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

consumption Bungay (17) and Whalen (141) treated the film as a homogeneous mass. For carbonaceous substrates, some investigators restricted the analysis to qualitative descriptions of the effects of film thickness on substrate uptake (55,109). The effect of mass transfer resistances have been well documented by several investigators (2,48,67).

Williamson and McCarty (146,147) and Williamson and Chung (145) studied substrate utilization by bacterial films and defined conditions for limitations of electron donor or acceptor. The biofilm model they presented could be used only when either the electron donor or acceptor is both substrate and flux limiting across the entire film. It is possible to have one of the species, electron donor or acceptor flux limiting and the other substrate limiting over a certain portion of the film, which cannot be described by the model. This is probably the situation in trickling filters or in natural environments.

If only one of the parameters is limiting throughout the film, the following relationship holds when the electron acceptor is substrate limiting (146):

$$S_{ca} < (K_{sa}/K_{sd})S_{cd} \tag{1}$$

where

S_{cd}, S_{ca} = Respective concentrations of the electron donor and acceptor at a specified film depth in mg/l

K_{sd}, K_{sa} = Monod half-velocity coefficients for the electron donor and acceptor, respectively, in mg/l.

Similarly for flux limitation which was based on a general metabolic reaction and Fick's Law, the following condition holds when the electron acceptor is flux limiting,

$$S_{oa} < \frac{D_{cd}\,\nu_a MW_a}{D_{ca}\,\nu_d MW_d} S_{od} \tag{2}$$

where

S_{oa}, S_{od} = electron acceptor and donor concentrations respectively in mg/l in the bulk liquid.

ν_d, ν_a = respective stoichiometric reaction coefficients for electron donor and acceptor.

MW_a, MW_d = molecular weights of the electron acceptor and donor respectively in g.

D_{ca}, D_{cd} = diffusivity coefficient of electron acceptor and donor respectively in the film.

Common examples of electron donor and acceptor are glucose and oxygen respectively. Neglecting convective terms, Lamotta (66) used the following material balance to analyze simultaneous diffusion and reaction.

$$\frac{\partial S_{ca}}{\partial t} = D_e \nabla^2 S_{ca} + r_v \qquad (3)$$

where

S_{ca} = substrate concentration at any point within the film.

D_e = effective diffusivity coefficient of S in the film.

r_v = rate of substrate consumption per unit film volume.

Assuming a zero order kinetics based on experimental evidence in the literature and under steady state conditions, he provided two rate expressions for the substrate uptake depending on the film thickness. For this purpose, a critical film thickness Th_c was defined.

$$Th_c = \frac{(2 D_e S_s)^{1/2}}{k_v} \qquad (4)$$

where

k_v = rate of substrate uptake (constant).

S_s = substrate concentration at the top of biofilm.

In the case of incomplete substrate penetration, the observed substrate uptake was found to be dependent on the magnitude of the depth of penetration and independent of the total thickness (66,129). For this case a correction factor called the effectiveness factor (λ) was defined as follows to account for internal diffusional resistances:

$$\lambda = Th_c/Th \tag{6}$$

where

Th = total film thickness.

Atkinson and Daoud (3) presented the biological rate equation based on diffusion with biochemical reaction. Several subsequent publications by Atkinson's research group (4,5) reviewed and showed how the biological rate equation presented earlier could be extended and used in the design of microbial film fermenters (5) and trickling filters (6).

The complete biological rate equations for microbial films were given as:

$$N = \lambda N_{max} \frac{(k_3 S)}{1+k_3 S} \tag{6}$$

where

$$\lambda = 1 - \frac{\tanh k_2 Th}{k_2 Th} \left(\frac{\phi_p}{\tanh \phi_p} - 1\right) \text{ for } \phi_p \leq 1 \tag{7}$$

$$\lambda = \frac{1}{\phi_p} - \frac{\tanh k_2 Th}{k_2 Th} \left(\frac{1}{\tanh \phi_p} - 1\right) \text{ for } \phi_p > 1 \tag{8}$$

$$\phi_p = \frac{(k_2 Th\,(k_3 S)}{\sqrt{2}(1+k_3 S)} [k_3 S - \ln(1+k_3 S)]^{-1/2} \tag{9}$$

$$k_2 = \left(\frac{a\ \alpha}{K_s D_e}\right)^{1/2} \tag{10}$$

$$k_3 = 1/K_s \tag{11}$$

S = substrate concentration in the solution.

Th = biofilm thickness.

a = area of viable organisms/unit volume

α, K_s = rate coefficients defined by the expression:

$$r = \alpha S/(K_s+S)$$

r = local rate of substrate uptake per unit area of viable microorganisms.

D_e = effective diffusion coefficient within the microbial mass.

N = rate of substrate consumption/unit interfacial area.

N_{max} = maximum rate of substrate uptake = $k_1 Th/k_3$

k_1, k_2, k_3 = biological rate equation coefficients.

