Organic carbon degradation in the East Gallatin River with biofilm kinetics
by Subramaniam Srinanthakumar

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Civil Engineering
Montana State University

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Chairperson, Graduate Committee

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NOTATIONS

Symbol		Units
A	= plan area of biofilm	L^2
A_c	= area of cross-section of flow	L^2
A_p	= plan area of study reach	L^2
a	= area of viable organisms per unit volume	L^{-1}
B	= coefficient describing a boundary effect by slime layers	T^{-1}
D_{ca}	= diffusivity coefficient of electron acceptor	$L^2 T^{-1}$
D_{cd}	= diffusivity coefficient of electron donor	$L^2 T^{-1}$
D_e	= effective diffusivity coefficient of S in the film	$L^2 T^{-1}$
D_L	= longitudinal dispersion coefficient	$L^2 T^{-1}$
D_o	= diffusivity of oxygen in the slime	$L^2 T^{-1}$
D_s	= diffusivity of glucose in the slime	$L^2 T^{-1}$
F	= flow rate in the channel	$L^3 T^{-1}$
F_c	= constant factor relating the quantities of glucose and oxygen utilized in the aerobic metabolism	–
f	= an empirical coefficient in determining longitudinal dispersion coefficient	–
H	= mean flow depth in the stream	L
j	= flux given by $D_L (\partial S/\partial Z) - U.S$	$ML^{-2} T^{-1}$
K_D	= coefficient for overall stream deoxygenation	T^{-1}
K_o	= coefficient for overall BOD removal	T^{-1}
K_{sc}	= coefficient for sedimentation	T^{-1}

Symbol		Units
K_s	= Monod half-velocity coefficient	ML^{-3}
k_1	= biological rate equation coefficient	T^{-1}
k_2	= biological rate equation coefficient	L^{-1}
k_3	= biological rate equation coefficient	$M^{-1} L^3$
k'_1	= laboratory determined BOD rate coefficient	T^{-1}
k_F	= rate coefficient in $R_{B20} = k_F S$	LT^{-1}
k_v	= rate of substrate uptake defined by $Th_c = \frac{(2D_e \cdot S_s)^{1/2}}{k_v}$	$M^{1/2} L^{-3/2} T^{-1/2}$
L	= length of study reach	L
M	= total attached biomass	ML^{-2}
M_A	= heterotrophic fraction of attached biomass	ML^{-2}
MW_a	= molecular weight of the electron acceptor	M
MW_d	= molecular weight of the electron donor	M
N	= rate of substrate consumption per unit interfacial area	$ML^{-2} T^{-1}$
N_{max}	= maximum rate of substrate uptake	$ML^{-2} T^{-1}$
n	= Mannings coefficient	—
O	= oxygen concentration in the film	ML^{-3}
p	= descriptive level of significance	—
Q	= stream flow	$L^3 T^{-1}$
R_B	= attached biomass production rate	$ML^{-2} T^{-1}$

Symbol		Units
R_D	= detachment rate	$ML^{-2}T^{-1}$
R_H	= hydraulic radius	L
R_S	= suspended biomass production rate	$ML^{-3}T^{-1}$
r	= local rate of substrate uptake per unit area of viable organisms	$ML^{-2}T^{-1}$
r_c	= individual cross-sectional area in a stream	L^2
r_j	= sources and sinks of S_j	$ML^{-3}T^{-1}$
r_v	= rate of substrate consumption per unit film volume	$ML^{-3}T^{-1}$
S	= substrate concentration	ML^{-3}
S_{ca}	= concentration of electron acceptor within the film	ML^{-3}
S_{cd}	= concentration of electron donor within the film	ML^{-3}
S_e	= effluent substrate concentration	ML^{-3}
S_i	= influent substrate concentration	ML^{-3}
S_j	= concentration of a water quality variable	ML^{-3}
S_{oa}	= concentration of electron acceptor in bulk liquid	ML^{-3}
S_{od}	= concentration of electron donor in bulk liquid	ML^{-3}
S_s	= substrate concentration at the top of biofilm	ML^{-3}
T	= water temperature	°C
Th	= biofilm thickness	L
Th_c	= critical film thickness	L
t	= time	T
t_f	= time of travel	T

