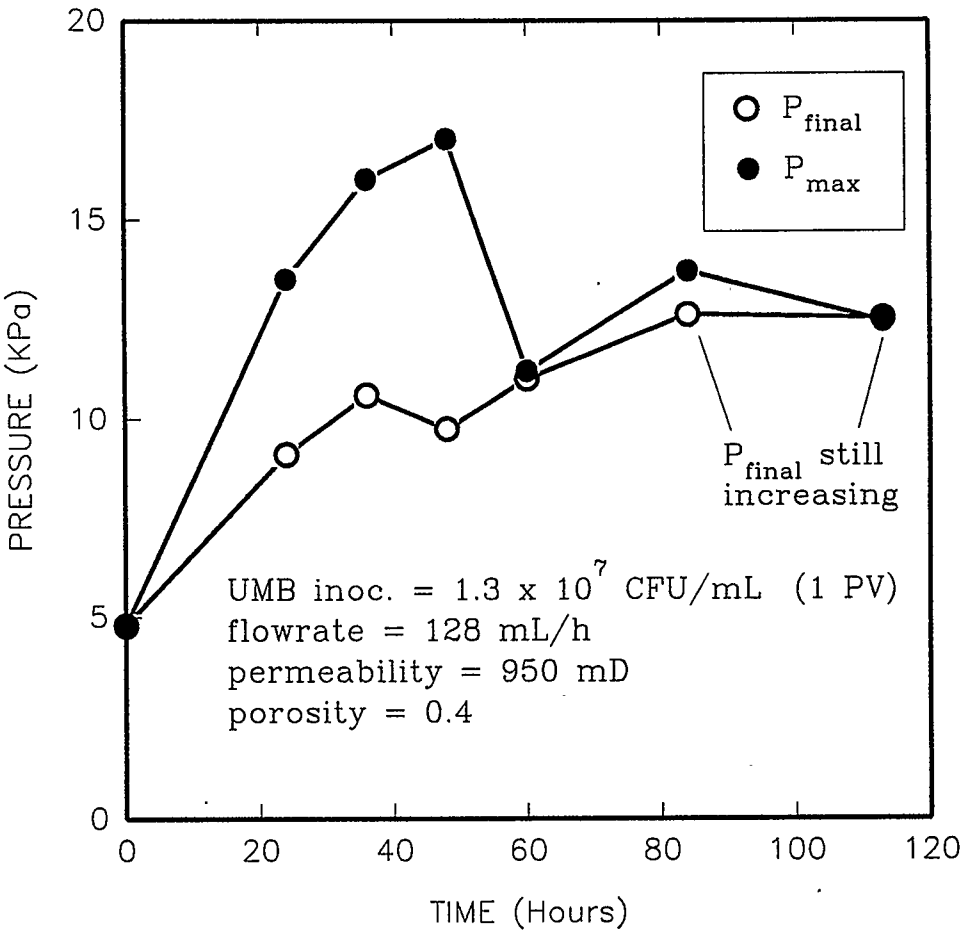

Figure 3.9 - Pressure Profile for Pack B

period was complete. Pack A showed a substantial increase in pressure drop but very little difference in the maximum value and the final value. On the other hand, pack B had a large maximum pressure drop but any plugging that had taken place must have been unstable as the final value was considerably smaller. At the peak of the growing phase (24 - 48 hours), the packs behaved similarly with large initial drops accounting for rapid biomass growth and then lower final pressures as biomass in the flow paths was eroded.

There are some minor differences in the last few data points that is not evident in the figures. The results from pack A remained constant in that the pressure drop maximized and then decreased somewhat and became stable. Pack B did not behave this way. The pressure drop would maximize and then decrease slightly but then it would slowly increase right up until the flood was completed. In fact, at the final experimental time a maximum was not reached but instead, the pressure climbed throughout the duration of the flood.

The variations between pack A and pack B must come from the difference in UMB injection strategy. It may be that pack A becomes more uniformly seeded with UMB due to the more dilute mixture and the higher injection volume. Since pack B has less injection volume but more UMB concentration, it may become seeded more erratically and many parts of the pack may

not be seeded at all. This would explain why there is a difference in maximum and final pressures right from the beginning. After the first shut - in period, growth has occured in both cores but while biomass is everywhere in pack A, pack B would have patches of growth here and there with clean flow channels in between. The subsequent nutrient flood (t = 24 h) will wash out a fair bit of biomass along the paths of least resistance. Pack A can afford this loss and still retain a large number of plugged pores which is why the maximum and final pressure drops are comparable. When pack B loses some biomass, it does not leave nearly as much plugging in other areas of the sand and so the pressure drop decreases to a much lower value when flooding is complete.

The differences in the final portions of the tests are harder to explain. Pack A can be assumed to have formed a uniform plug through the sand at this time and so the small pressure variations will be due to washing away of biomass infringing on the established flow region. While this would also be happening in pack B, there is also pressure increase to be accounted for. This increase is most likely due to a filter cake effect with biomass being stripped from the front of the pack and causing filter plugging further along. The reason this happens in pack B but not pack A must have to do with the types of pore spaces being plugged. If the large UMB concentration caused erratic seeding and plugging, perhaps the

remaining flow channels are small and numerous and so more susceptible to filter cake formation.

The effluent bacteria counts are plotted in Figure 3.10. It can be seen that counts are nearly identical for both packs. This is as expected because the bacterial growth cycle will be influenced by bacteria species and nutrient only and these were constant in both packs. It does show that the shut - in periods of 12 hours appeared to be sufficient for maximum bacterial growth. The counts do not decrease but seem able to replenish their losses each time. Of importance is the initial count values. Each pack retained all of the UMB that were injected. This can be compared to the consolidated cores in which effluent values were at least countable if not equal in magnitude to injection numbers. The concern here would be that the UMB were not permeating the packs but getting stuck at the opening and forming a skin plug. This does not happen and can be proven in two ways. Firstly, a skin plug effect would show up in pressure drop readings during initial flooding and the pressure stayed very constant throughout. Secondly, later times show a large number of bacteria in the effluent. Vegetative bacteria would certainly not be able to move through an area that UMB did not; therefore, skin plugging was excluded as a possible factor.