The advantage of these models is that they take into account the diffusional resistances in the slimes. The models, however, assume discrete viable organisms dispersed in the slime which is not true for filamentous organisms. Besides they do not consider oxygen limitations in the film. Harris and Hansford (49) emphasized the importance of this factor and formulated models to establish whether the performance of the microbial film is affected by limitation of oxygen, organic carbon or both simultaneously. They used two material balance expressions and a modified Monod expression to incorporate two reactants as given below:

$$D_s \frac{d^2 S_{ca}}{dx^2} = \frac{\mu_m X_f}{Y_A} \left(\frac{S_{ca}}{K_s+S_{ca}}\right) \left(\frac{0}{K_o+0}\right) \qquad (12)$$

$$D_o \frac{d^2 0}{dx^2} = \frac{\mu_m X_f F_c}{Y_A} \left(\frac{S_{ca}}{K_s+S_{ca}}\right) \left(\frac{0}{K_o+0}\right) \qquad (13)$$

where

D_S = diffusivity of glucose in the slime.

D_o = diffusivity of oxygen in the slime.

S_{ca} = substrate concentration in the slime.

0 = oxygen concentration in the film.

K_s, K_o = half-velocity coefficients for substrate and oxygen respectively.

μ_m = maximum growth rate of organisms

X_f = cell concentration in the slime.

x = distance measured into the slime from the interface.

Y_A = cell yield.

F_c = constant factor relating the quantities of glucose and oxygen utilized in the aerobic metabolism.

Kinetic parameters used in the model were taken from the literature. Below organic loadings of 300 mg/l COD, the oxygen profile remained positive while the substrate concentration profile dropped to zero. Within the loading range of 300 to 500 mg/l COD, both profiles fell to low levels. This was defined as the transition range before the limitation changed from substrate to oxygen.

Using an annular reactor Trulear and Characklis (129) developed material balance relationships giving rate expressions for substrate removal, biofilm detachment and accrual. Low fluid velocities were found to limit the transfer of glucose from the bulk liquid to the liquid film interface. This becomes diffusion limited. Biofilm detachment increased with fluid velocity and attached biomass.

Due to the complexity of the analysis involved and lack of parameter values the models of Williamson and McCarty (146,147) were not used widely as biofilm models in reactor designs. Meunier and Williamson (86,87) presented a simplified model so that these models could be used in the design of certain biofilm reactors. The model which could be solved in a programmable calculator first computes the limiting species of either the electron donor or acceptor and the flux. The design volume of the reactor is then determined on the basis of the calculated flux.

Reactor Studies of Substrate Kinetics and Biofilm Dynamics

Experimental channels or artificial streams have been used to study the impact of pollutants and organic enrichment on the structure and function of the periphytic communities. The idea of these channels and streams is to simulate the natural system in the best possible way and provide the controlled environment a complex system would require to study its responses to several perturbations. The successful application of continuous flow laboratory reactor systems in studying mixed populations has been reported by several investigators (31,43,66,94,149). Most of these studies have given considerable information on self-purification of streams even though they provided inadequate data for quantitative analyses. Steady state conditions for biomass growth and substrate consumption rates have been indicate in these studies. One study (94) concluded that substrate utilization was proportional to nutrient loading but at steady state, attached growth rate was limited by the available surface. Lamotta (66) found substrate uptake and biofilm growth rate to be defined by the initial substrate concentration during the early stages of growth and were zero order with respect to the subsequent concentrations.

Studies of microbiofilms in natural environments have been limited in scope. Investigations were carried out on kinetic studies of *Sphaerotilus natans* and related species in organic enriched waters as these organisms are predominant in river biofilms under polluted conditions (27,31,97,102). Based on these studies, *Sphaerotilus natans* was found to have a competitive advantage over other organisms especially at low DO, nitrogen, and high flow rates. Dias and Heukelekian (30) showed the utilization of inorganic nitrogen compounds by *Sphaerotilus natans* as readily as the organic nitrogen. Phaup and Gannon (97) determined the optimum concentration of sucrose for heavy growth of *Sphaerotilus natans* as 5 mg/l at a velocity range of 0.58 to 1.49 ft/s in the temperature range of 20 to 28° C. One mg/l organic carbon was found as a limiting concentration for the formation of slime by Curtis (27). Above this limit the slime growth was proportional to the concentration of organic carbon. By not accounting for sloughed material, they reported low yield coefficients. Stumm-Zollinger (122) discussed the implications with respect to the procedures in assessing in a laboratory the matabolic activity of a natural community. He concluded that laboratory mixed cultures grown on nonselective multisubstrate medium do not simulate natural populations. It can be inferred from his study that it is difficult, if not impossible, to grow and maintain a natural microbial population in the laboratory without any alteration. He concluded that bacteria in natural environments can utilize certain substrates concurrently.