Symbol		Units
U	= mean flow velocity	LT^{-1}
U_*	= shear velocity	LT^{-1}
V	= volume of reactor	L^3
W	= width of stream	L
x	= distance measured into the slime from the interface	L
X	= concentration of suspended biomass	ML^{-3}
X_f	= cell concentration in the slime	ML^{-3}
X_i	= influent concentration of suspended biomass	ML^{-3}
Y_A	= biofilm yield coefficient	–

ABBREVIATIONS

Ashfree Dry Weight	AFW
Centimeter	cm
Cubic Feet	cuft or ft^3
Cubic feet per second	cfs or ft^3/s
Degree(s) Celsius	°C
Dry Weight	DW
Feet	ft
Feet per second	fps or ft/s
Gallon(s)	gal.
Gallon(s) per minute	gpm
Gram(s)	g
Hour(s)	h
Inch(es)	in.
Micrometer(s)	μm
Milligram(s) per liter	mg/l
Milliliter(s)	ml
Minute(s)	min.
Pound(s)	lb.
Second(s)	s
Soluble Organic Carbon	SOC
Standard	Std.

Standard Deviation	S.D.
Standard Error	S.E.
Square meter(s)	m^2
Square feet	sq.ft or ft^2
Suspended solids	SS
Total Organic Carbon	TOC
Versus	vs
Volatile Suspended Solids	VSS

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ABSTRACT

The importance of sessile microbial populations in aquatic environments has been recognized for many years especially the heterotrophic slimes under polluted conditions. The extensive literature included in this dissertation review indicated that models proposed by previous researchers to predict substrate degradation in streams have been based on assumptions of first order or saturation kinetics incorporating mainly the substrate utilization by suspended biomass.

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INTRODUCTION

Mathematical models have been used widely in the past decade in simulating water quality and ecological interactions. The increased use and development of water quality models may be attributed to the Water Pollution Control Act amendments of 1972 (PL:92-500) which call for areawide wastewater planning across the United States. The Government Affairs Committee's Criteria and Standards Task Group, while recognizing the need for the re-evaluation of the goals of the 1972 Water Pollution Control Act, emphasized the importance of using calibrated models for realistic waste load allocations.

Mathematical simulation techniques are useful as long as the physical mechanisms involved are accurately reflected in the model. In this respect, river systems can be expected to behave differently depending on the category they belong to. They can be tidal, non-tidal, swift moving, shallow or sluggish deep streams. The significant physical and biological mechanisms involved in water quality modeling can be quite different in each class of river system. Shallow rivers for example, present problems in modeling quite different from that of deep rivers. The microbial population, sessile or suspended, dominating water quality modeling in various categories of rivers can be different. The importance of sessile microbial populations in aquatic environments has been recognized for many years. Natural biofilms growing on a river bed are composed primarily of algae and bacteria, appearing as a mass of slime. Under polluted conditions below a sewage outfall, the slimes are predominantly heterotrophic. In modeling organic carbon, biological slimes covering the stream bed play a major role, especially in shallow turbulent streams. The kinetics of substrate uptake may vary depending on the range of substrate concentrations

encountered in a study. In most studies involving modeling of natural systems, researchers arbitrarily assume the kinetics of substrate uptake, as it is difficult to determine it in each case. However, the critical problem is to determine which microbial population, suspended, attached or both play the dominant function in substrate assimilation. The appropriate kinetic expression may then be utilized in the stream model. Thus, identifying the appropriate kinetics of the processes becomes imperative in modeling any system.

This research is multidisciplinary in the areas of water quality and microbial ecology, elucidating biofilm effects on stream water quality. The study was designed to make use of the advances made in biofilm kinetics and couple it with mathematical modeling of river systems. The research approach was to carry out an artificial stream study to determine the kinetics of the substrate uptake and then use such information in the proposed stream models to study the applicability of such models in predicting organic carbon and biomass variations below a point source of pollution. The East Gallatin River which receives a partially treated sewage effluent from the Bozeman Wastewater Treatment Plant was used for field studies. The results of the study provide useful information in terms of assessing the important microbial population in shallow streams and the order of kinetics of substrate uptake by river films in a specified range of substrate concentrations.

GOAL, OBJECTIVES AND SCOPE OF STUDY

The research carried out encompasses theoretical, as well as field and pilot plant scale phases of study.

Modeling substrate and biomass in natural environments is complicated by the complex microbial communities and the presence of an undefined substrate. The goal of this research is to determine the growth and substrate utilization kinetics of river biofilms in multi-substrate natural environments.