Table 3.1 shows the number of bacteria per gram of sand at

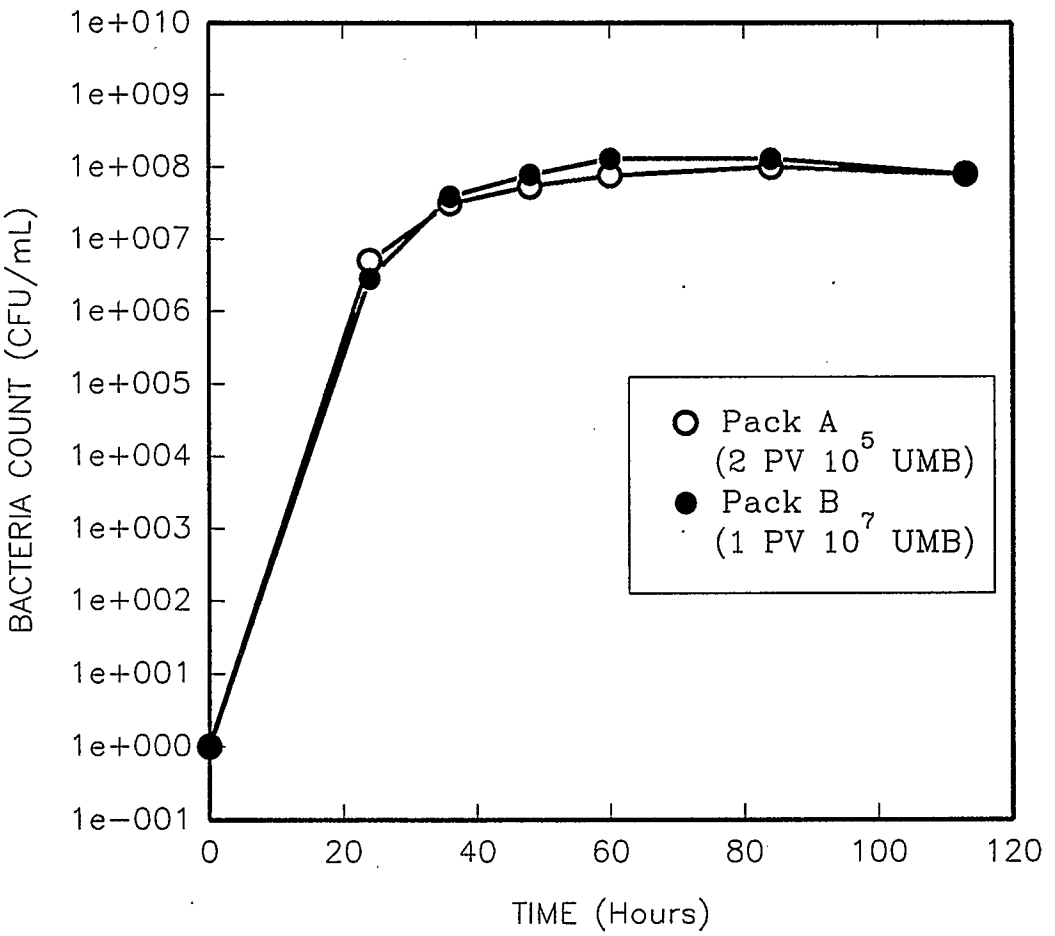

Figure 3.10 - Effluent Bacterial Concentrations for Packs A and B

Location Along Length of Sandpack	Pack A 10^6 CFU / g sand	Pack B 10^6 CFU / g sand
Inlet (0 inches)	127	266
2 inches	107	67
4 inches	155	129
Outlet (6 inches)	150	91

Table 3.1 - Bacterial Counts in Sandpacks A and B

different locations along the pack. All of these samples have very similar numbers. This is not surprising as the pack porosities were the same and sandpacks are generally very homogeneous throughout. Since every sample point would be expected to have approximately the same amount of pore space available for growth, the same bacterial species present and the same nutrient source, the bacterial numbers should correspond as well.

Variations in the counts could be due to a number of factors including experimental error but the order of magnitude is the important consideration. As all of these counts corrspond to approximately 10^8 CFU / g sand, the variation can be considered negligible.

3.4 Sandpacks with SCM

BHI is considered an extremely rich media and so additional experiments were performed using the leaner nutrient SCM. Three sandpacks were made and set up for UMB and SCM injection. All three packs were injected with one pore volume of UMB suspension containing 10^5 CFU / mL followed by one pore volume of SCM and shut - in. Pack C had a permeability of 1.3 D, pack D was 1.7 D and Pack E was 1.2 D. Figures 3.11, 3.12,

and 3.13 show the pressure responses for Packs C, D, and E respectively. It can be seen that for the first 60 hours of the experiments there was no apparent plugging in any of the packs. It was suspected that a problem had occured during UMB injection but this could not be checked against effluent counts as they take several days to appear particularly when the cells are in their UMB phase. At 60 hours the packs were reinjected with another pore volume of UMB and some procedural changes were made.

Pack C (Figure 3.11) was immediately followed by SCM injection as before. The pressure still did not react after another 24 hour shut - in period but subsequent floods did show favorable plugging results. It can be seen that there is a very large difference between the maximum pressure reached and the final pressure achieved when plugging starts. Also, even when considering the maximum pressure values, the permeability does not drop much below 40 % although it may have if the test had been run longer.

The lack of plugging is due to the use of SCM as the nutrient source. As was seen during the growth experiments described in Figure 3.1, cells grow considerably slower and reach lower final concentrations than when grown on BHI media. Less biomass or lower final concentration will result in less overall plugging. The slower growth would be partially

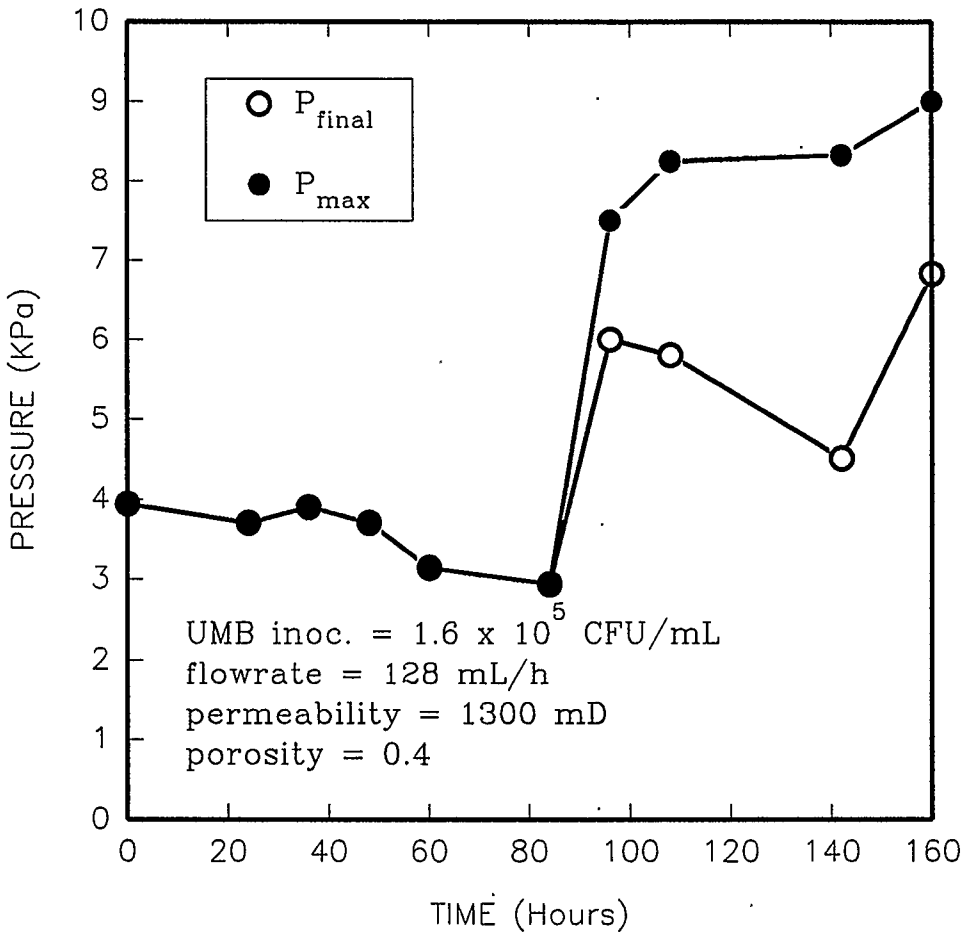

Figure 3.11 - Pressure Profile for Pack C

responsible for the lack of pressure response at the start of each test, but the difference in the growth curves of viable cells does not seem to be so great as to cause a dormant time of 60 hours. One explanation could be that UMB resuscitation magnifies the effect of the lower growth rate seen in viable cell profiles. Perhaps a media that is only slightly richer to vegetative bacteria is many more times so to UMB. Alternatively, it is known that bacteria often take time to become accustomed to new environments before beginning their exponential growth which would be illustrated by a longer lag phase. Perhaps it takes them much longer to awaken when the nutrient they are reintroduced to after starvation (SCM) differs from the one in which they last grew on (BHI). The small lag phases noticed in Figure 3.1 are for bacteria that have been growing on healthy, replenished media for two or three days while UMB are subjected to the new media with no preconditioning whatsoever.

