Transport of substrate and biomass in a packed bed bioreactor
by Ross Wade Lundman

A thesis submitted in partial fulfillment .of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University

Abstract:
Packed bed or porous media type systems are a unique environment for bacterial growth. The large surface areas, plug flow mixing, and laminar hydrodynamics may offer the attached organisms higher nutrient concentrations at lower shear stresses when compared to regimes such as plug, laminar, or completely mixed flows. Industries affected by biological accumulations in porous media range from oil recovery to waste water treatment. Perspective may be the only factor in deciding whether such an accumulation is useful or detrimental.

As industry has become more aware of the importance of such accumulations, more emphasis has been placed upon modelling the affected systems. Two areas where modelling has suffered from a lack of data are cellular detachment rates and substrate transport. The objectives of this research were 1) correlation of cellular detachment rate with glucose utilization rate and 2) observation of changes in transport of dissolved components through the porous media as a result of the biological accumulation.

To accomplish these objectives, two series of experiments were run. A differential reactor was packed with 1-mm glass beads, inoculated with Pseudomonas aeruginosa, and operated at a constant flow rate. In the first series of experiments the inlet glucose concentration was 18 g m"3. In the second series of experiments the glucose concentration was lowered to 2 g m-3. This change in influent glucose concentration provided for a wide range of accumulation rates and glucose utilization rates. Cell concentrations and glucose concentrations were measured daily at the influent and effluent of the reactor. Stimulus-response studies were also performed daily.

Results indicated detachment rate was a function of glucose utilization rate. Theory suggested the correlation may be linear; however, noise in the data made this determination impossible. The stimulus-response studies demonstrated the strong dependence of mixing characteristics upon the extent of the biological accumulation.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Packed bed or porous media type systems are a unique environment for bacterial growth. The large surface areas, plug flow mixing, and laminar hydrodynamics may offer the attached organisms higher nutrient concentrations at lower shear stresses when compared to regimes such as plug, laminar, or completely mixed flows. Industries affected by biological accumulations in porous media range from oil recovery to waste water treatment. Perspective may be the only factor in deciding whether such an accumulation is useful or detrimental.

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To accomplish these objectives, two series of experiments were run. A differential reactor was packed with 1-mm glass beads, inoculated with Pseudomonas aeruginosa, and operated at a constant flow rate. In the first series of experiments the inlet glucose concentration was 18 g m^{-3}. In the second series of experiments the glucose concentration was lowered to 2 g m^{-3}. This change in influent glucose concentration provided for a wide range of accumulation rates and glucose utilization rates. Cell concentrations and glucose concentrations were measured daily at the influent and effluent of the reactor. Stimulus-response studies were also performed daily.

Results indicated detachment rate was a function of glucose utilization rate. Theory suggested the correlation may be linear; however, noise in the data made this determination impossible. The stimulus-response studies demonstrated the strong dependence of mixing characteristics upon the extent of the biological accumulation.

INTRODUCTION

Industrial and Academic Relevance

Packed bed or porous media types of systems are a coupling of large surface areas, near plug flow mixing, and laminar flow hydrodynamics. These unique physical characteristics may offer attached organisms considerable advantages in the form of higher nutrient availabilities and lower hydraulic shear stresses when compared to true plug flow, laminar, or completely mixed systems. As a result of its existence, the biological accumulation alters both the chemical concentrations and flow regimes within the medium. The extent of profit or injury caused by the organisms is, as always, a matter of perspective. One thing is certain, biological accumulations in porous media or packed bed systems affect many industries.

The oil industry must respond to both the good and bad effects of these accumulations. Under the correct conditions, bacteria in oil formations (whether indigenous or introduced with the injection water) produce hydrogen sulfide, a process termed "souring". Souring decreases product quality and increases refining costs due to the complex handling procedures required for the toxic and strongly acidic hydrogen sulfide. To compound this problem, the bacteria may plug the formation (or the injection wells) once again raising recovery costs (McKinley, Costerton, and White, 1988).

Current research hopes to exploit this plugging phenomena. Bacteria injected into oil bearing formations may preferentially plug high permeability zones. This would enable waterflooding to liberate the oil trapped in zones of originally low permeability (Raiders,

Knapp, and McInerney, 1989). In addition, once in the formation, the bacteria may manufacture beneficial chemicals. The function of these chemicals could include dissolving of mineral deposits, acting as a surfactant, or altering fluid viscosities (Updegraff, 1990).

The ability of bacteria to degrade chemicals has long been known. Two industries making use of this knowledge are waste water treatment and bioremediation. Waste water treatment plants frequently employ trickling filters (downflow packed beds) to reduce chemical contents in municipal and industrial waste water to acceptable levels (Bryers and Characklis, 1990). Technology is currently being developed for both in situ (porous media) (Gannon, Manilal, and Alexander, 1991) and pump and treat (packed bed) bioremediation applications (Strandberg, Donaldson, and Farr, 1989).

Biological packed beds are also used for commercial chemical production. For example, E. coli immobilized in K-carrageenan beads are being used for the conversion of ammonium fumarate to L-aspartic acid (Hamer and Richenberg, 1988).