The theoretical objectives were to provide an extensive and critical review of the literature giving a background on microbial adhesion in natural environments, reactor kinetic studies done on microbial films and past modeling efforts, complete with a summary and a critique, and to: formulate substrate-biomass models based on theoretical considerations for organic carbon use and biomass variations below a sewage outfall in a stream.

An artificial channel was constructed on the river bank for the pilot plant scale study: (1) To determine the kinetics of organic carbon uptake rate by river biofilms under field conditions, (2) To compare the importance of the suspended microbial population to the sessile organisms in predicting the organic carbon uptake in shallow turbulent streams. Several runs were made, each for five hours to establish steady state conditions with attached biomass grown on plexiglass plates. Control runs at high and low substrate concentrations were made without using the attached biomass to assess the importance of suspended microbial populations. The following parameters were measured during each

run: pH, DO, temperature, TOC, SS, VSS. Chlorophyll, biomass and thickness measurements were also made on the attached films.

The information obtained from the experimental study and the field data collected were used to verify the substrate biomass models formulated under steady state conditions. The river data collected for the validation of the model included water quality and biofilm growth kinetic parameters measured at six sites, one above and five below the sewage outfall over three summers. The first summer period was used as a preliminary study period to formulate hypotheses. The general structure of the biofilms was also studied using electron microscopy.

LITERATURE REVIEW

The review of the pertinent literature is categorized into three main sections. The first section deals with background information on bacterial adhesion mechanisms and the role of attached bacteria as a major component of the assimilative capacity in river systems. Substrate kinetic studies of suspended biomass and biofilms, the transfer and transformation processes related to modeling distributions of substrates are reviewed in the second section in addition to published water quality models. The final section gives a summary and a critique of the relevant literature described in the previous sections. Several terms such as slimes, sewage fungus, periphyton, aufwuchs have been used in the literature to refer to the attached microbial growths in streams. In this study, for simplicity and convenience, the terms biofilm or slime layer will be used which refer to the gelatinous film formed on submerged surfaces such as rocks and includes both living and non-living materials.

Background Information

The occurrence of excessive biological growths in streams is of increasing concern to environmentalists because of their effect on aquatic life and oxygen resources. The microorganisms in rivers and streams can be attached to the streambed or suspended in the overlying water. The logical question to be asked at this point is why do microorganisms attach to a surface and how do they achieve attachment? It is imperative to understand the nature and the mechanisms by which microorganisms especially bacteria attach to the cobbles and rocks in streams before analyzing their effects on water quality.

Nature and Significance of Microbial Adhesion

A review of recent literature throws considerable light on the advantages that sessile microorganisms more specifically bacteria and algae have, compared to the suspended organisms in extracting food from fast flowing streams especially in nutrient limited conditions. Costerton (24) found that a square centimeter of an immersed surface might typically have as many as a million attached bacteria whereas a cubic centimeter of water flowing over that surface contained only a thousand bacteria. This was also revealed by Geesey (42) who demonstrated the significance of sessile bacteria over those free floating in unpolluted mountain streams. The attachment makes life easier for bacteria in a stationary location from where they could easily extract the organic molecules and nutrients from the passing water. Characklis (19) concluded from his review that sessile bacterial growths are an essential part of the assimilative capacity of rivers. Sanders (110) compared suspended and attached organisms in a river to batch and continuous cultures. The population that adheres, forms a thick slime layer on rocks in the streambed especially when there is organic enrichment. The East Gallatin River downstream of the sewage outfall in Bozeman, Montana with its cobbly bed was shown to have dense slime layers for a considerable distance (78,105). The nature of these slime layers above and below the sewage outfall varied due to the organic enrichment below the outfall. Costerton (25) found planktonic organisms favoring the "adherent growth habit" in aquatic systems. Hendricks (51) used respiratory and enzymatic data to establish that sessile microorganisms were more active than those suspended. The major role in recycling of substrates by slime layers has been long suspected by Zobell (150).

