Development of artificial biofilms for use in mass transport studies
by Michael Todd Abrahamson

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

Abstract:
Artificial biofilms are useful laboratory tools allowing fundamental biofilm research to be conducted under controlled and reproducible conditions. Unfortunately, these synthetic biofilms, which generally consist of uniformly distributed bacteria throughout a polymer matrix, lack the structural heterogeneity (voids, microcolonies) often seen in natural biofilms. Thus data obtained from this type of biofilm may not accurately describe the behavior of a natural biofilm system.

This thesis presents a procedure which can be used to construct an artificial biofilm that incorporates the structural heterogeneity observed in natural biofilm. Alginate was harvested from a mucoid strain of Pseudomonas aeruginosa, filter sterilized, and fixed to a glass slide where it served as the biofilm matrix. High densities of P. aeruginosa were injected into specific locations within the alginate matrix to represent microcolonies similar to those found in natural biofilms. The heterogeneous artificial biofilm was then used to study the effects of a single microcolony on the mass transport of dissolved oxygen. The artificial biofilm was probed using a dissolved oxygen microelectrode to obtain concentration profiles through the biofilm. Visual analysis of these profiles indicated that the surrounding dissolved oxygen field was affected by a single microcolony (150 μm diameter), up to a distance of 1400 μm away from the microcolony, and that the mass transfer resistance due to the biofilm matrix itself was negligible.

An artificial biofilm, containing uniformly distributed bacteria throughout an alginate matrix was used to evaluate a procedure used to extract the microbial respiration kinetics from substrate concentration profiles. Only those profiles which were partially-penetrated with respect to oxygen could be analyzed using this procedure. This indicates that the applicability of this procedure was highly dependent upon the shape of the substrate concentration profile. Those profiles which could be analyzed showed good reproducibility in determining a value for Ks. Observation of these profiles showed that the diffusion boundary layer was significantly influenced by the flow velocity. The diffusion boundary layer decreased from 0.048 cm to 0.004 cm when the flow velocity was changed from low to high.

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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.

November 8, 1995
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ABSTRACT

Artificial biofilms are useful laboratory tools allowing fundamental biofilm research to be conducted under controlled and reproducible conditions. Unfortunately, these synthetic biofilms, which generally consist of uniformly distributed bacteria throughout a polymer matrix, lack the structural heterogeneity (voids, microcolonies) often seen in natural biofilms. Thus data obtained from this type of biofilm may not accurately describe the behavior of a natural biofilm system.

This thesis presents a procedure which can be used to construct an artificial biofilm that incorporates the structural heterogeneity observed in natural biofilm. Alginate was harvested from a mucoid strain of *Pseudomonas aeruginosa*, filter sterilized, and fixed to a glass slide where it served as the biofilm matrix. High densities of *P. aeruginosa* were injected into specific locations within the alginate matrix to represent microcolonies similar to those found in natural biofilms. The heterogeneous artificial biofilm was then used to study the effects of a single microcolony on the mass transport of dissolved oxygen. The artificial biofilm was probed using a dissolved oxygen microelectrode to obtain concentration profiles through the biofilm. Visual analysis of these profiles indicated that the surrounding dissolved oxygen field was affected by a single microcolony (150 μm diameter), up to a distance of 1400 μm away from the microcolony, and that the mass transfer resistance due to the biofilm matrix itself was negligible.

An artificial biofilm, containing uniformly distributed bacteria throughout an alginate matrix was used to evaluate a procedure used to extract the microbial respiration kinetics from substrate concentration profiles. Only those profiles which were partially-penetrated with respect to oxygen could be analyzed using this procedure. This indicates that the applicability of this procedure was highly dependent upon the shape of the substrate concentration profile. Those profiles which could be analyzed showed good reproducibility in determining a value for K_S. Observation of these profiles showed that the diffusion boundary layer was significantly influenced by the flow velocity. The diffusion boundary layer decreased from 0.048 cm to 0.004 cm when the flow velocity was changed from low to high.