With the increasing interest in porous media and packed bed systems also comes an increasing value of predicting the extent and rate of associated biological processes. The modelling of these processes has been hindered by problems common to modelling biological accumulations in other geometries, 1) a poor understanding of the biological processes and 2) a poor understanding of the effect of the biological accumulation on hydrodynamics.

While the results of modelling are very sensitive to the process of biomass detachment, most times it is not directly measured. Modellers must use other measured variables to back calculate detachment rates. Thus, the accumulation must be largely described before it can be modelled and the utility of the model is diminished.

Substrate transport has suffered a similar fate. Due to the difficulty in determining mixing properties, most models assume completely mixed, laminar, or plug flow hydrodynamics. The effect of the biomass on hydrodynamics, and vice versa, is neglected.

Goal

The goal of this research was to quantify the effect of a biological accumulation on particulate transport and liquid mixing in a packed bed reactor.

Objectives

To accomplish this goal, the research was divided into two objectives. The first objective was to determine cellular detachment rate as a function of glucose utilization rate. The second objective was to determine the liquid mixing characteristics of the system and observe any changes in mixing as a result of the accumulating biomass.

Two series of monoculture, aerobic, packed bed experiments were run. For the first series, a high concentration of glucose in the reactor feed ensured that bacterial growth was limited by the concentration of dissolved oxygen. For the second series, a low concentration of glucose in the reactor feed guaranteed a glucose-limited system. The two extremes of growth provided for a wide range of mixing conditions and glucose utilization rates.

BACKGROUND

Transport Phenomena

The accumulation of biomass in any system is a complex product of two dependent phenomena, mass and momentum transfer. Whether the component of interest is particulate or dissolved, the system geometry significantly impacts the transport processes. Transport properties in porous media systems may deviate substantially from those seen in simpler geometries such as flat plates, closed conduits, or continuous flow stirred tank reactors (CFSTRs).

Particulate mass transfer in a porous medium may be described with the same general processes observed in other systems, with one addition, filtration. While filtration is theoretically possible in any system, it is usually negligible in systems other than porous media when compared to other transport mechanisms. Particle capture through size exclusion is an important feature of a porous medium. Variations in pore diameter may make it possible for a particulate of any size to be filtered. The effects are additive. As particles are filtered, more of the pore space is occupied by solids. Porosity is reduced, the average diameter of the pore spaces is decreased, and the rate of filtration is thereby increased.

Biomass may accelerate the filtration process quicker than abiotic particulates. As biomass is not a true solid, fluid may flow through the accumulated bacteria and extracellular polymeric substances (EPS). This is certainly true if the accumulation spans

the entire pore space. Any particle the same size as or larger than a bacteria could be captured.

Data from Rittmann and Wirtel (1991) and Sprouse and Rittmann (1990) suggest the capture efficiency of the media increases with increasing biomass regardless of mechanical filtration. Experiments conducted in a fluidized bed show that particle capture rates increased after the establishment of a biofilm. The biofilm therefore must demonstrate some degree of "stickiness" as size exclusion is nearly impossible in a fluidized bed.

The mass transfer of the dissolved components is also more complex in porous media. The path traveled by the fluid is highly variable in tortuosity, length, and diameter. This leads to a condition of enhanced longitudinal mixing when compared to closed conduit systems, a phenomena termed dispersivity.

Freeze and Cherry (1979) report dispersivity to be a function of three mechanical conditions resulting from the porous media geometry. First, mixing may be due to velocity distributions within a specific channel. Second, mixing may be due to variations in the pore velocities between channels of different size and roughness. Lastly, mixing may be due to the combining and splitting of channels. All three conditions contribute to a concentration profile within the system that may deviate significantly from what would be observed in a plug flow system.

The effect of a biological accumulation on these types of mixing characteristics is yet unknown. However, it may be expected that the intrusion of any particles into the pore spaces will distort the original fluid flow patterns, and thus biomass is expected to affect the mass transfer.

Conceptual Models of Biomass Accumulation

Two models are currently proposed to qualitatively describe the structure of cells and EPS within porous media. According to the first model, the biological accumulation is presumed to be a homogeneous mesh of polymer and cells completely filling the pores. Fluid in the reactor flows evenly throughout any given cross-sectional area. This model is unique in that it rejects the traditional view of fixed biological accumulations as necessarily being a film. No bulk fluid-biofilm interface exists. The true three dimensional nature of the media, bacteria, and bacterial products is highlighted.

The second model encompasses the more conventional model of biofilms. The reactor media may be visualized as numerous, parallel conduits with biofilm accumulations on the wall. Some points in a cross section may contain a very dense biofilm while others contain no biofilm. In this case, the biofilm may actually smooth and straighten the flow path. This model embodies the traditional bulk fluid-biofilm interface.

Taylor and Jaffe (1990) discuss both of these models in the first paper of their four-part series. They have named the two views the "closed" and "open" pore models, respectively. Results from their experiments indicate that flow through a porous media follows the "open" pore space model. Data from permeability reductions and dispersivity changes are used to support their conclusion.

Detachment Rates

Taylor and Jaffe (1990) performed mixed culture experiments in two identical, parallel columns packed with sand. The systems were then modelled. Data from the first

column was used to calibrate the model and data from the second was used to validate the calibration.