Figure 3.12 demonstrates a contrast with Figure 3.11. Pack D was injected with one pore volume of UMB at 60 hours but flooded with BHI instead of SCM. It can be seen that rapid growth occurs immediately and the permeability decreases to only 20 % once plugging starts.

Figure 3.13 shows that pack E was treated differently from either of the others. Originally it was shut - in for 48

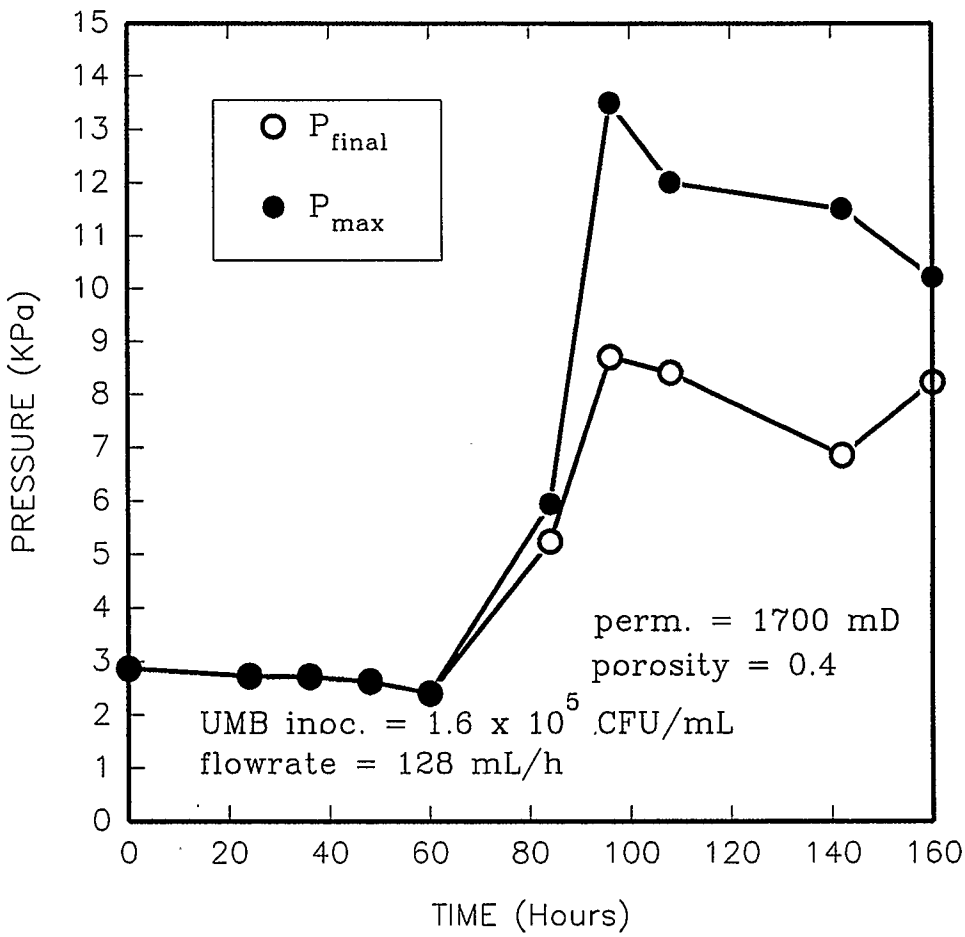

Figure 3.12 - Pressure Profile for Pack D

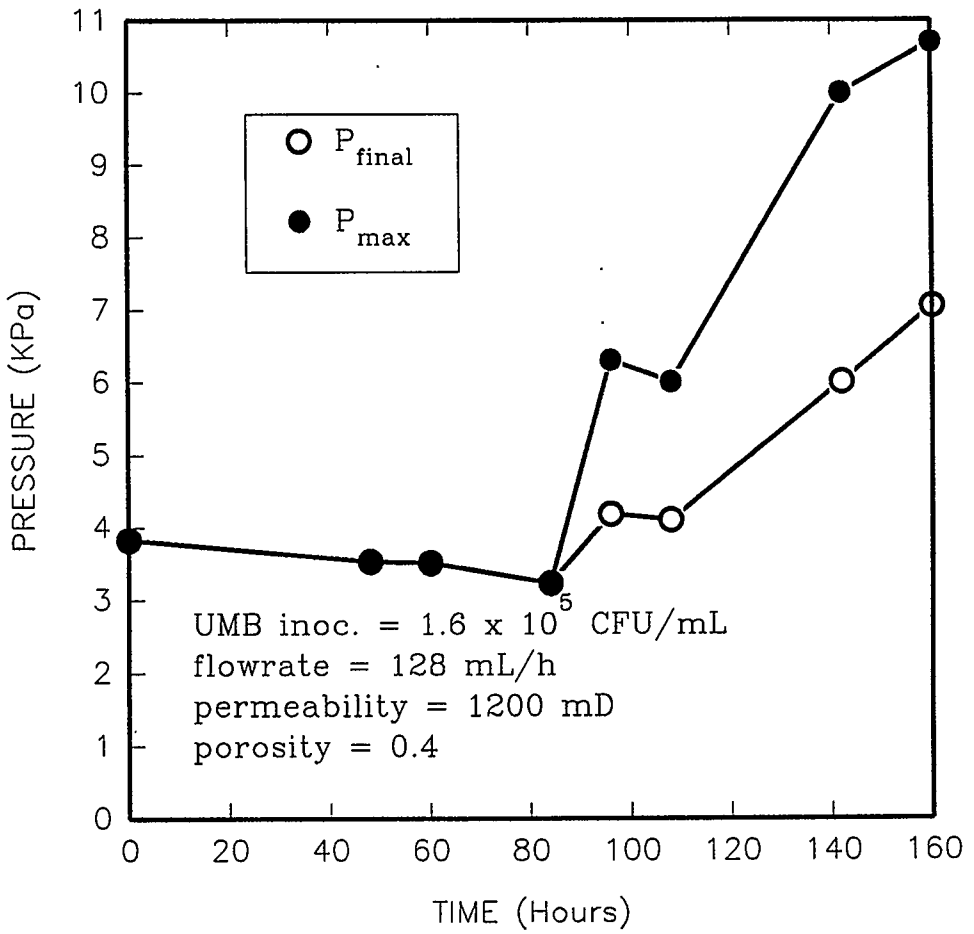

Figure 3.13 - Pressure Profile for Pack E

straight hours but as the growth was very slow, no conclusions could be drawn from this. At 60 hours, the pack was first injected with one pore volume of BHI and then with one pore volume of UMB and shut in. This treatment was performed in response to a conversation with an Imperial Oil researcher who mentioned that enough nutrient may remain in the pack even after washing through with the UMB solution. It can be seen that not enough nutrient remained as no pressure increase, and therefore little growth, was realized after another 24 hour incubation. The remainder of the floods for this pack used BHI and, as can be expected, growth was immediate and rapid and the pressure was still increasing when the experiment was completed.