Composition and Organisms of Slimes

Composition and colonization. Due to its hydrated nature which contains as much as 98% water, the capsular material surrounding bacteria is slimy and has almost the same refractive index as the medium (143). The slime composition has been reported to be predominantly carbohydrate with small amounts of nitrogen (19). Mackie (70) reported that the slimy material surrounding bacteria was composed mainly of polysaccharides and that the thickness varied according to nutritional and environmental requirements. Ward and Berkeley (133) reported that most of the bacterial polysaccharides are composed of more than one type of sugar residue often containing uronic acids and/or pyruvyl ketal groups which are responsible for the polymer having an overall negative charge. Using phase contrast and electron microscopy, Geesey (41) examined the in-situ distribution of cells revealing that they were enmeshed in an extensive "fibrous matrix." It was further determined that this material surrounding the bacteria was produced by the bacteria themselves. Using staining procedures on slime, Fletcher (38) and Jones (58) determined that it was composed of an anionic polymer with the characteristics of polysaccharides.

Several studies reported micro-colony development in slime layers (24,41) formed by a mass of tangled polysaccharide fibers suggesting that the glycocalyx may group bacteria in a somewhat organized community with several niches for different species. Such adherent populations tend to respond uniquely to changes in nutrient or environmental conditions. Most of the natural bacterial films are interspersed among algae forming a mixed attached population which was evident from several electron micrographs (25). Algae have polysaccharide fibers similar to bacteria and the initial colonization may be accomplished by either bacteria or algae. There is not enough evidence in the literature to determine

which of the two, algae or bacteria, colonize first in natural environments. Geesey (42) on the basis of electron microscopy showed that the attached algae provided a suitable surface for bacterial colonization. Algae would provide the surface and nutrients for bacterial growth. The electron micrographs obtained in this study support this finding as shown subsequently. Hendricks (51) considered a primary layer of bacterial growth as necessary for subsequent colonization by higher life forms. Baier (7) believed that the primary layer of bacterial growth changes the critical surface tension of the monolayer, helping higher life forms to colonize subsequently. Marshall (77) suggested zymogenous chemoorganotrophs to be the initial colonizers followed by oligotrophs and other higher forms. Whatever group of organisms colonize first, it certainly helps subsequent colonization by others. In general, only viable cells can colonize first as suggested by Meadows (83) because of their ability to withstand stresses.

Organisms of slime layers. In general, a slime layer would have micro and macro organisms consisting of procaryotes, eucaryotes and macroinvertebrates. In the context of this study, the macroorganisms are not considered. Aquatic biofilms generally are composed of phototrophs, heterotrophs and reducers (126). Sanders (109) reported that even though slimes in natural streams are composed of predatory, phototrophic and chemotrophic microorganisms, the main population was the heterotrophs. All these organisms live together in niches forming an interacting ecological community. The most prominent and important filamentous bacteria found in slimes was *Sphaerotilus* which grew as a chain of cells encased in filamentous sheaths (26,31).

The occurrence of *Sphaerotilus natans* in the East Gallatin River as far as six miles below the sewage outfall has been reported (78,105). It is not entirely certain what environmental factors allow *Sphaerotilus* to grow massively in competition with other organisms. Dias (31) found that *Sphaerotilus* would grow even at reduced DO levels whereas such an environment was less favorable to other attached bacteria in a mixed population. Curtis and Curds (26) examined and compared the composition of the slimes in different polluted habitats. The slimes were dominated by *Sphaerotilus natans* which require a continuous flow of nutrients and at least 1 mg/l DO, or zoogleal bacteria. The bacteria *Thiotrix*, *Beggiotoa* and the *Zoogleal* bacteria were found to become abundant with the development of slime (52). Most sewage fungus outbreaks were caused in situations when the soluble organic carbon concentrations were in the range of 6 to 20 mg/l and where daytime DO exceeds 8 mg/l (59). Nitrifying bacteria have been found in slimes in the presence of ammonia in rivers. Based on field measurements in shallow streams, Tuffey (130) concluded that the drastic decay of ammonia nitrogen was caused by nitrification by the attached population of nitrifying bacteria. The findings from this study and Curtis (28) indicated that substantial numbers of nitrifying bacteria were found on the mud surface on the river bed throughout the river with a considerably lesser concentration in the waterphase contributed by scour from the slimes.

