Macroscopic observation of hydrodynamics and pseudomonas aeruginosa bioflim processes in a porous media reactor
by Feisal Abedeen

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Engineering
Montana State University
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Abstract:
Microorganisms in the subsurface microenvironment can be made to play an active role in the solution of several industrial problems. A prior knowledge of the fundamental processes regarding cause and effect relationships is however essential in order to exploit these microorganisms effectively and safely. In order to study this, a simulation of the naturally occurring processes in the subsurface was attempted using porous media. Biofilm accumulation, mass transport of fluid and nutrients, biotransformation and reactor dynamics were studied inside thin rectangular glass reactors. Pseudomonas aeruginosa was made to adsorb and grow inside these reactors on packing media of varying dimensions. The substrate feed consisted of glucose, oxygen and micronutrient salts in distilled water, provided at a constant pressure drop across the system. Analytical techniques were invented or improvised for in situ measurement of spatial and temporal variations of the above variables. The experimental analysis yielded hydrodynamic and biochemical data, which was then integrated. Results indicated that these variables are highly inter-linked through mass balances over the respective compartments and phases.

MACROSCOPIC OBSERVATION OF HYDRODYNAMICS AND *PSEUDOMONAS AERUGINOSA* BIOFILM PROCESSES IN A POROUS MEDIA REACTOR

by

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A thesis submitted in partial fulfillment
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ABSTRACT

Microorganisms in the subsurface microenvironment can be made to play an active role in the solution of several industrial problems. A prior knowledge of the fundamental processes regarding cause and effect relationships is however essential in order to exploit these microorganisms effectively and safely. In order to study this, a simulation of the naturally occurring processes in the subsurface was attempted using porous media. Biofilm accumulation, mass transport of fluid and nutrients, biotransformation and reactor dynamics were studied inside thin rectangular glass reactors. *Pseudomonas aeruginosa* was made to adsorb and grow inside these reactors on packing media of varying dimensions. The substrate feed consisted of glucose, oxygen and micronutrient salts in distilled water, provided at a constant pressure drop across the system. Analytical techniques were invented or improvised for *in situ* measurement of spatial and temporal variations of the above variables. The experimental analysis yielded hydrodynamic and biochemical data, which was then integrated. Results indicated that these variables are highly inter-linked through mass balances over the respective compartments and phases.

INTRODUCTION

Background

A literature review shows that a number of similar studies have been made in packed beds or simulated porous media reactors. Microorganisms attached or adhering to solid surfaces have a tremendous advantage over suspended cells as they are assured of a continuous supply of nutrients and perhaps a more stable niche in which to proliferate. It also provides for a higher interaction between varied microbial communities. For instance, in a natural mixed culture system, the species found at the substrata are obligate anaerobes, followed by facultative aerobes and the surface population is likely to be an aerobic heterotroph species. Therefore, there is a strong driving force for microorganisms, especially those that occur in nature, to evolve as a biofilm species in preference to suspended cells.

Biofilms have been known to be useful in many situations while proving to be a nuisance in others. For instance, biofilms occurring on ship hulls and other submersed surfaces provide a significant loss of momentum by increasing the fluid energy loss at the surface. Inside pipes, tubes, on metal surfaces and on any electro-chemically suited surface such as the mammalian tooth, they cause a corrosion of the substrata leading to a deterioration and ultimate loss of the surface. They also provide significant heat transfer losses on surfaces that conduct heat in cooling or heating equipment. On the other hand, biofilms have been used for many decades to treat waste water as in trickling filters, for selective adsorption of toxic chemicals on activated surfaces in filtration-adsorption columns, as the final stage in small and medium scale water treatment plants with the biofilm growing naturally in the subsurface cores, and, for enzyme immobilization techniques. In general, they have existed for almost as long as man has existed. Recently, however, the active use of microorganisms for environmental applications is being viewed as a viable alternative to conventional chemical or mechanical

treatment methods. The Environmental Protection Agency has evolved its own program known as the Superfund Innovative Technology Evaluation Program (SITE, 1989) which is now in its fifth year. The program deals with evaluating treatment technologies necessary to implement new federal and state cleanup standards aimed at permanent remedies rather than short term solutions. Technologies undergoing review are listed in Table 1.