Pack E can also be compared to pack C where SCM was used throughout (Figure 3.11) because these packs had very similar initial permeabilities. Figure 3.13 shows a maximum pressure drop of nearly 11 kPa and rising after only 80 hours of BHI treatment. Figure 3.11 has a final drop of under 9 kPa after 160 hours of SCM treatment. For plugging concerns alone, it is obvious that BHI is the better media to use. However, long zones in which a deep plug is desired may not work with BHI as the UMB will start plugging before the zone can be completely permeated. SCM injection would allow a long time for the nutrient to penetrate the zone before the UMB at the injection point start to use the media and plug the finger.

Figure 3.14 shows the effluent concentrations for the three packs over the duration of the experiment. These counts show that suspicions of faulty initial UMB injection were unfounded. All of the UMB remained in the packs as was seen with packs A and B before. Over time, the bacteria did awaken but at a very slow rate as can be seen up until the 60 hour mark which is where the additional pore volumes of UMB were injected. From this point on, the effluent counts are very high. This may partially be due to the extra UMB injected. As counts show that growth was slowly occuring, many flow channels may have been plugged without noticably affecting the pressure drop. This would increase the non-plugged pore velocities and so many of the newly injected UMB would pass directly into the effluent instead of depositing on the sand, driving the effluent counts up.

Table 3.2 shows the viable cell counts in the sandpacks after they were dismantled. These results differ from the counts for packs A and B in that all of the bacteria grown on the plates was not uniform. Although the UMB and the vegetative bacteria are the same species, they can be differentiated on an agar plate because healthy bacteria will be visible after 24 hours of incubation while the starved bacteria take up to 5 days before counting is possible. There was also a contamination involved. This contaminant is an unknown

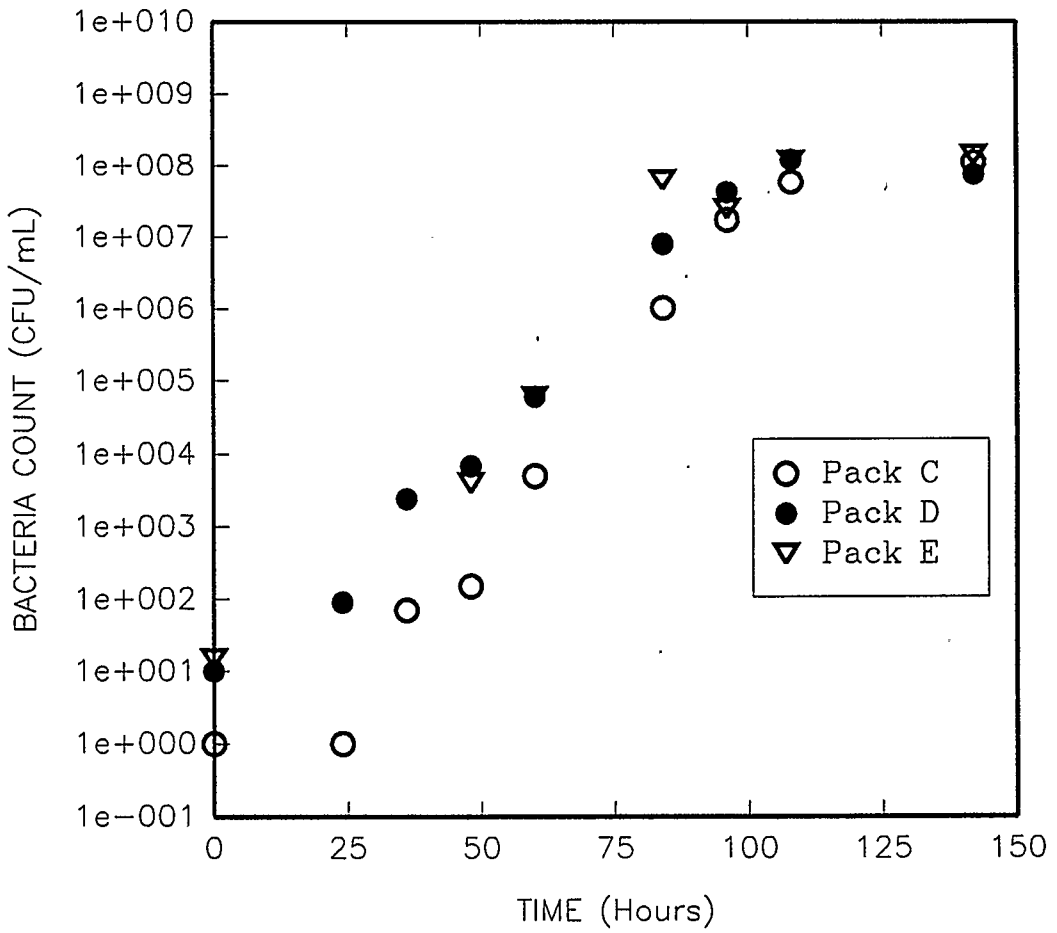

Figure 3.14 - Effluent Bacterial Concentrations for Packs C, D and E

Location Along Length of Sandpack	Bacteria Counts Measured in 10^6 CFU / g sand		
Pack C	Vegetative	UMB	Contaminant
Inlet (0 inches)	94	0	0
2 inches	48	0	0
4 inches	57	0	0
Outlet (6 inches)	87	0	0
Pack D	Vegetative	UMB	Contaminant
Inlet (0 inches)	179	0	0
2 inches	112	0	0
4 inches	72	7	0
Outlet (6 inches)	82	600	60
Pack E	Vegetative	UMB	Contaminant
Inlet (0 inches)	35	4	0
2 inches	60	44	148
4 inches	350	645	108
Outlet (6 inches)	105	518	52

Table 3.2 – Bacterial Counts in Sandpacks C, D, and E

species of bacteria that is present in the original UMB solution. It is not a factor in the experiments because it grows very slow even when compared to the UMB. Actually, it is very likely that this contaminant has become a UMB as well. Also, the cultures formed on agar are small, relatively slime - free and orange in color. They would not make a good plugging agent and they do not cause any problems with the counts due to their distinctive color and culture size. The contaminant can be thought of as a tracer as it does not effect the plugging but can be counted and its location noted as indicated in Table 3.2.

The UMB appear in these packs because they were re - injected in the middle of the experiment. At this time, there were already numerous vegatative bacteria present as can be seen from Figure 3.14 even though plugging had not started. When fresh nutrient followed the UMB injection, the viable cells used it for their exponential growth, leaving lower concentrations for the UMB resuscitation and so many bacteria remain starved.

Pack C, which was flooded with SCM throughout the experiment, had no UMB remaining after the test. This may be due to the fact that the growth rate in SCM is slow enough that the nutrient concentration is not depleted very quickly and so UMB resuscitation is hindered only slightly. Packs D and E were

both flooded with BHI after UMB re - injection. There was a 24 hour lag, however, in pack E as the UMB were injected after the nutrient in this pack and not the other way around as in D. This lag would account for the differences evident in Table 3.2. It is also interesting to note that the UMB and the contaminant appear in large numbers at the end of the packs with very few present at the inlet. This could be due to a couple of phenomena. Firstly, they may act as fines and just be pushed further along as more volume is pumped through. Secondly, because these organisms are alive, they could be stationary and slowly growing. The inlet bacteria obtain fresh media all of the time while further inside, the nutrient may already be depleted or diluted with old media depending on the flow patterns. This would account for the UMB at the front of the core disappearing into vegatative cells while the ones at the outlet still stay dormant. The contaminant bacteria may also be affected in this way. In laboratory growth experiments performed in flasks or tubes, these orange cultures disappeared very quickly. The vegetative *K. oxytoca* may produce some type of byproduct that is toxic to the contaminant and slowly kills it. Again, the contaminant can be seen to live much longer at the end of the pack where the growth would not be as vigorous and so the environment would not become lethal as quickly. Most likely, both cell transport and growth are responsible for the observed results. Taking packs D and E as similar situations at different time

steps, it would be expected that one or two floods later, all traces of UMB and contaminant would disappear.