CHAPTER 1

INTRODUCTION

Statement of the Problem

When growing biofilms in the laboratory, the researcher controls only the quality of the influent into the reactor, the kind of organism under study, the substratum material, and to a certain extent the hydrodynamic conditions of the reactor. Beyond these parameters, biofilm formation is no longer under the researchers control. One way a researcher can gain increased control over the experimental conditions, is through the use of an artificial biofilm. Artificial biofilms are useful laboratory tools, serving as simplified "models" for more complex biofilm phenomena. Artificial biofilms generally consist of uniformly distributed bacteria throughout a polymer matrix, and thus lack the structural heterogeneity (voids, microcolonies) found in some in natural biofilms. Therefore, the data obtained from this type of biofilm may not reflect the behavior of a natural biofilm system. In addition, some researchers consider cells immobilized in gel beads suspended in bulk fluid to be artificial biofilms. Because by definition "a biofilm consists of cells immobilized at a substratum..." (Characklis and Marshall, 1990), these gel beads can hardly be considered biofilm. The artificial biofilms

presented in this work were fixed to a substratum in an attempt to conform to this definition.

Goals and Objectives

The main goal of this research was to develop an artificial biofilm incorporating the structural heterogeneity seen in natural biofilm. This was accomplished through the immobilization of high bacterial densities in localized regions of a sterile matrix material, which was fixed to the substratum, to represent microcolonies. Furthermore, this artificial biofilm was compatible with microelectrode use since bacterial alginate was used for the matrix material of the biofilm. Such heterogeneous artificial biofilm would allow mass transport studies involving biofilm to be conducted under more controlled and reproducible conditions, while still maintaining some of the physical characteristics of natural biofilm. With use of this biofilm, it would be possible to produce data that more closely resembles the behavior of the natural biofilm system.

Two structurally different artificial biofilms were constructed and used in mass transport studies to demonstrate the usefulness of artificial biofilm. One contained a heterogeneous structure, the other a homogeneous structure. The heterogeneous artificial biofilm permitted the study of the effects of a single microcolony embedded in a sterile alginate matrix on the transport of dissolved

oxygen. The homogeneous artificial biofilm was used to evaluate the method currently used to extract the substrate diffusivity and microbial respiration kinetics from substrate concentration profiles. In addition the homogeneous biofilm was probed under two different flow velocities, allowing the effects of flow velocity on the microbial respiration kinetics and mass transport of oxygen to be examined.

The following objectives were set for obtaining the goal of developing artificial biofilm and evaluating their usefulness.

1) Select a biofilm matrix which was compatible with microelectode use to study the biofilm activity and mass transport.

2) Sterilize the biofilm matrix.

3) Attach the matrix to the substratum.

4) Immobilize high densities of viable bacteria into localized regions of the sterile matrix material to represent microcolonies.

5) Construct dissolved oxygen microelectrodes to monitor the transport of oxygen through the artificial biofilms.

6) Study the effects of a single microcolony embedded in a sterile alginate matrix on the transport of oxygen.

7) Evaluate the procedure currently used for the extraction of microbial respiration kinetics from substrate concentration profiles for its applicability and reproducibility.

CHAPTER 2

BACKGROUND

Natural biofilms

When nutrients in the bulk fluid become scarce, planktonic cells tend to attach to surfaces (Characklis and Cooksey, 1983 , Matilla-Sandholm and Wirtanen, 1992). Once here, these attached bacteria grow, reproduce, and excrete extracellular polymer substances (EPS) onto the surface resulting in the formation of biofilm (Characklis and Cooksey, 1983). The extracellular products are commonly polysaccharides which can be highly anionic, uronic, and acid containing polymers (Costerton, 1994). Due to their random block structure, many bacterial extracellular polymers bind large quantities of water. This makes the gel highly flexible and less susceptible to destruction by hydrodynamic forces, aiding in the survival of the microorganism (Gacesa and Russell, 1990). In addition to serving as a protective matrix, these polymers also provide cohesive forces, and adsorb nutrients for the microorganisms (Brown and Lester, 1980, Characklis and Cooksey, 1983, Matilla-Sandholm and Wirtanen, 1992).

At the microscale, the bacteria may be homogeneously distributed throughout the EPS matrix as seen in films of *Pseudomonas aeruginosa*, have an aggregated distribution as seen in films containing *Klebsiella pneumoniae*, or contain a mixture of both as seen in multiple specie biofilm (Characklis and Marshall, 1990). These observations have been supported by studies involving confocal laser microscopy. These studies have shown that the structure of some biofilm is complex and heterogeneous, containing bacterial aggregations (microcolonies) separated by interstitial voids within which the matrix is less dense. (Stoodley et al., 1994, Costerton, 1994).