Functional Aspects of Biofilms Including Their Activity

A natural biofilm because of autotrophic and heterotrophic groups of organisms mixed together present difficulties in compartmentalizing, in order to obtain quantitative information on the heterotrophs. Under polluted conditions, the heterotrophic fraction of the biomass is much higher than the autotrophic fraction. Common measurements of

periphyton involved: species diversity; indices of community structure; dry weight (DW) and ashfree dry weight (AFW); phytopigments; biovolume. Recent papers in the literature showed the interest of researchers to study the functional aspects of natural biofilms through the analysis of oxygen production and ^{14}C assimilation (59,88,103). More recently, there has been a trend towards the measurement of adenosine triphosphate (ATP) as an indication of the viable biomass (21,139). More realistic estimates of bacterial counts have also been reported using Epi-illuminated fluorescence microscopy (15,54).

Heterogeneous natural populations may be partitioned by use of estimates of dry weight, ash-free dry weight, Chlorophyll a and ATP (21). Weber and McFarland (135) emphasized the importance of Chlorophyll a as the primary photosynthetic pigment, the only form found in all algae and suggested a method of estimating the algal biomass. Geesey et al. (42) used a conversion factor of 60 to estimate cellular carbon from chlorophyll measurements. Others have provided methods for organic carbon estimates from chlorophyll measurements (73,79). Commonly employed values for ratios of mg cellular carbon to mg Chl. a range from 30 to 60 (21). By carefully combining the organic carbon estimates based on these different methods, the sample may be partitioned into autotrophic and heterotrophic components and viable and nonviable organic carbon. Problems of direct measurement of organic carbon in hetergeneous microbial communities have been mentioned by some investigators (21,42).

Mechanisms of Microbial Adhesion

The methods by which microbes adhere to surfaces have been of concern and consideration in the past few years. The problem of destruction and shearing of slimes,

especially the latter, requires a thorough knowledge of the modes of microbial attachment to surfaces. Experiments have demonstrated that due to some strong mechanisms of attachment, even extensive washing would not remove these attached growths (150).

Biocontact theories are presently based mostly on colloid stability theory (or DLVO theory). The colloid stability theory involves complex calculations of ionic double layer interactions and Van der Waals' forces. Pethica (96) reviewed the relationship of DLVO theory as applied to biocontact and presented a general theory based on the recent thermodynamic description of cell adhesion. Hall (47) presented a specimen calculation based on thermodynamic considerations which showed that changes in chemical composition are important variables as particles approach one another. This may mean that a shift in chemical composition would dominate the interaction. The attachment of bacteria to surfaces is influenced by the adsorbed organic substances which condition the surface for further attachment (75,76). However, some proteins have been found to inhibit attachment of bacteria to surfaces (96). The movement of the flagellates help overcome the potential barrier and contribute to their attachment. The use of pili extending from many organisms into the environment have been implicated in certain bacterial adhesion to inert surfaces (133). There is, however, no clear demonstration in the literature of the general involvement of these surface appendages in the attachment process.

Tadros (124) made a distinction between the two processes of particle attachment, namely deposition and adhesion. The difference between these two is determined by whether they are governed by short-range or long-range forces. Rutter and Vincent (107) described the long-range surface forces involved in particle deposition:

(1) Double layer interactions

(2) London–Van der Waals forces

(3) Steric interactions

(4) Bridging interactions

Deposition would be based on the balance of forces involved. Steric interactions predominate only when the surface and particle are highly covered with polymers. The long- and short-range forces were classified into various types by Tadros (124):

(1) Long-range attractive forces due to Van der Waals and electrostatic forces.

(2) Short-range forces are:

(a) chemical bonds

(b) dipole interactions

(c) hydrophobic bonding

(3) Interfacial reactions

Based on the phenomena of hydrophobic interactions, Rutter and Vincent (107) showed that microorganisms being hydrophilic adsorb to a hydrophilic clean glass surface stronger than to a hydrophobic teflon. Interfacial reactions are important with microorganisms capable of secreting polysaccharides which would condition the attaching surface. Ward and Berkeley (133) mentioned the possibility of the polysaccharides being produced only after the microbial adhesion had occurred.

Fletcher (39) divided the accumulation of microorganisms onto a surface into three stages:

(a) Adsorption of the organisms to a surface.

(b) Attachment by forming polymer bridges.

(c) Growth and division of organisms on the surface.