Table 1 Comparison of treatment technologies considered for remediation of hazardous compounds in the groundwater by the USEPA.

	Method of treatment	percentage
1.	Biological	11.3
2.	Fixed biofilm	3.8
3.	Physical/chemical	53.1
4.	Solidification/stabilization	18.9
5.	Thermal	17.0

Of relevance in the above table is the deceptively small number of treatment methodologies based on biofilms grown on surfaces. The main difference between this and other treatment methods is the absence of any secondary problems associated with or a consequence of the method.

A number of physical, chemical and biological processes occur on any surface which contains microorganisms, in the presence of adequate amount of energy and microenvironmental conditions (Figure 1). The transfer, transport and transformation rates are unique to every microorganism and many of the kinetic and stoichiometric coefficients remain constant under similar influencing factors (such as pH, temperature, pressure and concentration).

A general macroscopic material balance for a single component is,

E1..NET RATE OF ACCUMULATION = NET RATE OF TRANSFER OR TRANSPORT + NET RATE OF TRANSFORMATION

In order to construct the balance, the first two terms may be experimentally measured while the last (function of an intensive property - changing only with factors that describe the environment) is calculated. It is relatively difficult to directly measure transformation processes.

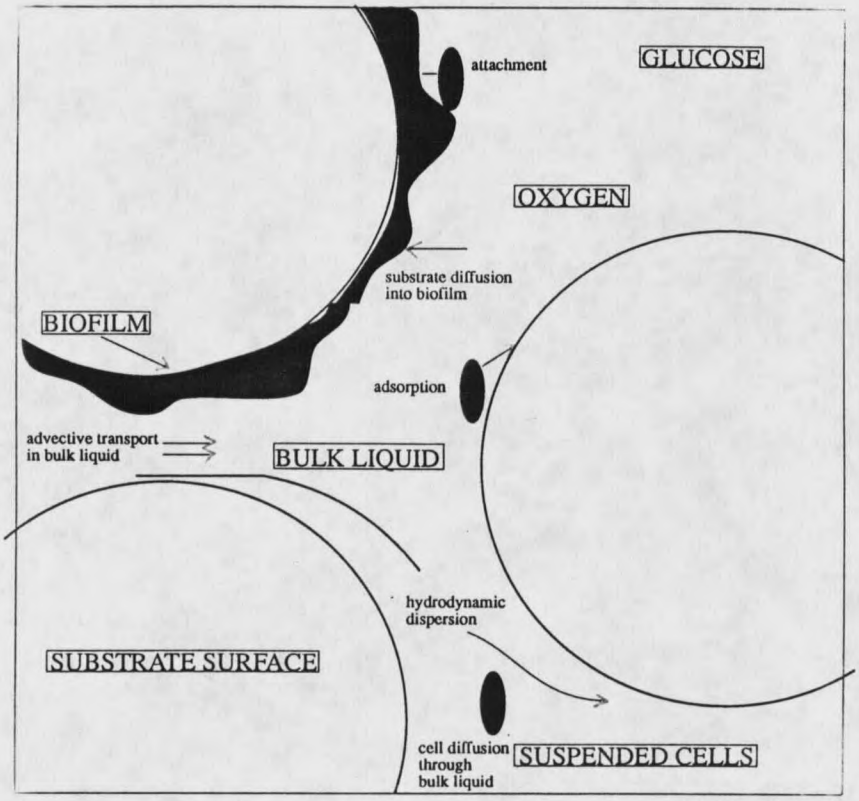

Figure 1. Biofilm accumulation patterns observed in the subsurface are caused by transport, interfacial transfer and transformation processes, including filtration, exclusively seen in porous media.