Artificial biofilms

Microorganisms intentionally immobilized or entrapped in a polymer matrix layer fixed to a surface are often thought of as artificial biofilm. The construction generally consists of a polysaccharide matrix material (i.e. kelp alginate, agar, K-carageenan, etc.) into which bacteria are uniformly distributed. (Woodward, 1988, Jouenne et al., 1994, and Whitham and Gilbert, 1993). Several papers have been written which detail the procedures used for immobilizing bacteria in various types of polymers (Woodward, 1988, Smidsrød and Skjåk-Bræk, 1990). Artificial biofilms are popular for use when studying biocide and antibiotic performance against biofilm (Whitham and Gilbert, 1993, Jouenne et al., 1994).

The microbial cells in these artificial biofilms expressed similar responses as those in natural biofilm systems, showing a decreased susceptibility to biocides in comparison to planktonic cells.

Artificial Biofilm Development

Biofilm Matrix

Kelp (brown seaweed) alginate has been widely used for the immobilization of bacteria in artificial biofilms as it is commercially available, its gels are highly durable, and the technique used is considered to be mild and easy to perform (Hiemstra et al., 1983, Chen and Huang, 1988). Kelp alginate is also similar in its chemical composition to bacterial alginate which may be found in natural biofilm. Table 1 lists the types of polysaccharides produced by potential biofilm forming bacteria. Alginate is an unbranched, copolymer of 1,4 linked α-L-guluronic acids and β-D-mannuronic acid residues which occur in varying proportions and sequential arrangements (Martinsen et al., 1989, Smidsrød, 1970). The uronic acids are monosaccharides which have been oxidized at C6 to produce a carboxylate group and therefore are negatively charged. (Gacesa and Russell, 1990). The monomers can occur in homopolymeric blocks or in alternating sequences. The relative proportion of the two uronic acids varies from alginate to alginate and is a major factor in

Table 1. Microbial Extracellular Polysaccharides.(Modified from Biofilms by Characklis and Marshall, 1990)

Polysaccharide	Microorganism
Xanthan	*Xanthomonas campestris*
Dextran	*Aerobacter spp* *Streptococcus bovis* *Streptococcus viridans* *Leuconostoc mesenteriodes*
Alginates	*Pseudomonas aeruginosa* *Azotobacter vinelandii*
Gellan gum	*Pseudomonas elodea ATCC 31461*
Zanflo	*Erwinia tahitica*
Polytran	*Sclerotium glucanicum*
Pullulan	*Aureobasidium pullulans*
Curdlan	*Alcaligenes faecalis*

determining the properties of the polysaccharide (Gacesa and Russell, 1990). Alginate can be found in all kelp where it serves as a structural component of the cell wall (Cottrell and Kovacs, 1977, Smidsrød, 1974). In addition to kelp, some bacteria are able to synthesize and excrete alginate. It was observed by Linker and Jones (1966), that *Pseudomonas aeruginosa* was able to produce an alginate like polysaccharide. They later confirmed that an O-acetylated alginate was a major component of *P. aeruginosa* slime. Alginate has also been found to be

produced by the bacteria *Azotobacter vinelandii* and by several other pseudomonads. (Gacesa and Russell, 1990, Smidsrød and Skjåk-Bræk, 1990).

There are some important differences in the compositional and structural detail of bacterial alginate in comparison to the kelp alginate. The first is that kelp alginate contains poly-guluronic acid blocks (i.e. consecutive guluronic acid residues) while the alginate synthesized by *P. aeruginosa* does not. Alginate which contains guluronic acid blocks produce strong, rigid gels (Martinsen et al., 1989, Smidsrød and Haug, 1972) while alginate rich in mannuronic acid blocks, such as that produced by *P. aeruginosa,* tend to form gels that are more voluminous and elastic (Gacesa and Russell,1990, Smidsrød and Haug, 1972). The second difference is that the mannuronic acid residues are acetylated in bacterial alginate. Skjåk-Bræk et al. (1989b) determined that the acetylation of mannuronic acid residues decreases the gel strength, and increases the water binding capacity of the alginate when compared to non-acetylated kelp. The absence of guluronic acid blocks and the presence of acetylated mannuronic acid residues in the bacterial alginate results in a weaker, more flexible gel in comparison to kelp alginate (Gacesa and Russell, 1990). Use of the bacterial alginate for the matrix of the biofilm would allow the biofilm to be easily penetrated with microelectrodes without causing damage to its fragile sensing tip.