Detachment rates were incorporated into the model as a complex function that included terms to account for both the biological state of the organisms as well as the effects of physical shearing. No detachment rates were measured experimentally. Values were calculated from other measured variables.

The model indicated that significant errors occurred when detachment rates (shearing rates) were ignored. This was especially true for their system. It was demonstrated that the length of the column allowed the detached biomass to be filtered or reattach downstream. They noted the distribution of the biofilm on the particles largely influenced the detachment and reattachment of biomass. This influence was attributed to a nonuniform pore size distribution and thus nonuniform hydraulic environment.

Howell and Atkinson (1976) modelled trickling filters for use in the waste water industry. The model was unique for the time as it included sloughing. Their attention was drawn to the sloughing phenomenon after noticing that published data showed a high degree of variability. The efficiency of the filters, mass of biofilm in the filter, and biofilm thickness on the support particles cycled with time.

Their trickling filter model built upon a traditional tanks-in-series approach. Howell and Atkinson realized that while large parts of the film would slough, the entire accumulation would not slough simultaneously. To mimic this phenomenon, the tanks-in-series model incorporated a cyclic but random sloughing factor that affected only a small number of tanks at any one time. The results of this study came much closer than previous models to predicting the unsteady behavior of actual trickling filters. The overall conclusion was that the average values routinely employed in the design of trickling filters

were inadequate. Consideration of sloughing was essential as sloughing induced channeling and dead zones which significantly impacted the bed efficiency.

Rittmann (1982) proposed a steady state detachment rate model based entirely on the mechanics of shear stress. The model was born from criticism of a previous modelling effort by Rittmann and McCarty (1980), in which no account was given for biomass loss due to mechanical processes. In his treatise, Rittmann used a first-order model with parameters calculated using the data of Trulear and Characklis (1982) obtained in rotating annular biological reactors (RABRs). The model was extrapolated from the geometry of a rotating annulus to that of both packed and fluidized beds. As was expected, results indicated that shear losses were of increasing importance as shear stresses increased.

Rittmann also noted that use of a model with a first-order detachment rate coefficient gave numerical results similar to those given by existing models incorporating first-order endogenous decay coefficients as the two models were of essentially the same form. This numerical similarity was especially true at low shear stresses where the endogenous decay coefficient and the detachment rate coefficient could not be statistically differentiated.

Speitel and DiGiano (1987) noted that while detachment rates were definitely dependant upon shear stresses, higher detachment rates occurred during periods of high growth or high biodegradation. They experimentally determined rates of biomass loss in beds packed with granular activated carbon. The reactors were liquid-saturated and fed with either phenol or paranitrophenol. The inoculum was an undefined microbial population. The system was modelled building upon the work of Rittmann (1982). The detachment equation not only included a first-order term for shear loss due to mechanical

processes, but also included a Monod-type component which accounted for growth-based biomass loss. Results of the modelling effort agreed quantitatively with the experimental work. Comparison of the first-order equation of Rittmann to the data of Speitel and DiGiano showed very poor correlation at transient conditions. Higher than needed estimates of detachment at steady state, although qualitatively correct, were also noted.

The conclusion was once again that biomass loss was extremely important to the modelling process. In the model, biomass loss due to growth-based detachment accounted for 33% and 67% of new biomass formed, depending on the substrate. Based on this, Speitel and DiGiano concluded the parameters in the detachment rate equation, and thus detachment rates, were highly substrate specific.

Chang and Rittmann (1988) studied the effects of surface irregularities on detachment rates in packed columns. Two types of media, spherical and irregular granular activated carbon, were tested. Reactor design made independent measurement of important system parameters such as detachment rates impossible. A model previously developed by Chang and Rittmann (1987) calculated detachment rates using the first-order equation of Rittmann (1982).

For both reactor runs, Chang and Rittmann observed qualitatively the same detachment phenomena following biofilm establishment. However, at times early in the biofilm development, the irregular surface provided the attached cells better protection against shear stresses. The detachment rates were lower from the irregular surface when compared to detachment rates from the smooth surface. As the accumulations approached steady state, however, the detachment rates became very comparable. In fact, the detachment rates for both systems approached those predicted for smooth

surfaces. A step function best modelled the transition in detachment rates at early times to detachment rates at steady-state. Data within the time frame of high biomass accumulation (the transition phase) rates was neglected.

In a later study Chang, Rittmann, Amar, Heim, Ehliger, and Lesty (1991) studied the effects of abrasion on biomass loss. As the original intent was to demonstrate the advantages of fluidized beds over packed beds concerning plugging and channeling, a liquid-fluidized bed utilizing glass beads as support material was chosen for the study. Once again, a model was used to calculate variables that were not directly measurable. Results indicated that as the concentration of the glass beads in the reactor was increased, the detachment rate increased due to increased abrasion. However, the results were somewhat confounded by a simultaneous decrease in biofilm thickness and an increase in biofilm density, both of which partially offset the effects of increased abrasion. Most importantly, detachment rates in these systems were higher than those expected in non-fluidized systems. Abrasion was attributed to be the controlling factor in loss of bacteria from fluidized particles.