At the usual pH range found in natural habitats, Marshall (77) determined a net negative charge associated with most bacteria on the basis of electrophoretic studies. Marshall (77) and Scheraga (113) described two different types of sorption in the adhesion process: Reversible sorption where application of a shear force or flagellar action would remove the bacteria and irreversible sorption caused by the extracellular polymers produced by bacteria and anchoring them to the surface. Bacteria and natural solid surfaces have been shown to be predominantly negatively charged which causes electrostatic repulsion. However, the Van der Waals attractive forces operate when the cells get close to the surface and may provide a weak net attraction at the secondary minimum. It is possible therefore for the cells in the initial reversible attachment to be held at a finite distance from the surface in equilibrium by the balancing of attractive and repulsive forces. At this point, irreversible attachment is accomplished by the organisms excreting extracellular polymers which overcome the electrostatic repulsion barrier and attach by bridging directly to the surface (75). This has been further supported by Zobell's work (150) and by bacteria forming colonies on submerged surfaces (41,58). Wardell and Brown (134) looked at another aspect of colonization. Under limitations of carbon, the free receptor sites available on the surface and cells can be used by cells to adsorb with the small amount of polymer produced. When there is an excess carbon, large amounts of polymer may be produced which would cover all the available binding sites and possibly hinder attachment (74). The importance of this aspect can be readily seen in aquatic environments with low nutrients. During the adhesion random or perpendicular orientation of the bacteria depends on whether the extracellular polymers were produced around the entire bacteria or only at one pole. Costerton (24) found the polymeric fibers termed glycocalyx to be

negatively charged. The mechanism of attachment of these glycocalyx to the surface appear to be similar to the bridging mechanism of the polyelectrolytes in coagulation (64,137). The attachment bond is stronger than the connecting fibers because shearing off the organisms on a surface leaves a print of attached polymers (66). Fletcher (39) defined passive and active bacterial attachment. Passive attachment is caused by molecular adsorption. Two types of physiological activity required for active bacterial attachment are:

(a) mobility

(b) synthesis of polymers required for bridging

Motility helps increase the momentum and the statistical chance with which the bacteria can reach the surface. This shows clearly that attachment is dependent on physiological processes.

Factors Affecting Attachment, Growth and Nutrient Removal

Effect of the attaching solid surface. Many cells do not divide unless in contact with biological or non-biological surfaces (96). Several surface properties are important in the formation of a primary film (52). The influence of solid surfaces on attachment and growth have been reported by many researchers (42,51,75,150). The solid surfaces concentrate nutrients and thus enhance attachment. The relationship between the surface area of a laboratory container and bacterial activity was demonstrated by Zobell (150). Solid surfaces in addition to concentrating nutrients aid in controlling the diffusion of exoenzymes from the cell. However, low molecular weight nutrients that are concentrated are not responsible directly for attachment (150).

Dexter et al. (29) listed the effects of several parameters of solid surfaces other than toxicity on the microbial attachment growth:

(a) The surface texture of the surface

(b) The surface charge

(c) Wettability of the substrate

After analyzing the influence of substrate wettability on the attachment of marine bacteria to various surfaces including microscope slides, polystyrene and polyvinyl fluoride (PVF), they found the "bioadhesive range" in terms of surface tension. If the surface tension of these materials was greater than a critical surface tension, they were defined to be in the 'bioadhesive range'. Usually natural substrates like cobbles and artificial substrates like glass slides were found to be in this range. They described the formation of a film in two stages initiated by an organic conditioning film, which meant that it was unlikely that the wettability of pure clean surfaces and the texture had any direct influence on the attachment process after formation of the conditioning film. The difference between low-energy surfaces such as teflon and high-energy surfaces such as clean glass in bioadhesion was demonstrated by Weiss and Blumenson (138). There are several examples found in the literature in agreement with the critical surface tension concept. In natural environments, the attaching surfaces of microbes are rough and therefore there will be several zones of contact. Short-range forces such as chemical and hydrophobic bonding become stronger in these contact zones compared to long-range interactions such as Van der Waals and electrostatic forces which make adhesion sensitive to the detailed geometry of the surfaces near contact (124). This may give rise to a range of adhesive strengths even for an apparently uniform population.