Biofilm Matrix Preparation

Several methods exist for the extraction of extracellular polymers from bacterial cultures (Brown and Lester, 1980), but not for polymer purification (i.e. removal of bacteria while maintaining polymer integrity). With use of these extraction methods (physical and chemical) the bacterial cells are separated from the polymer, usually by centrifugation, leaving the polymer dissolved in the supernatant. In this form, biochemical analyses can be performed on the polymer, but it is of little use in serving as an artificial biofilm matrix. To maintain the polymers physical integrity a filter sterilization method was developed.

Immobilization of Microorganisms

Alginate gel beads containing microorganisms are commonly used in bioreactors. They have been widely studied to determine the mass transport rate to immobilized microorganisms. Large diameter (>1 mm) kelp alginate beads containing cells, show a significant mass transport resistance of the gel matrix which often limits the reaction rates of immobilized cells (Kurosawa et al.,1989, Hiemstra et al., 1983, Ogbonna, et al. 1991, Chen and Huang, 1988). In large beads, active cells were found to a depth of only 50-200 μm (Stormo and Crawford, 1992, Kuhn et al., 1991). This corresponded to the penetration depths of oxygen for immobilized cells as well (Chang and Moo-Young, 1988). Several methods exist for improving oxygen transfer to immobilized cells and have been

classified into three groups 1) oxygenation of the bulk medium 2) use of oxygen carriers and *in situ* oxygen generation (algae cells) within the matrix and 3) immobilization of cells in microgel beads with diameters of 50-200 μm (Ogbonna et al. 1991, Kurosawa et al., 1989). To produce beads on this scale, several instruments have been developed which can make small beads from kelp alginate solutions (Stormo and Crawford, 1992, Matulovic et al. 1986, Ogbonna et al., 1991). With these instruments a solution of kelp alginate is forced through needles or nozzles of various size where vibrations or a concentric air stream shear the carrier droplets off the tip producing a fine aerosol of cell suspension which settles into a stirred solution of $CaCl_2$. These methods produce small beads 10-100 μm but most can only utilize low viscosity alginate. Due to the high viscosity of bacterial alginate, microgel beads made of this alginate can not be made using these instruments.

Biofilm Systems Modeling

To describe the mass transport of a substrate through a biofilm system, models to analyze the data generated in these systems are needed. These models can provide insight into the growth and substrate utilization kinetics within microbial films. This in turn, leads to a better understanding of biofilm processes, allowing the design and operation of reactors to be optimized (Harremoes, 1978,

Rittmann and McCarty, 1980, Muslu, 1993, Lewandowski et al., 1991). A number of different biofilm processes may be rate limiting for the overall reaction of the biofilm including the transport of nutrients from the bulk solution into and through the biofilm, the reaction itself, or the transport of by-products (Harremoes, 1978, Fan et al., 1990). The diffusivity of nutrients through the biofilm has been identified as the controlling process for the substrate uptake rate in most biofilm systems (Lewandowski et al., 1991, Kurosawa et al., 1989, Patel and Bott, 1991).

Most models tend to describe an idealized biofilm. This biofilm has a uniform cell density and thickness. Substrate concentrations change only in the z-direction normal to the surface of the biofilm. It also assumes that all required nutrients are in excess concentration, except the rate-limiting substrate. (Rittmann and McCarty, 1980, Lewandowski et al., 1991, and Hannoun and Stephanopoulos, 1986). The models commonly used to describe the behavior of a biofilm system are as follows.

Molecular diffusion, the only means of mass transport within the biofilm described above, is related to the substrate concentration by Fick's second law.

$$\left(\frac{\partial C}{\partial t}\right)_{Diffusion} = D_e\left(\frac{\partial^2 C}{\partial x^2}\right) \qquad (1)$$

where D_e is the diffusion coefficient of the substrate in the biofilm, C is the substrate concentration at the distance x from bottom of the biofilm, and t is time.