3.5 Comparison with Published Work

As mentioned in Chapter 1, UMB technology is still relatively new and so experimental work is not widespread. The lab of Dr. Costerton at the University of Calgary has provided the only published data of UMB plugging in MEOR research. Comparisons are difficult as their work varied in some very fundamental ways.

Firstly, much of their research was done using very small cores (5 cm long, 1 cm diameter). Because of the small sizes, injection of hundreds or thousands of pore volumes of UMB and nutrient could be performed (Lappin - Scott et al., 1988). This will have the effect of creating much more of a permeability decrease due to UMB filtering before growth even occurs. In a field situation, injections would be limited to just one or two pore volumes. As seen earlier in this chapter, this was the injection scheme used in the research presented in this thesis.

Comparing the same two sets of research, another experimental

difference can be found. While the research in this thesis used constant flowrate and measured pressure drop, the other work used constant pressure drop and measured flowrate. The problem with using constant pressure drop is that measurement of the flowrate can be quite inaccurate. The effluent is simply collected at the core outlet and then divided by the flooding time to determine the rate of flow. This will give a broad average over the duration of the experiment. When using constant flowrate and measuring pressure drop, variations in the flood can be monitored and analysed, immediately. This will give a better idea of what is happening during plugging. It is very beneficial to have the ability to measure pressure drop at any time rather than getting just one value for the whole experiment. For example, this extra analytical ability was instrumental in realizing the clay fines problem.

Rather than follow previous work, it was decided to change the procedure as mentioned above to examine different facets of UMB plugging. Even so, some broad comparisons can be made with the published data. UMB were found to fully penetrate cores and provide plugs through the entire porous media in all of the published work as well as in this work. Also, bacterial effluent counts were similar and cell numbers in dissected cores were also comparable. These comparisons help lend validity to this research even though most of the results

and plugging profiles have not been previously published.

3.6 Application of Results

The experimental research performed in this study can provide invaluable information with regards to application of porous media plugging in the field. The results may be linked to expected field trials in many ways.

The time frame involved would be the major concern when scaling up a procedure of this type. As the nutrient in this study was not anaerobically prepared, sufficient oxygen was assumed to be present to support aerobic growth of the bacteria. In a reservoir, the nutrient would not be as rich and would not be replenished as often because of the larger volumes and longer times involved. Because of this, anaerobic bacterial growth would dominate and, generally, this type of growth is much slower. This would lead to longer shut - in times for inoculation. Also, a reservoir must be able to transmit fluid from injection well to production well relatively quickly to avoid any chance of skin plugging. The fluid transport should take no longer than one week and each shut - in time would be one or two weeks leading to an overall procedural time of two or three months. Of course these are

approximate times as the process will be very dependent on bacterial species, reservoir conditions and nutrient source.

One positive factor in scaling up is that fewer nutrient injections would be necessary in the field. This is due to the fact that high permeability zones will be surrounded by tighter, alternate pathways in an actual reservoir. As the plug is formed, some flow will go around the biomass into these tighter regions and so the bioplug will not be eroded each time to the extent observed in laboratory tests. After the original UMB / nutrient injection, one or two subsequent injections may be all that is required to plug the zone.

Expense is always a factor in tertiary recovery methods but UMB plugging can be relatively inexpensive. Firstly, the injection volumes would be expected to be low as the zone targetted for plugging would only be a small percentage of the total reservoir volume. Also, many nutrient sources may be waste products from other industrial procedures and so would cost very little to obtain. Lastly, UMB are very inexpensive to produce in high numbers after which they can be easily diluted to the desired concentration for injection.

The experiments in which clay fines were present provided interesting results with respect to the stabilization of the clay fines. In this study, the plugging action of the clay

fines was simply replaced by plugging due to biomass. Perhaps a procedure could be developed in which biomass may be grown enough to stabilize the fines but to allow flow to pass through, as well. Alternatively, the biomass plug may be treated after it is formed to allow flow while still retaining the clay fines. Further research into these procedures would be necessary to determine if they are feasible for this type of work.

CHAPTER 4

MEOR PLUGGING SIMULATION

As demonstrated in the experimental discussion, the process by which porous media becomes plugged with biomass may be affected by many parameters. It would, therefore, be very useful to have a simulator that would predict how well an MEOR procedure would work when certain variables are changed. A number of simulating attempts were referred to in the Chapter 1, but none of these described the particular strategy used for UMB plugging.

4.1 Important Concepts in the Simulation

Some simulators treat bioplugging as a continuous flow system. In reality, this would be much too expensive to use in the field. As long as nutrient is supplied to all of the desired areas, the well can be shut in while the bacteria grow. UMB, in particular, may take up to a week before resuscitation and plugging occur. Since much of this time involves the bacterial lag phase, nutrient is necessary but not used in large quantities and so one slug of media will last for quite some time. Even after the UMB have grown into vegetative

cells, nutrient would only be needed at intervals and can be injected in slugs and then the well can be shut in again.

One of the more important stages in MEOR plugging is the initial injection phase. More specifically, the rate of adsorption of bacteria needs to be predicted. If UMB are adsorbed very quickly, there could be a danger of skin plugging or a lack of full penetration through a zone. If, on the other hand, they adsorb very slowly, very high concentrations or injection volumes would be needed. UMB retention may be due to physical filtration or physical - chemical interaction such as attraction due to surface charge (McDowell -Boyer et al., 1986). These factors are very hard to predict and so simulation becomes difficult and inaccurate (Cheneviere et al., 1989).

Parameter estimation is necessary to model adsorption alone as well as when describing physical filtration. It would be easier just to observe the number of UMB adsorbed in an experimental situation that models the reservoir conditions. One overall parameter may then be developed that will take into account all of the factors relating to UMB retention. This parameter will be a type of seeding variable that can be calculated by determining the number of UMB retained in the rock after injection and nutrient flooding. This number may be expressed as a fraction of the total pores in a cross -

section that are seeded by a UMB as compared to the total number of pores available.

Also necessary will be a reseeding parameter which will be used after the first shut - in period. During the next nutrient injection, newly vegetative bacteria will be stripped away from their former pores and forced into previously unseeded pores. Again, reseeding may be expressed as the fraction of clean pores that become seeded during flow divided by the total amount of clean pores remaining. This value cannot be easily calculated but instead can be varied in the simulator to observe its significance. In order to determine an actual reseeding value, back - calculating from experimental results could be performed.

Using seeding and reseeding parameters provides a way to describe a very complex flow phenomenon with two simple values. The UMB plugging procedure can now be modelled based on the static bacterial metabolism. This allows for focus to be placed in the area which will be most responsible for the success of an MEOR treatment, bacterial behavior.

4.2 Program Equations

The program is based on UMB flooding of a high permeability zone immediately followed by nutrient injection. The reservoir is then shut in to allow enough time for the UMB's to be stimulated and to produce cell mass and slime sufficient to plug the pores they inhabit. After this time, nutrient may be injected again to replenish the supply and allow for cell movement. The well will be shut-in once more and plugging will continue. The important phases of the simulation and the useful equations are described below.

Firstly, the core seeding must be estimated. As mentioned before, this seeding parameter defines the fraction of pores in a core cross-section that contain a UMB after injection. When the core is shut-in, only these pores will be plugged until subsequent nutrient injection takes place.