Biofilm Dynamics

Biofilms influence heat, mass and momentum transfer losses on surfaces when accumulated to a sufficient thickness. They also cause a metal loss when involved in corrosion. Advantages of immobilized cell systems include: ability to maintain a high reactor cell concentration at a range of residence times, and, an insignificant loss of biomass due to washout or hydraulic upsets caused by unexpectedly high dilution rates. Several investigators have observed that an initially adsorbed organic biofilm will sufficiently modify the inert surface so as to enhance bacterial adsorption. Cell transport to a surface is probably dominated by

molecular or eddy diffusion and is gravity assisted in preference to motility or chemotaxis.

Bacterial cellular sticking efficiency has been found to be proportional to shear stress (Characklis et al., 1984). Also, the maximum number of adherent cells were observed to be much less than the total surface monolayer coverage (about 1 - 3 % under non-growth conditions.)

As the fundamentals of biofilm technology were established, the development of fixed film waste water treatment and fermentation processes brought about the need for a model to describe substrate utilization and biomass concentration.

Recently there has been a large amount of research on extra cellular polymeric substances (EPS). Theories vary about EPS production as being a process to attach cells onto a surface, to being a mechanism for transport away from unfavorable nutrient conditions. On the other hand, there is strong evidence to suggest that EPS production is higher in viable cells as compared to starved cells. Research also suggests that EPS has highly dynamic physical and chemical characteristics which are dependent on the environmental conditions, and is supervised by the cell.

Bryers and Characklis (1982) have found that a reduction in suspended biomass concentration results in a decrease of measured deposition rate. This also indicates that rate of suspended biomass deposition is limited by the rate of bacterial particle transport to the surface rather than the rate of cell adhesion at the surface. Sometimes the overall deposition rates may decrease with increasing Reynolds number because of decreasing cell adhesion rate (sticking efficiency) even though the particle transport rate is increasing. Biofilm production due to cell growth and EPS production was found to be the major contributor to net biofilm accumulation. Also, the biofilm decay rate for thin biofilms had a negligible influence on net biofilm accumulation. Biofilm detachment rate due to shear forces was the major negative factor contributing to net biofilm accumulation. When biofilm thickness was greater than the viscous

sublayer in a flow system, the frictional resistance undergoes a large increase.

Reactor Theory

In an ideal Plug Flow Reactor (PFR), A) mixing is lateral and not axial and is incomplete, B) the residence time is high and there are no statistical fluctuations, and, C) there are changes with respect to time, and, axial and radial distance for nutrients and biomass. Many of these characteristics are in direct contrast to those found in a well mixed reactor such as a CSTR. Further, unlike a pipe flow regime where molecular diffusion, created by concentration gradients, dominates; in porous media there are pressure or velocity gradients creating hydrodynamic dispersion. This dispersion is several times in magnitude compared to molecular diffusion. The material balance mentioned earlier may be written for each component:

Substrate Balance

$$E2.. \quad \frac{dS}{dt} = D_s \frac{d^2S}{dZ^2} - v \frac{dS}{dZ} + R$$

SUBSTRATE ACCUMULATION DIFFUSION ADVECTION SUBSTRATE CONSUMPTION

where S is the bulk liquid substrate concentration, t the reactor operation time, v the axial velocity, D_s the effective dispersivity for transfer of substrate from bulk liquid into biofilm and R the net rate of substrate metabolism as given by the mass balance shown below. This is the equation for axial substrate plug flow neglecting advective and diffusive flow in x and y directions. D_s includes lateral, transverse and molecular diffusion and accounts for the higher dispersion in porous media. Assuming that the EPS non growth-associated transformation rate is negligible and that growth is limited by concentration of a single substrate S, the transformation or consumption term may be separated into terms for the cells and EPS. The term R includes both biomass in suspension and suspended material.