As the substrate diffuses into the biofilm, the substrate utilization at any point in the biofilm is assumed to follow a Monod relation;

$$\left(\frac{\partial C}{\partial t}\right)_{\text{Re action}} = -\frac{V_{Max}C}{K_S + C} \tag{2}$$

where

$$V_{Max} = \frac{\mu_{Max}X}{Y_{\frac{X}{S}}} \tag{3}$$

where C is the substrate concentration at the distance x from bottom of the biofilm, V_{Max} is the maximum substrate utilization rate, K_S is the half-saturation coefficient, μ_{Max} is the maximum growth rate, X is the biomass concentration, $Y_{x/s}$ is the biomass yield, and t is time.

Combining these two processes of reaction and diffusion results in the following reaction-diffusion model which can be used to describe biofilm activity;

$$\left(\frac{\partial C}{\partial t}\right)_{Biofilm} = D_e\left(\frac{\partial^2 C}{\partial x^2}\right)_f - \frac{V_{Max}C}{K_S + C} \tag{4}$$

When consumption equals transport, a steady state will occur which simplifies the equation through the removal of the partial derivatives, resulting in:

$$D_e\left(\frac{d^2C}{dx^2}\right)_{Biofilm} = \frac{V_{Max}C}{K_S + C} \tag{5}$$

Several researchers have determined the diffusivity and the rate of substrate consumption using a diffusion-reaction model similar to that in equation

(5). However, there are difficulties with this models use. The first difficulty is that this equation has no analytical solution due to the non-linear Monod-type reaction term. The second difficulty is to determine the microbial kinetic parameters and substrate diffusivity from the same experiment. In modeling studies researchers may use a numerical analysis to solve equation (5). Numerical methods are often used to give an approximate prediction and are usually calculated using algorithms on computers. With the numerical method, researchers may choose diffusivities or kinetic parameters from literature which were determined under similar experimental conditions for use in their model, (Tang and Fan, 1987, Rittmann et al., 1986, Revsbech et al., 1986) or design experiments which separate the effects of consumption (kinetic parameters) and transport (diffusion) to determine the starting parameters.

When determining the kinetic parameters of the Monod expression, batch, chemostat, or respirometry reactors with suspended cultures are generally used. This allows external and internal mass transport effects to be ignored (Fan et al., 1990). As Rittmann et al. (1986) points out, a serious drawback of the conventional chemostat or batch culture techniques used to estimate kinetic parameters for microorganisms is the possible alteration of cell physiology. The differences in kinetic parameters between suspended culture and the immobilized culture have been noticed by researchers (Fan et al. 1990, Hooijmans et al. 1990a, Hiemstra et al., 1983). For example, Toth et al. (1988) discovered that

upon encapsulation into a gel, *Escherichia coli* cells exhibit enhanced respiration. Hiemstra et al. (1983) found the apparent $K_S(O_2)$ of the immobilized cells was dependent upon the density of the cells in the alginate beads as well as the bead radius. Finally, Hooijmans et al. (1990a) conducted a study in which the intrinsic kinetic parameters of immobilized enzymes showed a decrease with a factor 2.3 for V_{Max} value and with a factor 2.7 for the K_M value when compared to the parameters for the free enzyme. Thus the immobilization process or the carrier material itself, not only can have an effect on the maximum intrinsic conversion rate (V_{Max}) but also on the affinity of the enzyme (K_M) for oxygen (Hooijmans et al., 1990a).

When determining the diffusivity of a substrate through a polymer matrix, the procedure generally used is to deactivate the biological material (biofilm) with a chemical (i.e. mercuric chloride) or use a sterilized polymer. This will allow biological kinetics to be ignored (Revsbech et al., 1986, Sun et al. 1989, Hulst et al. 1989).

Another alternative used by some is to simplify the reaction-diffusion equation by assuming zero order kinetics for which analytical solutions can be derived (Bungay et al.,1969, Rittmann and McCarty, 1980, Onuma et al., 1985, Shuler and Kargi, 1992, Larsen and Harremoes, 1994). This assumption is not without merit as the K_S values of oxygen are low in comparison to the oxygen concentrations in the dominant part of the active biofilm. The saturation constant of

oxygen (K_{ox}) for most bacteria is small (<0.5 mg/L) (Tang and Fan, 1987, Larsen and Harremoes, 1994, Chang and Moo-Young, 1988). It is not surprising that the K_S for aerobic bacteria is so low for oxygen as its value represents the affinity of the organism for the growth supporting nutrient. The lower the value the greater the bacterium's affinity for that molecule (Alexander and Skow, 1989, Wiseman, 1983).