Zero time is assumed to be the time at which the UMB's become vegetative and begin to grow exponentially. The UMB lag phase is neglected for the simulation as nothing occurs during this time. It would be simple to add on the required lag phase time at the end of a simulation study when considering an actual field test.

At each time step the following parameters are calculated:

A. Bacterial Growth - The vegetative cells are assumed to grow at an exponential rate whenever nutrient is present. The kinetics of exponential growth follow the Monod equation:

$$\mu_b = \mu_{max} * \frac{[S]}{K_s + [S]} \qquad (4.1)$$

μ_b is the specific growth rate (related to doubling time) defined before in Chapter 1. K_s and μ_{max} are parameters that are specific to the bacteria and substrate type that is used while [S] is the substrate concentration and can be expected to decrease during growth as it is utilized by the organisms. Regular growth profiles can be used to determine specific growth rates for different concentrations of substrate as mentioned in Chapter 1. A plot of these growth rates versus [S] will then allow for determination of the two parameters μ_{max} and K_s. The value of K_s will be indicative of the reliance of the bacteria on substrate concentration. A low value means that the cells will grow at nearly the same rate until there is very little nutrient remaining. High values demonstrate a very high dependence on nutrient amounts.

Once the rate is determined, bacterial counts can be calculated according to the exponential growth equation where:

$$\frac{N_{cell}}{N_{init}} = e^{\mu t} \qquad (4.2)$$

B. Substrate Utilization - As the bacteria grow, the substrate concentration will gradually decrease as it is used for microbial activity. The kinetics of this process are as follows:

$$\frac{dS}{dt} = -\alpha * \mu_b * N_{cell} - \beta * N_{cell} \qquad (4.3)$$

In this equation, α and β are parameters specific to the organism and the type of substrate used. The rate of nutrient depletion will directly affect the growth if K_s is large in the Monod equation. If K_s is relatively small, equation 3 will only be useful to calculate when the nutrient is entirely used up.

C. Slime Production - As the bacteria grow, they produce a slime that greatly enhances their plugging ability. This slime is produced at a rate that is dependent on the cell count as well as the cell growth rate:

$$\frac{dP}{dt}=\alpha_2*\mu_b*N_{cell}+\beta_2*N_{cell} \qquad (4.4)$$

Again, α_2 and β_2 are parameters specific to the organism and the nutrient type. Low slime - producers will have very low parameters while high slime - producers may have values that allow for the bacteria to produce ten times their volume in slime.

4. The Carman-Kozeny equation is used to relate the permeability and the porosity. Since biomass will choke off pore space, corrected porosities can be calculated and then the following equation can be used.

$$k=\frac{\phi^3}{5*S_o^2*(1-\phi)^2} \qquad (4.5)$$

The parameter, S_o, in this equation is calculated using initial porosity/permeability data and then kept constant throughout the remainder of the plugging procedure.

4.3 Program Assumptions

The program uses the equations from above to solve the

plugging system. Before explaining the simulation method, some of the prevailing assumptions should be explained.

1. It is assumed that there is no nutrient loss due to adsorption. It is assumed that enough nutrient is originally pumped through the rock so that it becomes adsorption saturated. Nutrient depletion is through bacterial activity only.

2. The seeding and reseeding parameters assume that, initially, there is only one UMB or bacteria present in each occupied pore. Results will then give maximum times that should be allowed to completely plug each pore.

3. The lag phase is ignored in the growth kinetics. This is standard practice for microbial simulation and is justified as the lag phase observed in experimental results was quite small when compared to the exponential phase (see Figure 3.1).

4. If the nutrient concentration or shut - in time is not sufficient for total plugging of a pore, the simulation is halted and the variable is changed. Partial plugging would be very difficult to simulate as flow would only be partially diverted. Also, total plugging is the target affect and so partial simulation is of little interest.

5. It is assumed that the injected fluid has an alternate flowpath should the high permeability zone become plugged. This assumption is true to form in an actual field situation in which a waterflood will be shunted around a plugged zone and into previously untouched, tighter rock. This assumption does, however, make it very difficult to compare one dimensional experimental core results with simulation profiles. In the experiments, the flow forces its way through the plug because there is nowhere else for it to go. This will, of course, prevent complete plugging in the core as bacteria are pushed out of the pore space and into the effluent.

4.4 Program Algorithm

The program describing MEOR plugging of a high permeability zone uses the aforementioned assumptions and equations and is structured as follows:

1. Initial parameters and constants are set. These include initial permeability, initial porosity, pore size, seeding and reseeding parameters, bacterial growth parameters, nutrient uptake parameters, slime production parameters and initial nutrient concentration. The seeding parameter determines how

many pores will be affected by growth for the first shut - in period.

2. The specific growth rate is calculated and the number of bacteria present in each pore due to growth is determined. From these values, nutrient depletion and slime formation can also be calculated.

4. The volume of cells and slime can then be determined and the porosity of the rock can be modified to reflect the biomass being formed. Permeability can then be calculated from this porosity and the Carman - Kozeny equation.

5. A check is done to see if either the pore is plugged or the nutrient is used up. If all of the nutrient is utilized before plugging is complete, the program stops and recommends a higher concentration be used. If the pore is plugged, the program will skip to the next injection time.

6. At the next injection time, the reseeding parameter is used to determine how many clean pores will obtain bacteria. The nutrient concentration is changed back to its original value as the core has been replenished and then steps 2 to 5 are repeated.

7. Eventually, the entire core will become seeded and then

plugged and the permeability will effectively drop to zero as the flow is diverted elsewhere.

This algorithm is written in FORTRAN and compiled using WATFOR77 on an IBM compatible personal computer. It accounts for the important factors in MEOR plugging and provides profiles of all of the changing variables. Initial parameters can be varied quite easily to examine their effects on the final profiles as the run time is very quick.

4.5 Simulator Results

As mentioned before, the main purpose of the simulator is to examine the effect that different parameters will have on the plugging of porous media. As there is an infinite combination of parameters that can be tested, only a few will be mentioned to demonstrate the simulator results.

Table 4.1 shows the basic parameters used for the simulation. Values for parameters such as seeding and reseeding were chosen based on the plugging profiles they generated. Other numbers were taken as typical values and are not specific to any particular rock or organism.

Permeability	500 mD
Porosity	0.2
Seeding Fraction	0.2
Reseeding Fraction	0.2
μ_{max}	0.69 (t_d = 1 h)
K_s	1 g / L
$[S]_o$	1000 g / L
α	2.0
β	0.284 h^{-1}
α_2	1.83
β_2	0.155 h^{-1}

Table 4.1 – Basic Parameters Used in the Simulation

Figure 4.1 shows the porosity and permeability profiles throughout the simulation using the base parameters. The relationship between the two is due to the Carman - Kozeny equation and can be seen to be less drastic as the permeability becomes closer to zero. Originally, with a seeding of 20 % of the available pores, the permeability drops to just under half of its original value. After two more nutrient injections, the permeability is less than 5 % of its original value. This would be considered sufficient to shunt flow into other channels in a reservoir.

Figure 4.2 demonstrates the general trends of the substrate concentration, bacteria counts, slime production and overall pore plugging for the duration of one shut - in period. The substrate can be seen to drop considerably during this time although some still remained after plugging was complete. Also, the shapes of the slime and plugging profiles are very close to the bacteria profile. This is because slime production is directly related to cell growth and so will increase accordingly. Plugging, of course, will be dependent on the rate of overall biomass that is produced and since both biomass curves are exponential, plugging will also occur in this manner.