$$E3.. \quad R = -\mu_{max} X \frac{S}{(K_s + S)} - \mu_{max} X K_g \frac{S}{(K_s + S)} Y_{X/S}$$

SUBSTRATE CONSUMPTION RATE = CELLULAR CONSUMPTION + EXTRA-CELLULAR CONSUMPTION

μ_{max} is the maximum specific growth rate of the species, X the total concentration of cells in bulk liquid and biofilm, K_s the Monod half-saturation constant for the growth limiting substrate, K_g the EPS growth associated coefficient and $Y_{X/S}$ the growth yield of the biomass.

Biomass Balance for Suspended Cells

$$E4.. \frac{dX_s}{dt} + v \frac{dX_s}{dZ} = D \frac{d^2X_s}{dZ^2} + \mu_{max} X_s \frac{S}{(K_s + S)} + R_d$$

ACCUMULATION ADVECTION DIFFUSION NET GROWTH NET DETACHMENT

where R_d is the net detachment rate of cells from biofilm into bulk liquid and X_s the concentration of planktonic cells.

Biomass balance for Biofilm Cells

$$E5.. \frac{dX_b}{dt} = \mu_{max} X_b \frac{S_b}{(K_s + S_b)} + \mu_{max} X_b K_g \frac{S_b}{(K_s + S_b)} + R_d + R_{ad}$$

BIOFILM ACC. CELL. BIOFILM GROWTH EXTRA-CELL. BIOFILM GROWTH DETACHMENT ADSORPTION

where X_b is the concentration of biofilm cells, S_b the substrate concentration in the biofilm and R_{ad} the net rate of adsorption of suspended cells from bulk liquid to the substratum. The other assumptions are made that growth is single substrate limited and the net decay rate is negligible compared to the net growth rate. The EPS growth term is omitted from the biomass balance for suspended cells as it is traditionally associated with biofilm formation on substrata. In reality, this might not hold true.

Energy (Momentum Transfer) Balance

The above balances are mass balances. The system is assumed to be at steady-state with respect to temperature and thus there is no need for a heat balance. The energy loss across the reactor system (due to pressure drop) is the result of viscous and inertial forces, given by the equation of the form:

$$E6.. \quad \frac{P_1 - P_2}{L} = a \frac{\Omega v}{g_c} + b \frac{\sigma v^2}{g_c}$$

where P is the pressure at points 1 and 2 (influent and effluent respectively), L is the reactor length, a the viscous resistance coefficient (inverse hydraulic permeability), b the inertial resistance coefficient, Ω the absolute viscosity of the bulk fluid, σ the absolute density, v the superficial velocity and g_c the Newtons law proportionality constant. a and b may be determined experimentally for different kinds of porous media. If pure viscous flow is assumed, the above energy equation converts into the Darcy equation.

The Darcian approach is limited to Reynolds numbers less than 10. Hence, considering the relative importance of the suspended cells and/or the impaction of substrate medium on the particles, it is likely that the Darcian approach is limited in that it completely neglects inertial forces. These could play an important role in increasing biofilm accumulation rates in the porous media reactors.

Significance of Research

Petroleum Formation Plugging

This is an area in which biofilms are a definite menace. Oil is recovered from the subsurface through producing wells by providing sufficient pressure into injection wells. These wells are strategically located at varying distances from each other for the most hydraulically and economically efficient recovery of crude oil. Either fresh water or sea-water may be used for the purpose and often a part of this is recycled

after a preliminary treatment. Even though the injected water is pre-treated, inorganic salts are present in the soil and the hydrocarbons in the crude oil provide the carbon source. Microorganisms are introduced into the system either through injected water or they may exist *in situ*. These cultures begin to grow and adhere to the soil surface. Depending upon the porosity of the aquifer, they may penetrate the saturated zone along with transport of water and hydrocarbons. Eventually, they block the pores of the aquifer and prevent efficient hydrocarbon removal by bringing about high pressure drops and low flow rates.