Peyton (1992) studied the effects of shear stress and glucose utilization rate on cellular detachment rates of Pseudomonas aeruginosa in RABRs. Results showed that over the narrow range of shear stresses studied, shear stress did not significantly affect detachment rate. Detachment rate was concluded to be most strongly correlated to the growth rate of the film. Therefore, a model was proposed which incorporated the variables influencing growth rate. Variables included in the proposed equation were yield, biofilm thickness, and substrate utilization rate. Detachment rates calculated from this equation gave significantly better results than those of Rittmann, Chang and Rittmann, Speitel and DiGiano, and others.

Stimulus-Response Experiments

Ever since Danckwerts' (1953) solidification of residence time distribution theory from vague concepts into a practical technology, the residence time study has received widespread application in flow systems as a tool for determining flow characteristics. In fact (and by his own admission), Danckwerts' original journal article has reached the stature of "a primary reference which is seldom cited" (Danckwerts, 1981). It shall remain so here.

Regardless of the lack of citations from his original article, Danckwerts did unite several ideas to form the foundation of a technology which is currently considered standard practice. The residence time of a tracer in a system or part of a system can be measured by introducing the tracer at the influent (the stimulus) and recording the concentration of the tracer as a function of time at the effluent (the response). Three methods of tracer injection have been effectively used as stimuli; step, pulse, and cyclic. All three methods can yield equivalent information, but the step and pulse methods are the more common. While cyclic tracer injections are theoretically equal to pulse and step injections, the mathematics and qualitative interpretation of the responses are considerably more complex.

If the pulse or step method of tracer injection is employed, the concentration of tracer at the influent as a function of time is usually assumed to form a "perfect" pulse or step respectively. For a pulse injection, this implies the influent concentration profile is a Dirac delta function. Often, if the time taken to introduce the tracer is very short compared to the residence time of the system, the injection is assumed to be perfect. For the step function, "perfection" implies the concentration of the tracer at the influent

is originally a constant and instantaneously changes to its final value. Step functions are also approximated as perfect if the time taken to complete the step is short when compared with the residence time.

A difference should be noted between the hydraulic residence time and the tracer residence time. Hydraulic residence time is the time the fluid spends in the system. This may not be equal to the time the tracer spends in the system. The residence time of the tracer will always be measured using the stimulus-response method. It then becomes a question of how well the tracer resembles the fluid or particulate for which the residence time is desired. The choice of the tracer is an important consideration in interpreting results. A pertinent example of this would be a system in which the tracer is absorbed.

Naor and Shinnar (1963) reiterate this theme by stating that if the tracer does not adequately represent the fluid of interest, the results will represent a response function to a change in concentration. They also explain that this may not be totally undesirable as it may provide important information, but the effect on the results should be noted. Difficulty in choosing an adequate tracer may be the main reason residence time studies have not received more use in studies of biological systems.

Naor and Shinnar also point out that the response of the system to the stimulus must be linear if the step and pulse inputs are expected to yield identical information. A linear response is always received if the tracer is nearly ideal; however, if a less-than-ideal tracer is employed, a non-linear response must also be noted. Swaine and Daugulis (1988) cover, among many other relevant topics, the process of tracer selection in some detail.

For systems in which residence times are short, the time needed to inject the tracer may be significant when compared to the mean residence time. The pulse can no

longer be considered as a perfect Dirac delta function, but it is still possible to calculate a residence time by measuring the concentration of the tracer as a function of time at both the inlet and outlet of the reactor. This technique has been named the imperfect pulse method.

The main disadvantage to the imperfect pulse method lies in tailing. Tailing occurs when the concentration of tracer at the point of measurement maintains a low, but non-zero, value for an extended period of time. Any tailing in the inlet signal will cause an even larger tailing effect in the outlet signal.

If the traditional analysis of moments is performed, tailing will severely skew the results. The region where the measurements are generally least accurate is the region of lowest tracer concentration, the tail. This is also the region where the largest time values occur. The mathematical equation used to calculate residence times is strongly influenced by large time values if the concentration of the tracer is not zero. Large time values translate into long residence times if tailing is present.

Advanced mathematical techniques such as Laplace transforms (Ostergaard and Michelsen, 1969) or Fourier transforms (Condoret, Riba, and Angelino, 1989) must be used to improve the accuracy of the calculations. Anderssen and White (1971) state that even the use of these methods is not foolproof as the choice of the weighting parameters for such techniques will influence the results. Not surprisingly, optimization of the weighting parameters may be more than trivial.

Tracer studies have been applied to a myriad of abiotic systems; however, the application of the same technology to biological systems has been lacking. Without doubt, the presence of a biological component complicates the implementation of the experiment and the interpretation of the results. This lack of application has been

especially tragic considering the large effect such a biological accumulation may have on the flow regime. Most published material involving tracer studies in biological systems concern three large industrial applications; waste water treatment, immobilized enzyme catalysis, and fermentation.