Another property of this system not shown on the graph involves the method of plugging. When the seeded pores become

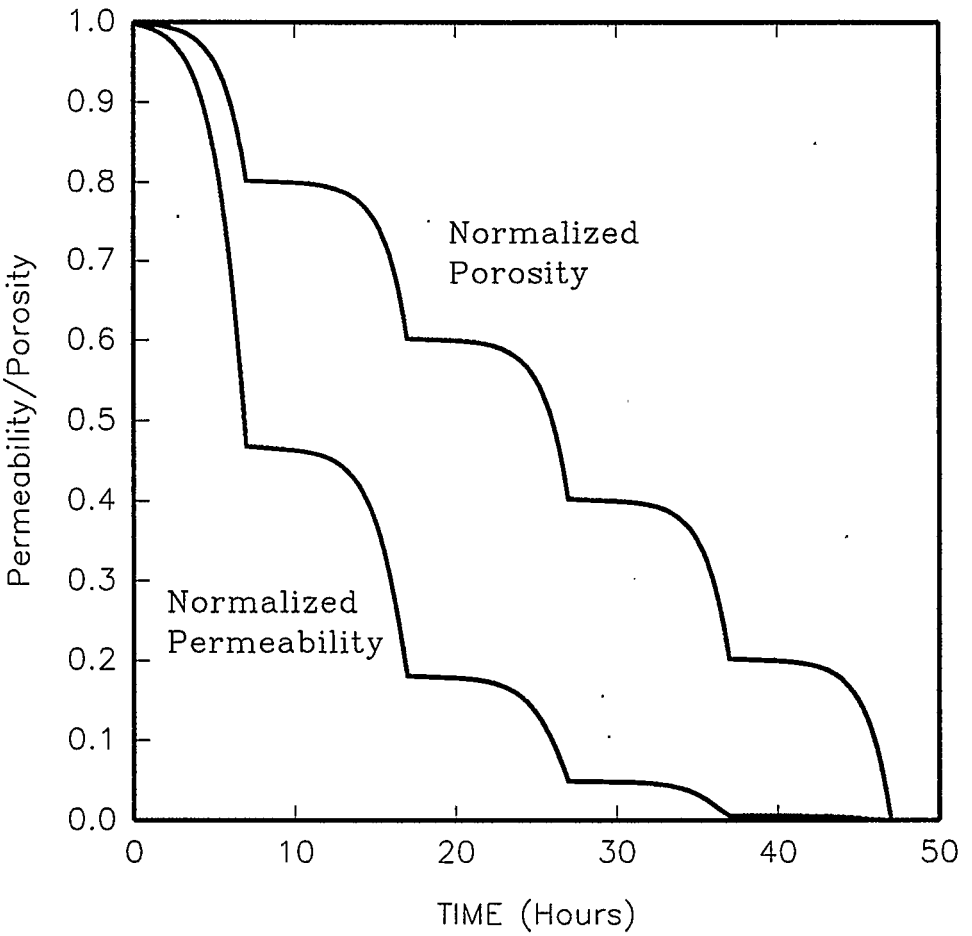

Figure 4.1 - Permeability and Porosity Decreases Using the Basic Simulation Parameters

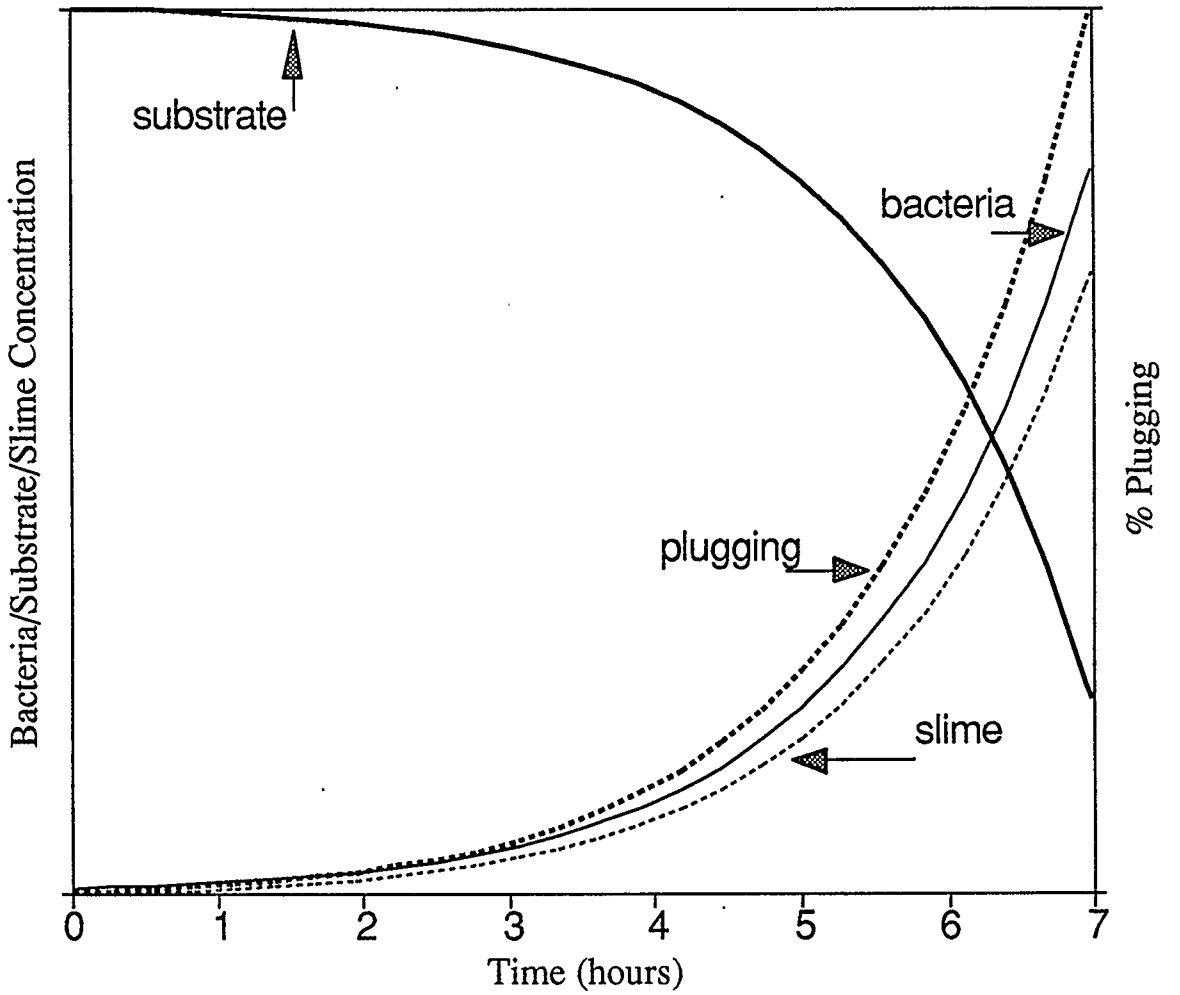

Figure 4.2 – Profiles for Substrate Concentration, Bacterial Growth, Slime Production and Pore Plugging During a Single Shut – In Period

completely plugged, cell mass itself accounts for just over 30 % of the biomass with slime causing the majority of the blockage. This indicates that for these parameters, the bacteria produce over twice their volume in biopolymer. This indicates that the basic parameters describe an average slime - producer as some bacteria will produce much more than this.

Simulator parameters may now be varied and the results can be compared to the base results. The differences between the results is tabulated in Table 4.2.