Effect of shear forces and velocity. The influence of flow velocity is seen in transporting nutrients to the attaching surface and in shearing the biomass building up. Higher

velocities over film surfaces enhanced slime growths due to better transfer of nutrients from the overlying water to the surfaces of bacterial cells (52,109). This was also supported by Hartmann (50) in his study on the influence of turbulence on bacterial activity. Since very high velocities would promote high scour rates and low velocities would be unable to transfer food molecules adequately, an optimum range of velocities for growth can be delineated. Experimental investigation in this connection by Sanders (109) and Characklis (18) on biofilms grown in the velocity range of 0.1 to 1.0 fps showed a velocity around 1 fps giving maximum growth. Sanders (109) showed that high velocities produced a dense and tough slime in contrast to the low density and more fragile slime mass at low velocities. Characklis (18) showed that biofilms can withstand high shear forces exceeding 15 dyn cm^{-2}. Shear forces become very important in determining film thicknesses because of the physical removal and the transfer of nutrients to the film. Trulear and Characklis (129) supported the assertion that increased shear stress caused greater scour rates.

Effect of pH and temperature. Reid (99) suggested an optimum pH of 7.2 for slime growth. Close to neutral pH, maximum production of polysaccharide occurred (143). This meant that a pH range of 6.5-8 would be optimum for bacterial growth. Environments more acidic than pH 3 to 4 or pH greater than 10 are not common. The different species of microorganisms isolated at various extreme pH environments and their life have been reported by Langworthy (68).

Green (45) reported that the percentage of dry matter in slimes varied between 3.5 to 6.5%, in the temperature range of 5 to 30°C; but higher temperatures increased the dry weight. The bacterial polysaccharides are synthesized at a larger rate at temperatures lower

than the optimum for bacterial growth (36). This may explain the lower optimum temperature for slime growths compared to suspended growth. *E. coli* was reported to produce about 25 times the amount of polysaccharide material at a temperature 15 to 20°C than at the optimum temperature of 37°C. Fletcher (39) suggested it was difficult to make any general prediction of the temperature effects on physiology other than their basic influence on reaction rates. Only a few reports are available on the activity of river microorganisms at very low temperatures such as below 5°C. Baross and Morita (10) summarized stream data showing the effect of temperature on microbial growth rates which indicated that 8 to 20 times higher generation times are needed during the winter (0 to 5°C) compared to the summer (16 to 21°C).

Effect of dissolved oxygen (DO). DO is obviously an important factor from the point of view of metabolism of organisms. Depending on the diffusion of oxygen, there will be aerobic and anaerobic zones in the biofilm. The cells in the anaerobic zone or below the limiting thickness for the diffusion of oxygen die or metabolize anaerobically (109). The mass of organisms in the top aerobic zone is considered to be active. Sanders (109) reported that the maximum nutrient removal occurred when the slime thickness reached the limiting thickness which had a minimum value of 21 microns. The active film thickness was found to be independent of DO (63). Tomlinson and Snaddon (127) and Kornegay and Andrews (62) have shown that the active film depth is about 100 μm. The extent to which oxygen would penetrate the film depends on the diffusivity coefficient, the type of film and the stoichiometry of the reaction.

In the presence of anaerobic conditions in the lower part of slime layers, product formation in those layers become important. It is however, difficult to establish the role of

these anaerobic decomposition products in varying the substrate concentrations by diffusing through the top aerobic layer. Sanders (109) showed an indication of a reduction in BOD removed from the supernatant substrate after reaching the limiting depth due to either the anerobic products released or the utilization of these products by the organisms in the top layer. Oxygen was found below the active layer in some studies showing that it was not rate limiting (111,141). Using a nutrient broth of 20 mg/l and a heterotrophic film, Whalen (141) found high concentration of oxygen throughout the slime mass stabilizing at 75 μm depth. However, when a 500 mg/l nutrient broth was used, the DO profile stabilized at 0.25 mg/l below 150 μm. Variations in DO did not produce chemical compositional variations in the slimes (63).

Effect of substrate and nutrients. Substrate and nutrients being directly involved in the metabolism of the cells have a very significant effect on attachment and growth. It has been suggested in several studies (8,75,129) that an organic film is formed initially on the attaching surface. This would be influenced by the chemical composition of the liquid media. The organic film, which Baier (7) suggested as a prerequisite for attachments, conditions the surface by enriching it with organics and latering the surface tension. The organic substances in the medium were found to promote attachment and in some cases inhibit the attachment process (39). Wardell and Brown (134) based on their study of a continuous flow culture found increased adsorption of cells to a surface under carbon limitation due to the larger number of free receptor sites available on the cell envelope and the surface. When there was glucose limitation, a small amount of polymer was found to be sufficient to act as an adhesive between those receptor sites on the cell envelope and the surface. This factor may become very significant in natural environments with low

nutrients. Excessive carbon promoted larger polymer production and by covering all binding sites inhibited attachment (74). This would mean that, with carbon excess there will be more polymer coated to the surface than the number of bacteria attached.