Kennedy and Droste (1985) described the factors affecting the startup of anaerobic downflow trickling filters for treatment of waste water. As part of the research, stimulus-response studies were performed on the reactors after establishment of a mature biofilm. A pulse of tritium was injected into the recirculation line and aloquots of effluent were taken at short intervals for approximately 3 hydraulic residence times. The experiment was replicated for three different feed flow rates (varying by a factor of 17). The recovery of tracer was reported to have been in excess of 90% for all cases. The results of this study indicated the flow regime in the reactor was that of a CFSTR. The CFSTR type behavior was attributed to biogas production. They proposed the biogas may have acted as a gas lift pump causing a large degree back mixing.

Suschka (1985) reviewed previously published residence time studies on biological percolating filters. Experimentally measured residence times were found to be at least three times longer than those predicted from falling-film theory. To explain this large disparity, a reactor model was devised in which two distinct liquid layers existed. The first liquid layer was free flowing over the surfaces. The second was captured within the biofilm. Under this scheme, the liquid volume maintained in the reactor would be much larger than values calculated from the older flat-plate, falling-film model.

This two-layer model was used to explain high effluent oxygen concentrations in a seemingly anaerobic reactor. The free flowing layer could have maintained high oxygen

concentrations because the diffusion of oxygen into the film was slow when compared to the residence time of the liquid in this layer.

Guzy, Saidel, and Lotan (1990) applied a tanks-in-series model to an immobilized enzyme packed bed reactor fed with two differing substrates. Tyroglubin, a high molecular weight (669,000 Daltons) protein was pulsed into the reactor. The high molecular weight minimized liquid diffusional effects and eliminated diffusion of the tracer into the pores of the packing. The concentration of tracer in the effluent was monitored continuously via absorbance measurements.

The number of tanks-in-series needed for modelling a particular flow rate was calculated from the residence times and variances of the response curves. The residence time curves indicated that the number of tanks required to model the range of flow rates was not a monotomic function of the flow rate, but was the result of two competing processes, mechanical agitation and diffusion.

The problems associated with the perfect pulse injection method were seen in this study. At the highest flow rate, the residence time was approximately 12 seconds while the tracer injection time was 2 seconds, not a negligible difference. Thus, the results may have been confounded by a poor method of analysis. No effort was made to deal with the imperfect stimulus.

Anselme and Tedder (1987) reported tracer studies for determination of the flow regime in a packed bed bioreactor. Yeast cells were immobilized and fed glucose to produce ethanol. Of the various types of support media investigated, crushed brick provided the best performance and was therefore used in most of the experiments, including the residence time determinations. A step change in ethanol concentration was introduced at the inlet of the column. The effluent was collected and ethanol

concentrations determined with a gas chromatograph. Results indicated that axial dispersion was important to modelling the flow. It was also noted that improved culture stabilization techniques were needed to prevent plugging of the pores from cell debris.

Similarly, Godia, Casas, and Sola (1987) reported on the fermentation of glucose to ethanol in a bioreactor packed with yeast immobilized in carrageenan gel beads. A tracer study was conducted via a step introduction of lactose at the column inlet. Results showed the packed column had flow characteristics best represented by a tanks-in-series model. This CFSTR behavior was once again attributed to a large evolution of carbon dioxide with the degree of backmixing too large to be modelled by dispersion alone.

Gonzalez, Caminal, de Mas, and Lopez-Satin (1989) operated a column packed with ground wheat straw which was enzymatically hydrolyzed to sugar. To study the bed hydrodynamics a step perturbation of potassium chloride was introduced at the inlet. The conductivity of the reactor fluid was then measured near the outlet. Axial dispersion in the reactor was found to be negligible. The reactor was subsequently modelled as plug flow. Porosity of the system was calculated from the measured response curves. The liquid volume of the reactor was computed as the flow rate divided by the residence time. Liquid volume then divided by the total reactor volume yielded the porosity.

Hamamci and Ryu (1987) investigated the use of a tapered bioreactor filled with yeast immobilized in carrageenan to produce ethanol from glucose. A tapered column was chosen to alleviate the mixing problems associated with the evolution of large quantities of carbon dioxide. The dispersion parameters for the hydrodynamic model, however, were determined in a cylindrical column. This allowed the authors to calculate the dispersion parameters from the already well established equations for flow through a round conduit. The tracer was blue dextran. Once again this tracer was chosen

because of its high molecular weight which reduced diffusional problems. The concentration of the blue dextran at the effluent was determined via a spectrophotometer. Over the range of flow rates employed in the study, it was determined that the dispersion was significant, but relatively constant.

MATERIALS AND METHODS

Laboratory Techniques

Experimental Design

Two modes of operation, constant flow rate and constant pressure drop, are commonly seen in liquid-saturated porous media systems. However, setting often dictates the frequency of occurrence. In natural environments, constant pressure systems are prevalent. Obvious examples include aquifers and oil reservoirs. In man-made locations, constant flow systems such as packed columns are predominant.

For reasons related to laboratory operation, this project employed a constant flow system. From preliminary work, it was concluded that constant pressure drop systems suffered from catastrophic plugging problems associated with the biological accumulation. The extremely low flow velocities, resulting from the plugged media, created significant contamination and sampling problems. A constant flow rate system was chosen because of the ability to maintain higher flow velocities.