Kolbel and Hush (1989) found that when a natural system was analyzed, biofilm cells were mostly gram positive pigmented fermentative glucose degraders while the suspended cells were mostly gram negative non-pigmented anaerobic rods. Regardless, it must be remembered that the diversity of soil microorganisms is very great. A large variety of bacteria and fungi exist in any soil sample due the abundant availability of nutrient and microenvironmental conditions. This microbial diversity has been extensively studied and a number of resulting microbial community interactions have been observed (Ward et al., 1987).

At the field site, the soil types are also very varied in terms of size, structure and nutrient content. This soil heterogeneity enhances the complexity. These two factors (microorganism diversity and soil heterogeneity) make the characterization of any soil sample a challenging task. In addition, there is an absence of a standard technique for characterization of a mixed cultures enrichment. Most enrichment methods are biased - you usually find the microbe that you look for.

Understandably, the predictive modelling of a field site, with respect to bioremediation, is no mean task and is always subject to a question of interpretation. For initial success of a theoretical prediction, the methodology for writing the model is more critical than the accuracy obtained for one field situation.

Crude oil may be present in a wetted porous rock in the form of oil

droplets. In order to decrease interfacial surface tension, surfactant is either introduced into the reservoir or produced *in situ* by enhancing bacterial activity, the latter being utilized to enhance oil recovery. Soil microorganisms themselves help in oil recovery by reducing viscosity and through the process of selective plugging. Recovery of oil was found to increase from 20 % OIP (oil in place) to 40 % OIP when bacteria were introduced into cores. Thus the results demonstrate the surfactant effect of bacterial cultures. Bubela (1984) found that for cores having similar porosities and permeabilities, the extent of plugging is decided by the nature of pore size distribution i.e. the concentration of suspended cells decides whether plugging occurs or not. Therefore the authors claim that pore size distribution is more important than the average pore size. Additionally, when a low concentration of bacterial cells was introduced into a porous medium, the permeability did not significantly decrease, thus providing the concept of 'limiting bacterial concentration'.

Adhesion and Filtration of Bacterial Cells

Loosedrecht et al., (1987) attempt to measure adhesion as a function of the contact angle of water using a micro-filter grown biofilm. The contact angle is related to hydrophobicity, which increases with decreasing surface energy. In the same study, hydrophobicity of a *Pseudomonas aeruginosa* biofilm was measured by two other methods (contact angle was found to be about 25.7 degrees), and compared to the affinity of cells to the hydrocarbon phase. The other methods were based on adhesion of cells to polystyrene and the distribution of cells between a Dextran/PEG phase respectively.

In another study, the same authors relate electrophoretic mobility to adhesion. Solid and bacterial surfaces are commonly negatively charged and these are counterbalanced by opposite charges. Between like charges, electrostatic repulsion is compensated by van der Waals attraction. In chemostat experiments it was observed that bacteria became more

hydrophobic during the exponential growth phase (high μ_{max}). These results may explain the mechanism of flocculation in suspension and adhesion to surfaces in reactors with high growth rates.

Bouwer (1987) has measured the deposition efficiency of small particles in a packed bed. For 1 mm size particles in a 0.5 mm bed, the removal is effected primarily by means of interception (transport caused by velocity gradients), giving a collection or removal efficiency of 0 to 0.1. The rate of effective particle capture, R_p may be defined:

E8.. $R_p = \alpha K_d c$

where α is the sticking efficiency ranging from 0.1 to 0.001, K_d is the overall particle transfer coefficient, and, c is the bulk liquid particle concentration.

The rate of effective particle transfer is the ratio between rate of particle sticking to packed bed element to rate of particle approaching the same. Sticking efficiency is defined as the ratio between the number of contacts between particle and biofilm leading to successful attachment and removal, to the total number of contacts.