Substrate Diffusivity and Kinetic Parameter Analysis from Substrate Concentration Profiles

As mentioned it can be difficult to obtain both the diffusivity of a substrate and microbial kinetic parameters from the same experiment. However, one way this can be achieved is with the use of microelectrodes. Microelectrodes sensitive to dissolved oxygen have seen considerable use for determining both the microbial respiration rate and diffusivity of oxygen through biofilm (Bungay et al., 1969, Revsbech et al. 1986, Hooijmans et al., 1990a, Lewandowski et al., 1991). Biofilm systems are diffusion limited, and as a result the concentration of substrates vary with depth forming concentration gradients. (Patel and Bott, 1991, Lewandowski et al., 1993). Using ion-selective and gas sensing microelectrodes with tip diameters less than 20 μm, direct measurements of these chemical gradients in biofilms can be made creating substrate concentration profiles. The

small tip allows measurements in microenvironments to be made without causing much disturbance or destruction to the biofilm structure. As Lewandowski (1994) states, the shape of the profile is simultaneously influenced by these three factors:

1 - microbial substrate uptake rate which is a function of microorganism concentration and their affinities for the substrate,

2 - substrate transport rate through the film which depends on substrate diffusivity through the biofilm, and

3 - substrate transport rate to the biofilm which is a function of microbial substrate uptake rate, substrate diffusivity through the water, and hydrodynamics near biofilm surface.

A major advantage of using microelectrodes is the ability to obtain data normally not available such as detailed chemical analysis of the bulk water as well as chemical measurements inside the biofilm (Lewandowski, 1994). Another advantage of using microelectrodes includes its fast response and the reduction of very small amounts of oxygen making measurements in stagnant medium possible, such as that found in biofilm (Revsbech and Ward, 1983, Hooijmans et al., 1990b).

The mass transport coefficient and kinetic parameters for oxygen in the artificial biofilm can be determined from substrate concentration profiles using a procedure developed by Lewandowski et al. (1991). With this procedure, a dissolved oxygen microelectrode is used to measure the dissolved oxygen

concentration above and along the depth of the biofilm. The biofilm-water interface is located on the substrate concentration profile allowing the profile to be divided into two parts: the bulk, which contained information about substrate transport to biofilm, and in the biofilm, which contained information about the microbial activity (Lewandowski et al., 1991). Using a microprobe, which simultaneously measured the substrate concentration and optical density, Lewandowski et al. (1991) were able to show that the interface can be located where a discontinuity (inflection point) occurs in the substrate concentration profile. The discontinuity arises because the mass transfer of substrate in the water and the biofilm are governed by different equations that are tied together by the requirement that the substrate flux at the biofilm-water interface be continuous at a steady state. The external mass transport resistance (bulk fluid profile) was modeled by the following empirically derived equation:

$$\frac{C - C_s}{C_b - C_s} = 1 - Exp[-A(x - x_s)] \qquad (6)$$

where C is the local substrate concentration, C_s is the substrate concentration at the surface of the biofilm; C_b is the substrate concentration in the bulk fluid, x is the distance from bottom of the biofilm, x_s is the biofilm thickness, and A is an experimental coefficient determined graphically from the slope of a linearized form of equation (6).

The profile in the biofilm was described using a third order polynomial regression and modeled using equation (5) and its integrated form

$$\left(\frac{dC}{dx}\right)_{Film} = \sqrt{2\frac{V_{Max}}{D_f}(C - C_o - K_S \ln\frac{K_S + C}{K_S + C_o})} \quad (7)$$

where C is the substrate concentration at a distance x from bottom of the biofilm, C_o is the substrate concentration at the substratum, D_f is the diffusion coefficient for the substrate in the biofilm, V_{Max} is the maximum substrate utilization rate, K_S is the half-saturation coefficient.

The advantages of using this technique is that it does not require extensive sample preparation, simplifying assumptions, (Lewandowski, 1991) nor a time rate of change measurements as many other techniques do (Bungay et al., 1969, Sanders et al., 1970).