As the results from this research were to be applicable to other projects, Pseudomonas aeruginosa, an aerobic, Gram negative, bacteria was chosen for study. The bacteria had already received considerable attention from the research community and as a result was better characterized than most species. It was hoped that information from these experiments could be added to a large data base already receiving use in predictive modelling. Following this same theme, the substrate chosen was glucose (the

substrate for which the most information regarding growth of Pseudomonas aeruginosa was available).

In order to test extremes of bacterial growth, the experiments were divided into two series. In the first series the influent glucose concentrations were maintained at 18 g m^{-3}. This high concentration of glucose implied the bacteria would experience oxygen-limited growth as their oxygen requirements would exceed the solubility of oxygen in water at the operating temperature. Thus, the biological activity would be at a maximum for an aerobic system in which the oxygen was supplied by means of dissolved air. The second set of experiments were run with an influent glucose concentration of 2 g m^{-3}. This left the bacteria glucose limited and growth rates were lower.

The reactor was sampled approximately once per day. Samples were taken from the influent and effluent of the reactor for determination of glucose and cell concentrations in the bulk fluid. Pressure drop across the reactor was recorded. Stimulus-response curves were collected using the method of imperfect pulse tracer injection with a salt solution as the tracer. The concentration of salt was proportional to the conductivity of the liquid. The progress of the pulse through the system was measured by conductivity probes at the inlet and outlet of the reactor.

Three runs were made for the first series and two runs for the second series. At the end of one run from each series, an experiment was performed to determine the permeability of the reactor (including the accumulated biomass) to cells in the bulk fluid (microbead penetration experiments). At the end of the other runs, the reactor was disassembled and the quantity of biomass weighed. Not until after the first two runs of the first series were the microbead penetration experiments determined to be necessary.

Therefore, a third experiment was run for this series. No other measurements were taken from the third run except the microbead penetration data.

To determine the permeability of the system to bacteria, fluorescent latex microbeads (Polysciences, Inc., #15705) were injected as a pulse into the reactor. The fraction of beads leaving the reactor and distribution of beads captured in the reactor after 10 minutes were counted. After 10 minutes, the reactor was disassembled. Sections of the glass beads inside the reactor were distributed into beakers based upon distance from the inlet. The beakers, along with the reactor contents, were dried. To enable counting, the microbeads were resuspended with a known quantity of water and the beakers placed in an ultrasonic water bath for a minimum of 30 minutes. The ultrasound treatment insured the microbeads were completely removed from the surface of the glass beads.

It was assumed from physical similarities between the bacteria and the beads, that the performance of the beads while passing through the reactor imitated that of the bacteria. The beads were spherical with a 1 μm diameter, a specific gravity of 1.05, and a negative surface charge (Drury, 1992).

Reactor Design

To obtain meaningful data, a reactor in which the biofilm properties remained constant as a function of axial length was desired. Due to a lack of a better measurement, thickness was chosen as the characteristic on which the criteria of "constant" biofilm properties was applied. From previous efforts by Abedeen (1990) and

Crawford (1987), the maximum length over which the biofilm thickness could be expected to remain constant was 50 mm. Thus, 50 mm was chosen as the reactor length.

The final reactor design is shown in Figure 1. The reactor housing was cut from cylindrical glass tubing 31 mm in diameter. The diameter was chosen to yield a minimum bed diameter to particle diameter of 30. This was the smallest ratio recommended by Cohen and Metzner (1981) (for newtonian fluids) allowing wall effects on packing geometries and fluid flow to be neglected. The minimum ratio was exceeded as the packing was previously chosen to be 1-mm glass beads.

Brass gauze (#30) over each end of the reactor retained the beads in the bed. Flow was dispersed by funnels attached to each end of the reactor with silicon sealant. The influent funnel contained a stainless steel ball to further insure even distribution of the fluid. Pressure taps, liquid sampling ports, and conductivity probe ports were provided at each end of the reactor at the gauze-bead interface.

For the first run, the conductivity probes were cemented directly into the reactor. This resulted in poor data because the probes could not be calibrated. The reactor design was subsequently changed. For the remaining three runs a septum was built into each end of the reactor through which a conductivity probe was aseptically inserted and removed as needed for calibration.

Support Apparatus

The support systems for the reactor are shown in Figures 2 and 3. Dilution water was pumped to the reactor from a 0.125 m^3 aerated supply tank. This tank provided for a 2-day reserve of distilled water if the house water supply were interrupted. Particulates,