Filtration has also been known to enhance plugging. When biocide is introduced into the aquifer in order to control souring, microorganisms are either killed or inhibited. As a consequence, sloughing may occur at the biofilm-substrata interface causing large pieces of biofilm or aggregates of cells to break away. These may be embedded in the pores of the formation at a distance from the point of biocide addition. Also, at a large enough distance, the biocide residual may approach zero and microorganisms may begin to grow and form a dense biofilm, creating formation plugging problems anew. Filtration might also be mechanically caused as a consequence of souring, by the precipitation of iron sulfides in pores.

Both detachment and attachment of bacteria to surfaces may be

beneficial during starvation. For instance, in the soil environment, detachment would cause transport of the cell and attachment to small particles would also increase the velocity of transport.

Shaw, Bramhill et al. (1985) studied the significance of the polysaccharide glycocalyx. In the past, studies on plugging of rock cores dealt mostly with dead bacteria and the importance of the exopolysaccharide glycocalyx (EPS) was underestimated. Recently, it has been shown that surface colonization of live bacterial populations in cores leads to the production of large quantities of EPS, which can cause a decrease in permeability as high as 99 %. This EPS is the result of an auto-mechanism of bacteria to adapt to low nutrient environments. It contains a hydrated matrix of mostly anionic polymers, which form chemical bonds with organic and inorganic nutrients through ion-exchange processes.

The authors observed that the slowest rate of plugging occurred in media that contained dead bacterial cells and inert silicon carbide particles, while the fastest rate of plugging occurred in media that contained both live bacteria and inert particles. In the former case a final permeability was reached when the filter cake formed its own pores and inherent permeability.

The other interesting observation was made by injecting biocide into cores that had been previously plugged with bacterial cultures. Biocide addition of a type which kills bacterial cells did not increase permeability as rapidly as an oxidant, which kills bacteria and also dissolves EPS. The authors conclude that biofilm formation is an ecologically predictable reaction of cells in order to restrict flow and entrap nutrients for use by the population.

Reservoir or Product Souring

When sulfate is present as a nutrient, sulfate reducing bacteria metabolize it to organic sulfur which causes souring. Souring has been defined as "The formation by bacterial activity of sufficient hydrogen

sulfide in the reservoir (reservoir souring) or product (product souring) to materially affect the properties of the reservoir or product fluid (e.g. oil, gas or water)" (W. Subcasky, Chevron - personal communication). Even if sulfate and short chain hydrocarbons are not initially present in the system, there may be microorganisms such as general fermentative heterotrophs (GAB) that can metabolize the nutrients into those that may be useful for SRBs. The oil industry has determined that at some time period after the initial breakthrough, souring will occur, regardless of the reservoir conditions and little can be done to prevent it entirely. The important requirements are a liquid phase and a reducing environment - conditions that are almost always prevalent in most oil recovery operation environments. The situation is far worse for sea-water flood systems as the fluids are rich in sulfates and assimilable organic carbon. Sulfur must be mechanically or chemically removed or separated from the hydrocarbons to meet crude standards. The hydrogen sulfide or sulfur di oxide gases, formed mainly in formations and injection/production wells as a result of bio-chemical or chemical reactions must be continuously disposed. Souring is therefore expensive to control especially since EPA imposes stringent air and effluent discharge quality standards.

Bioremediation

This is a field wherein the positive influence of microorganisms may be utilized. For instance, at contaminant spill sites, chemical/mechanical treatment strategies are adopted for the saturated zone where transport of fluids is easy and the contaminating hydrocarbons may be pumped out, treated and pumped back to the fluid stream. However, when microscale levels of contaminant concentrations (decided by regulatory agencies) are desired, these methods may no longer be cost effective. At very low concentrations, the process may be replaced by microbial degradation.