Results of Substrate Diffusivity and Microbial Respiration Kinetics Reported in Literature

A summary of the kinetic parameters and diffusivities found in biofilm and 'artificial biofilm' using different procedures are presented in Table 2. As seen in Table 2 a wide range of diffusivities have been reported in literature using similar polymers. The diffusion coefficients of solutes in gels are often inconsistent and can seldom be compared with one another, in part due to the considerable variations in the experimental conditions that are used. (Martinsen et al., 1992, Ruiz-Bevia, et al., 1989). The diffusivities of various substrates through biofilm vary

from as low as 2% to as high as 122% of the molecular diffusivity in water. Several factors have been shown to have an effect on the diffusivity of a solute through biofilm. Among these factors are a biofilm's structure, cell density, amount of extracellular compounds present, age, and roughness of the surface, which increases the mass transfer into the biofilm near the surface (Tang and Fan, 1987, Fan et al., 1990, Siegrist and Gurjer, 1985). Furthermore, there are also inherent inaccuracies in the experimental methods used. For example, the change in substrate concentrations through the film is not simply a mass transfer effect, but it is also being consumed by the cells in the biofilm. It is difficult to separate these effects and estimate the contributions due to mass transfer resistance and substrate consumption (Patel and Bott, 1991). Finally, as Hulst et al. (1989) points out, the differences seen in the effective diffusivity through purified polymer matrices may be due to the degree of gel inhomogeneity. It was shown by Skjåk-Bræk et al. (1989a) that alginate gels have neither a homogeneous polymer concentration nor structure throughout them due to the kinetics of gel formation.

Table 2. Summary of respiration kinetics and oxygen diffusivities reported in literature.

Matrix Material / Method of Analysis	Oxygen Half-Saturation Coefficient (K_S)	Oxygen Utilization Rate	Oxygen Diffusivity Through Matrix $\left(\frac{D_{Matrix}}{D_{Water}}\right)\times 100\%$	Reference
Biofilm / Microelectrode	0.25 mg/L	0.05 mg/L-s	88	Lewandowski et al. (1991)
Biofilm / Microelectrode	0.025 mg/L	0.037 mg/L-s	66	Lewandowski (1994)
Biofilm / Microelectrode - Reactor	ND	0.17 mg/L-s	122	Larsen and Harremoes (1994)
Biofilm / Microelectrode	ND	0.025 mg/L-s	2-5	Bungay et al. (1969)
Biofilm / Microelectrode	ND	ND	51	Revsbech (1989)
Biofilm / Microelectrode	ND	0.34 mg/L-s	33	Chen and Bungay (1981)
Alginate Bead / Diffusion model	ND	ND	54-76	Hulst et al. (1989)
Agar Bead/ Microelectrode	0.57 mg/L	0.158 mol/kg-s	80 (Literature)	Hooijmans et al. (1989)
Alginate Bead / Reactor - Diffusion Model	0.15 to 0.768 mg/L	3.5 to 13.3 mmol/g cell-h	88-99	Kurosawa et al. (1989)
Alginate gel / Diffusion Cell	ND	ND	84-86	Sun and Furusaki (1988)
Agar gel / Diffusion Cell - Reactor	0.075 mg/L (Batch Reactor)	0.135 mg/L-s	70	Sato and Toda (1983)
Biological Aggregates / Diffusion Cell	ND	ND	20-100	Matson and Characklis (1976)

ND - Not Determined. Experiments used zero-order kinetics or deactivated biofilm.

CHAPTER 3

MATERIALS AND METHODS

Media

The following media were used in association with work on the artificial biofilm and will be made reference to in this chapter.

Mineral Salts Medium

(grams/Liter)

K_2HPO_4, 7.0; KH_2PO_4, 3.0; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.1; Glucose, 2.0; Distilled H_2O, 1 Liter. [pH 7.2.]

Preparation:

1) Add phosphates to 500 mL of distilled water. In a separate flask add sulfates to 500 mL distilled water. Autoclave phosphates and sulfates solutions for 25 min at 20 psi pressure 121 °C.
2) Let solutions cool to 25 °C and aseptically combine.
3) Filter sterilize glucose into combined solution. Mix thoroughly.