When the value for maximum bacterial growth rate is halved, the time necessary for plugging doubles. Also, the cells utilize 7 % more substrate over this longer time duration. Lastly, a lower cell to slime ratio can be seen to exist once the pore is plugged but this difference is not really very significant.

The Monod kinetics may also be altered by varying the value for K_s to make it more significant. A change by a factor of 10 does not have very much impact as this parameter is still too small when compared to the value of substrate concentration. However, an increase by a factor of 100 has an incredible impact on the system. The plugging time increases to over 17 hours from the base value of 7.5 hours. The final substrate concentration also changes from 20 % for the

Parameter Change	Plugging Time	Cell to Slime Ratio	Remaining Nutrient
Base case	7.4 h	0.484	21 %
μ_{max} (1/2 X)	14.3 h	0.436	14 %
K_s (10 X)	8.3 h	0.471	20 %
K_s (100 X)	17.2 h	0.381	6 %
α_2 (2 X)	6.7 h	0.256	50 %
β_2 (2 X)	7.3 h	0.429	27 %
No Slime (excess media)	9.0 h	1.000	----
Pore Size (1/2 X)	6.4 h	0.484	21 %

Table 4.2 – Sensitivity Analysis of Selected Parameters in the Simulation

original parameters to just 6 % using the much higher K_s. The cell to slime ratio decreases slightly but again, this change is not substantial.

Slime production kinetics can also be varied. When either of the two parameters are doubled, plugging time changes very little. Changes are noticed, however, in the cell to slime ratio, which is as expected. In particular, doubling α_2 leads to a decrease in the ratio from 48 % to just 25 %. Also, over half of the initial substrate remains when plugging is complete. This is because the substrate utilization kinetics are based on cell numbers and growth rates and much fewer bacteria are needed for plugging with the higher slime production.

Without slime production, the simulation stops due to a shortage of substrate. The base nutrient concentration is not high enough to support the necessary growth of bacteria. If the nutrient concentration is increased, plugging will occur in only 1.5 hours more than was necessary with slime production. This is a result of the exponential growth ability of the cells. Once a large number of bacteria have accumulated they can plug a pore space by themselves very quickly as the doubling time is only 1 hour. The concern would be how stable this plug would be as opposed to one that consisted of both cells and slime.

When the pore size is halved, the plugging time decreases by only 1 hour. Again, this is because the initial 5 or 6 hours are used to build the biomass up from one simple bacteria. After this, with cell doubling occuring so rapidly, it takes only a small time increment to plug larger and larger volumes as long as nutrient is available.

These types of sensitivity analyses can be very useful when designing an actual field test. For example, it can be seen that growth kinetics mostly affect plugging time while slime production kinetics have the greatest effect on substrate concentrations. When varying parameters, a type of optimization can be reached from which bacteria may be chosen for their specific properties and parameters to help ensure success in a tertiary recovery process.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Experiments have been performed that test the plugging ability of a MEOR process. Concentrations of *K. oxytoca* were starved so that they would provide ultramicrobacteria or UMB for injection.

Firstly, consolidated sandstone cores were tested. Peculiar pressure drop profiles led to further examination of the validity of the marked core permeabilities. It was found through SEM photography that there was a considerable clay fines problem in these cores. Most likely, the sandstone was tested and erroneously labelled based on results from air flow experiments which would not be expected to mobilize the clay fines to the same extent as liquid flow would.

UMB plugging was tested in these cores anyways and results were presented as pressure increases during each flood. UMB effluent concentrations were initially very small as most of the starved bacteria were retained in the core rock and clay. Initial pressure drop readings corresponded with those for a control core that had not been inoculated with UMB, however

once bacterial growth occured, the response of the inoculated cores changed drastically. The biomass seemed to actually stabilize the clay fines and the plugging reached a maximum beyond which any extra biomass was simply washed through the core and into the effluent.

When these bioplugged cores were backflushed, another variation was noticed. The core that was supposed to be the tighter of the two (300 mD as opposed to 400 mD) seemed to have a less stable bioplug. This could be due to less uniform seeding of the UMB or erratic nutrient flow patterns. The higher permeability core may have larger and more uniform flow channels which would minimize the flow irregularities and form a more stable plug of biomass.

One sandstone core did not experience this clay fines problem and had a 700 mD permeability. Pressure profiles for this core were steady and consistent during initial flow testing. Initial UMB retention in this core was much less than observed in the clay - filled cores. The UMB effluent concentration was only slightly smaller but of the same magnitude as the injection concentration. Bacterial growth caused the pressure drop readings to be somewhat erratic at the beginning of each flood but once flow channels were established through the biomass, the readings stabilized. The maximum initial pressure drop gave an indication of the extent of growth

through the core and the permeability dropped to about 30 % of the original value. Backflowing washed out extra biomass and decreased the pressure drop slightly but the permeability remained only half the original value.

Further tests were performed using sandpacks. Firstly, the effect of UMB injection volume and concentration was examined. Two pore volumes of 10^5 CFU / mL were injected into one pack and one pore volume of 10^7 CFU / mL was injected into another pack. The low concentration, high volume injection caused a larger permeability decrease than in the other sandpack. This, again, would be due to uniform UMB seeding forming a stable plug. Injecting just one pore volume of high concentration UMB leaves the seeding process much more susceptible to erratic and non - uniform behavior.

Nutrient selection is another important MEOR factor. BHI is considered to be a rich nutrient while bacteria grow much slower on SCM media. Sandpack experiments were run in which each of these nutrients was used and the corresponding plugging profiles were observed. BHI caused plugging within 48 hours of inoculation while SCM plugging was not apparent until nearly 100 hours had passed.

When all of these sandpacks were dismantled, bacterial counts were determined along the length of each pack. These counts

were similar for all of the samples, proving that bacteria were present throughout the length of each pack and skin plugging was not a factor.

These results demonstrate that UMB plugging is a feasible manner in which to redistribute flow patterns in reservoirs containing high permeability zones. The results also show the sensitivity of the procedure to a variety of factors which will allow for MEOR strategies to be developed for specific recovery situations.

Finally, a MEOR plugging simulator was developed and discussed. This simulator used only two parameters to model bacterial transport which allowed for emphasis to be placed on the biological factors involved in such a process. Bacterial activities such as growth, substrate utilization and slime production were studied and their effects on the MEOR process were discussed. The program provided an ideal opportunity to observe the sensitivity of the plugging process to many biological parameters not easily observed in experimental work.

5.1 Recommendations

MEOR technology is slowly becoming better understood but further experimentation is still needed. There are so many variables involved in a MEOR process that new tests can always be designed that will provide valuable information. Tests using different nutrients and bacterial species would be the most helpful in attempting to optimize bacterial growth while minimizing nutrient expense.

Also, many of the concerns mentioned in Chapter 1 still have not been properly addressed. Highly structured experiments need to be developed to closely simulate actual reservoir conditions. These tests could be modelled after the three - dimensional simulator described in Chapter 1 (Cusack et al, 1990) which was well designed but obtained very few results. Experiments may also be designed based on results obtained from the one - dimensional floods presented in this thesis and in previously published papers.

It is understandable that producers do not want to risk productive oilfields for MEOR field tests but without this type of work, this technology may never gain feasibility. Laboratory experiments are necessary and very informative but realistic field test trials are even more important. These

large - scale, well - planned tests would provide very convincing data as to whether MEOR procedures can be considered inexpensive and efficient alternatives to existing tertiary oil production techniques.

Finally, MEOR simulation must be further refined. The simulation attempts to date do not fit experimental data well enough to be used to design full - scale processes. While simple simulators are fine for sensitivity analysis, there is much work to be done before a commercially viable simulation can be developed.

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