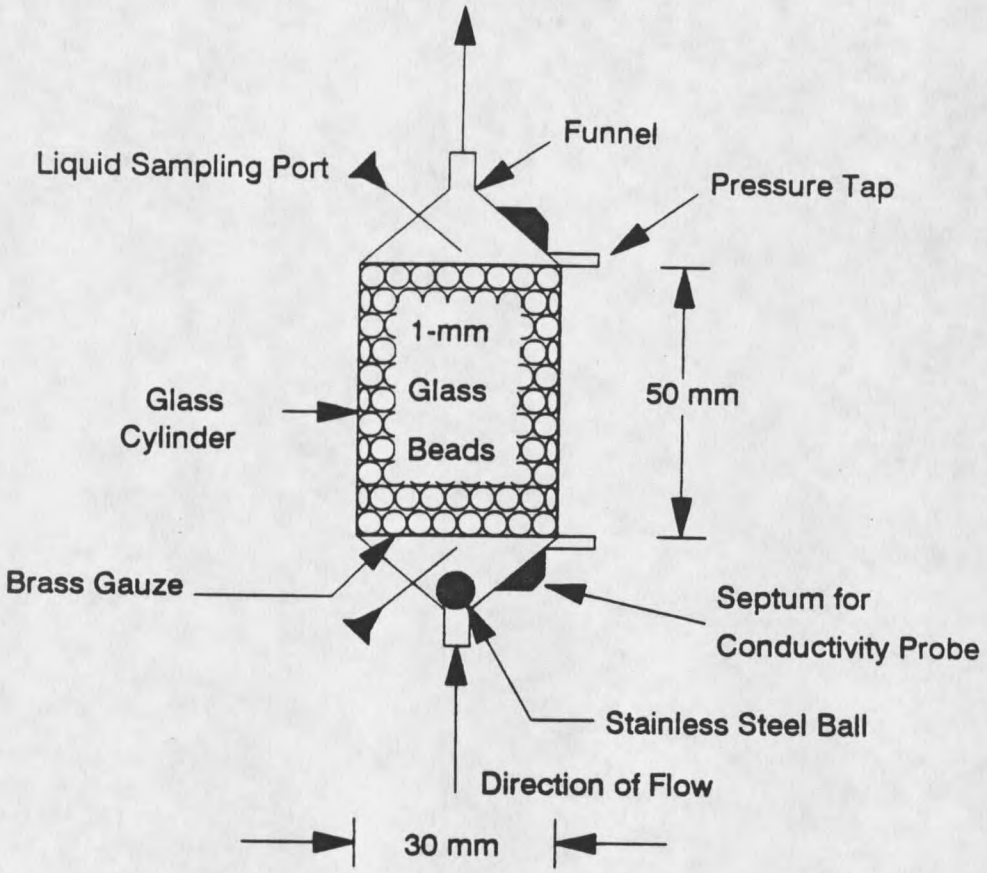

Figure 1. Detailed diagram of final packed bed reactor design.

including bacteria, were removed from the dilution water by two in-line 0.2 μm cartridge filters (sterile). After filtration, the sterile dilution water flowed through a 1.5 m stainless steel coil submerged in a constant temperature (20°C) water bath. Nutrients were added to the bulk liquid just prior to the reactor influent.

To prevent contamination, the minerals were divided into two carboys. The glucose and phosphates were isolated from the nitrates and other minerals in an attempt

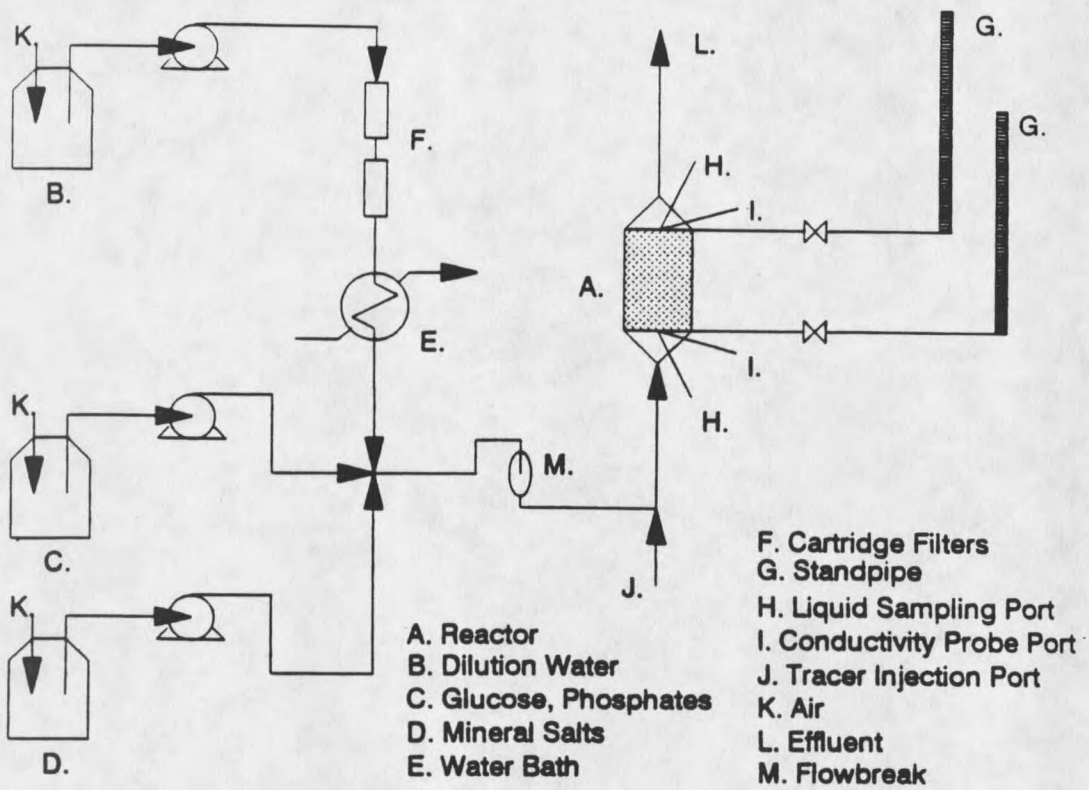

Figure 2. Schematic of apparatus used in high glucose loading rate experiments.

to produce a nutrient-limited environment in both containers. Should the bacteria contaminate either feed container, the extent of growth would be minimal.