The concentration of nutrients in general has been found to vary the amount of slime directly (99). Easily sloughing films were found to be characteristic of growths in liquids having high amounts of oxidizable material (52).

Effects of film thickness. In the literature, there has been a striking similarity in the concepts of film development, even though there have been disagreements on other aspects (33,63,109). Biofilm thickness is an important parameter in the metabolism of the slime community. McKinney (81) stated that the trickling filter efficiency would be maximal with a thin layer of organisms. This was supported by several investigators (63,108,127) who showed that the effective depth of film ranged up to 120 μm. There was disagreement, however, among these investigators on the changes in nutrient removal rates beyond the effective film depth. A literature review indicated two different theories, one based on Sander's work (109) and the other on Kornegay and Andrews (63) and Tomlinson and Snaddon (127). According to the first theory, the nutrient uptake rate is reduced after the limiting biofilm thickness had been reached due to the fermentation products from the bottom layers diffusing into the aerobic layer and providing additional nutrition. The second theory postulated that there was a limiting thickness corresponding to a maximum nutrient removal rate but this rate became constant with increasing thicknesses. This condition remained until sloughing occurred with higher thicknesses. The anaerobic layer forming at the bottom is assumed not to change the nutrient utilization rates of the

films. Hoehn and Ray (55) made a comparison of these two theories by studying the nutrient removal capacities of films in relation to their thicknesses and attempted to correlate these data with changes in physical characteristics. They reported that the two theories were not mutually exclusive because as films grew, there was a limiting thickness when the nutrient removal rates declined. However, with more time the films adjusted to the changes in the internal environmental conditions after which they recovered, giving the original nutrient removal capabilities. When the films were about 300 to 400 μm thick, a steady state nutrient utilization rate was achieved. These results were supported by Kornegay and Andrews (63) Tomlinson and Snaddon (127) and Lamotta (66). The pattern of variation for biodensity was similar to nutrient removal with film thickness. The density increased up to the limiting thickness and declined reaching a steady density beyond about 300 μm thickness (55). This variation was found not to have been caused by the succession of bacterial types.

Substrate-Biofilm Kinetics and River Modeling

Theoretical Developments on Substrate-Biofilm Kinetics

Attempts have been made by several researchers to elucidate the mechanisms of substrate removal by biofilms and study its kinetics in reactor systems. Emphasis in sanitary engineering research was directed towards developing a better understanding of the kinetics of growth and substrate utilization of biofilms. In the previous section, the studies by several investigators on biofilm growth and nutrient removal characteristics have been described. These experimental conclusions provided an impetus for the models developed subsequently. This section will describe the theoretical considerations which formed the

basis of substrate-biofilm models. The reaction scheme in these models involve substrate and nutrients, biomass, an exogenous electron acceptor and products. Organic substrate in homogenous systems flows through the microbial population enabling reaction with cells at all the points in the liquid phase whereas in a heterogeneous system it flows over the biofilm with reaction taking place only at the biomass surface. In a series of publications, Atkinson (1) described the process firstly as a pseudo-homogeneous reaction system which is reaction rate limited and secondly as a heterogeneous system in which substrate diffusion in the liquid phase or reaction rate became rate limiting (123). Considering only the rate limitation by dissolved organic matter and unlimited by the exogenous electron acceptor oxygen, Atkinson (2) subsequently incorporated diffusional resistances in both liquid and microbial mass. Considerable theoretical developments backed by experimental investigation followed (18,63,66,84,112). In all these cases, only one reactant was considered to be rate limited. Three major steps may be identified in describing the overall process of substrate uptake by biofilms:

(a) Diffusion of substrate from bulk liquid to the interface between the liquid and biofilm.

(b) Diffusion within the biofilm.

(c) Biochemical reaction within the film.

Lamotta (65) studied step (a) in detail by experimentally defining the reaction controlled region. The true kinetics of reaction can be studied by the proper choice of a fluid velocity. This would eliminate the external diffusional resistances. Muller (90) and Baillod (9) after studying steps (b) and (c) demonstrated that internal diffusion became very significant at low oxygen concentrations or carbonaceous concentrations. For diffusion and oxygen