Bouwer and Cobb (1987) show how a biofilm model may be useful in predicting the most likely electron acceptor and donor (or most likely

metabolic pathway) in a heterogenous environment. Since specific microorganisms biodegrade a specific micro-pollutant the model may help in two ways: A) it could predict which nutrient needs to be added into the subsurface for enhancing biodegradation (or predict the micronutrient most likely to be biodegraded) or, B) it could predict which microorganism species needs to be introduced into the soil. Either of the methods eliminate guesswork in designing expensive injection/extraction wells and pumping systems.

In their model, the basic modelling equation is derived by simultaneously solving equations for the following: A) flux of substrate across fluid boundary layer (Ficks first law), calculated as a function of axial distance, B) steady-state substrate utilization by Monod kinetics and diffusive transport within the biofilm, and, C) steady-state biofilm thickness formation related to maintenance energy of biomass by Monod kinetics.

A similar theory applies to the vadose zone where most of the contamination problems occur. Since there is not much transport in this zone, treatment becomes difficult and there is a stagnation of contaminants. Land farming has been conventionally practiced as an effective treatment method.

In one study (Raymond et al., 1976), several kinds of oils were introduced into soils under optimum conditions for biodegradation. The average reduction in concentration varied between 49 and 90 % depending on oil and soil types. The average rate of degradation of oil was found to be 2.4 $m^3/4 \times 10^3 m^2$ per month.

Selective Plugging

Like the above microbially enhanced solution options above, this method for improving recovery of hydrocarbons from formations is in its research stage. Often, during oil recovery operations in injection/production well systems, the pressure drop created by the

injected water is lost due to losses in the recovery zones. If these zones are "selectively plugged", the hydrocarbon loss will be lower and the required pressure drop would be sustained. Introduction of chemicals into these loss zones has been successfully used to form precipitates and block the pores. However, microorganisms form a suitable alternative with the advantage of naturally selecting for the larger pore size first.

McLeod et al. (1988) found that the percent plugging of cores is a function of pore volumes of injected bacteria and is of greater importance for viable cells as compared to dead or starved cells. The starvation of *Klebsiella pneumonae* was observed to cause a reduction in the glycocalyx content of the biofilm. A reduction in EPS content theoretically implies