The mineral salts and glucose were prepared to be 100 times the recommended concentration (Sieble 1987) and mixed with the dilution water in a ratio of 1 part feed to 99 parts water. A complete table of minerals and concentrations is given in the appendix. While the temperature of the minerals and substrate was not controlled, the effect on the

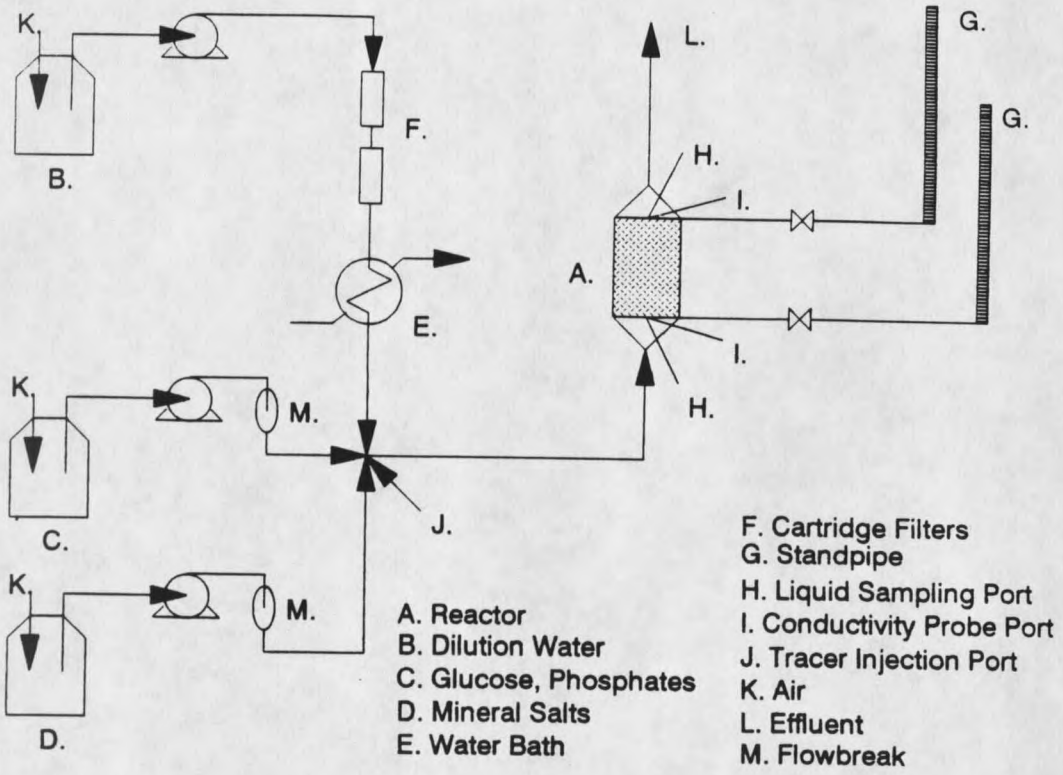

Figure 3. Schematic of apparatus used in low glucose loading rate experiments.

overall fluid temperature was negligible.

For the first series of experiments (see Figure 2), the nutrients were mixed with the dilution water before the flow break. The flow break was included to ensure that bacteria from the reactor did not migrate upstream into the nutrient reservoirs. An injection port for the salt tracer was located approximately 15 cm ahead of the reactor (down stream of the flow break) for the first series of experiments.

For the second series of experiments (see Figure 3), the tubing configuration was altered. Flow breaks were added to both feed lines and the flow break was removed from the dilution water line. The nutrient and tracer injection points were moved much closer to the reactor. This reduced cell concentrations at the reactor influent by eliminating a significant portion of the surface area (tubing wall) previously available for colonization.

All the tubing used in the experiment was Masterflex™ silicone (oxygen permeable) tubing. The pumps used were Masterflex™ peristaltic pumps (1-100 rpm).

Conductivity Measurement

The system for measuring liquid conductivities was originally used by McCready (1977) to determine the mixing characteristics of static mixers. Unfortunately, McCready's conductivity probes were much too large to use in this study. For this experiment, probe size was extremely important. First, the conductivity probes needed to be small enough to insert through a septum without causing leakage upon removal. Second, the probes needed to be small enough to not significantly disturb flow through the reactor.

As a solution, the probes (see Figure 4) were constructed from stainless steel syringe needles. The probe design was originally forwarded by Lamb, Manning, and Wilhelm (1960). However, the design was subsequently miniaturized by Larsen and Keck for work presented by Keck (1987).

Probe operation was based on the flow of electricity through a fluid separating two electrodes. One electrode acted as a current source, while the second received the fraction of the current which was able to pass through the liquid. Any change in liquid