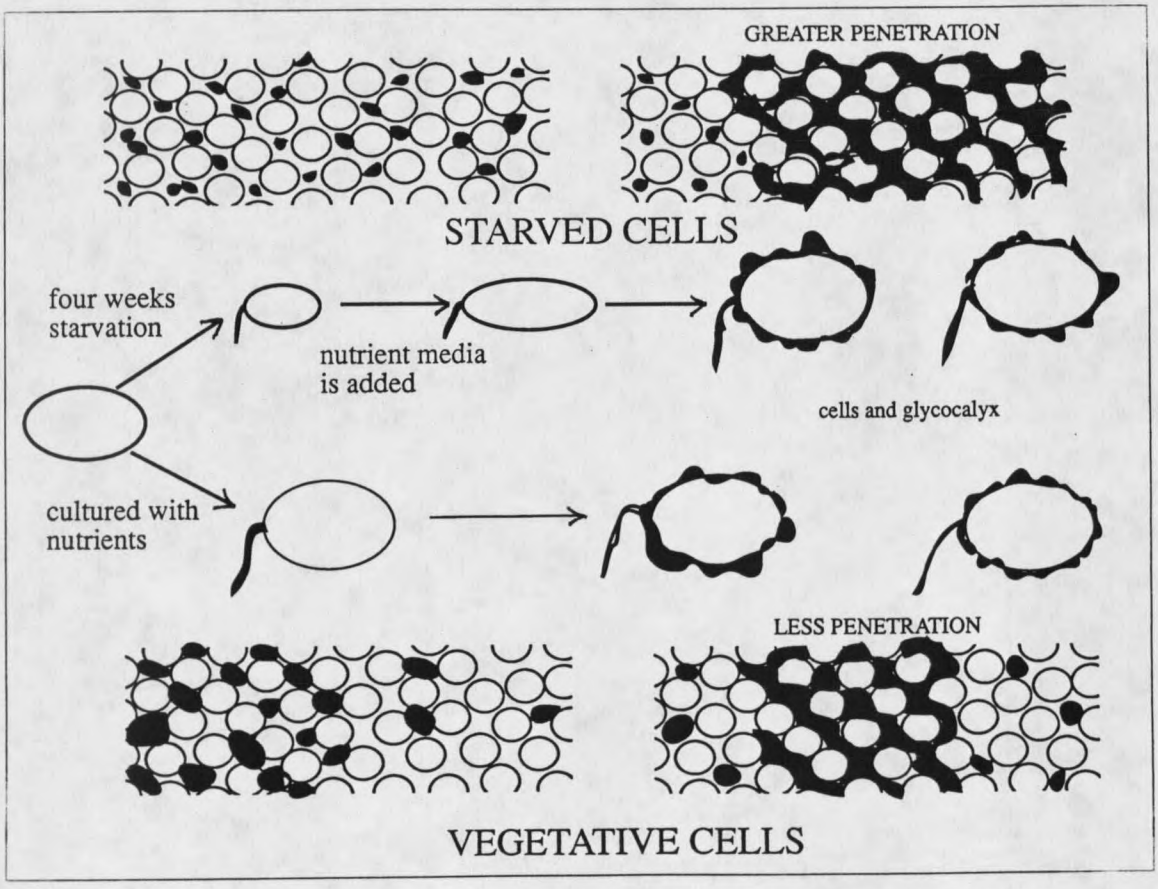

Figure 2. Studies have shown that starved cell cultures contain submicron sized bacterial cells, which when stimulated with nutrients, gradually attain normal size and growth. Lappin-Scott et al., 1988 measured deeper penetration into cores when starved cell suspensions were injected.

a reduction of bacterial adhesion and core plugging. Another observation was that respiration per vegetative cell was highest in the middle section of the core. It is stipulated that the biomass, aggregated at the inlet end, may have limited either substrate.

Lappin-Scott et al. (1988) use similar theory with sandstone cores in place of glass media. Their results show that when starved *Klebsiella pneumonia* were injected along with nutrients, the cell size and shape changed from small cocci to larger rods. The authors used 200 - 400 mDarcy for permeability, which is representative of oil well formation data. The injection of finely dispersed solids in oil or water, or *in situ* chemical reactions leading to insoluble precipitates are methods that are currently in use for plugging. This study indicates that bacteria offer a feasible alternative owing to deeper penetration and selective plugging of permeability zones.

Starved cells penetrate deeper into cores and form a better resistance to fluid flow (Figure 2). If these cell cultures were injected at selected locations, they would be first transported into the larger pores. With the provision of nutrients they would block the pores. The present study has shown that the larger the particle diameter (or pore size), the higher the biofilm accumulation (due to higher substrate flux).

Research Purpose

Goal

Develop a fundamental understanding of processes which control transport, attachment, growth and activity of microorganisms in porous media.

Objective 1:

Develop experimental methods for monitoring temporal and spatial distribution of biofilm and related variables.

Objective 2:

Conduct a process analysis including all relevant biochemical and hydrodynamic variables.

Experimental Tasks

Preliminary work was accomplished by Crawford (1987). One of first objectives was to confirm the consistency of the previous experimental procedures. A confirmatory method, using dye tracer study, was also developed for measurement of biofilm thickness *in situ*. The analytical techniques were standardized to avoid misinterpretation of experimental data in the future.

Six sets of experiments were run in an orderly manner shown (Figure 3), each with a specific objective. The progression of these experiments was such that a preceding experiment formed a basis for designing the succeeding one. The modelling work was not completed and is thus not mentioned in the thesis.