Clark et al. (22) successfully used artificial streams to study the structural and functional responses of the attached biological communities to disturbances. Benthic community photosynthesis and respiration and the effects of some important environmental factors such as light intensity, CO_2 supply, DO and temperature were studied in some laboratory

streams by McIntire (80). Several other studies using a controlled environment in artificial streams to study the physical variations and toxicants on biological communities have been reported (72,95,144). Based on an extensive literature review, it can be said that artificial streams provide a very useful means of studying natural microbial communities as long as the limitations are clearly recognized.

Water Quality Modeling of Rivers

Water quality models. A general framework in the formulation and application of simple mathematical models to water quality analysis is described in Hydroscience (57) for EPA water programs. Several processes govern the degradation of organics discharged into rivers and other water bodies. The basic mechanisms of self-purification have been described qualitatively in the literature (98,132). DO and BOD have been used as the two overall parameters of water quality for a long time. In addition to biooxidation, several factors such as sedimentation, scour and biological extraction have been reported as important in studying in-stream BOD removal rates (32,92,125,148). The overall removal of BOD may be given as:

$$K_o = K_D + K_{Sc} + B \tag{14}$$

where

K_o = coefficient for overall BOD removal.

K_D = coefficient for overall stream deoxygenation.

K_{Sc} = coefficient for sedimentation.

B = coefficient describing a boundary effect by slime layers.

Overall observed deoxygenation coefficients (K_o) for 23 river systems were reported by

Wright and McDonnel (148) which varied from 0.08/day to 4.25/day under steady state flow conditions. In order to estimate stream deoxygenation coefficients associated with oxidation of carbonaceous BOD, empirical relationships were developed relating them to stream hydraulic geometry. The main point to note from this study was that the observed in-stream deoxygenation rates were much higher than the laboratory BOD reaction rates, especially when stream flows were less than 800 cfs. Velz and Gannon (131) introduced the coefficient B for which Bosko (14) and Novotny and Krenkel (92) subsequently formulated expressions to account for BOD removal by slime layers in European and American streams. Models have been proposed and used by several investigators for DO and BOD (12,89,93,101,114) since the original Streeter-Phelps model (120). These models were modifications of the single term first order kinetic model. The schemes required for parameter estimation of these models have been reported over the last few years (13,101). Several researchers have criticized the first order models which are based on a number of simple biochemical reactions (34,40,100). In the most recent stage of development, models utilizing saturation kinetic expressions have come into use (34,40,106). They successfully used Monod kinetic expressions in defining the utilization of substrate uptake by suspended biomass and showed their application in streams. Gates (40) concluded that laboratory batch reactors could be used with considerable advantage in studying biological processes in a river. The problem with using Monod type expressions in river modeling lies in the difficulties of selecting and using the appropriate coefficients. Rutherford and O'Sullivan (106) used curve fitting procedures to determine the coefficients to be used. Several water quality parameter compilation and modeling techniques for water bodies from small shallow streams to larger rivers in different geographic regions have been

reported in the literature by various investigators (11,23,53,60,61,71,89,92,114,117,142). The important observation to be made from these modeling efforts is the emphasis placed on the sessile microbial communities in small, low flow streams (92,114) even though some studies did not consider them (11,71).

Transfer processes. In general, advection and dispersion are the major transport processes in rivers. Advection is very important in streams and rivers whereas dispersion becomes predominant in estuaries (57). A very useful parameter is the longitudinal dispersion coefficient, D_L which combines the effect of diffusion and dispersion; diffusion in this respect refers to mixing produced by turbulence and Brownian motion and dispersion by the variation of velocity across the stream. This parameter provides an easy method of determining the spread of pollutant over long distances in streams. The useful application of this parameter and its determination for modeling efforts have been discussed in the literature (35,37,69,82,106).

A Summary and Critique

In general, microbial adhesion in streams provides the microorganisms especially the bacteria with a stationary location exposed to a continuous supply of nutrients and protection against many sources of stress in aquatic habitats. The sessile microorganisms were found to be enmeshed in a polysaccharide matrix. There was no clear agreement on whether algae or bacteria colonize first even though there was evidence to show that attached algae provided a suitable surface for subsequent bacterial colonization. Massive growths of *Sphaerotilus natans* were found predominating under polluted conditions even

at reduced DO levels, possibly by their successful competition with other organisms. Often heterotrophs predominate the slime layers in polluted areas.

Several theories have been presented by researchers on the mechanisms of microbial adhesion. The accumulation of microorganisms onto a surface may take place in three stages:

(a) Adsorption of organisms to the surface

(b) Attachment by polymer bridges

(c) Growth and division of organisms on surface

There has been no clear demonstration in the literature of the general involvement of pili and flagellates in the adsorption process, even though obviously the hydrodynamics of organisms with these surface appendages should contribute in some way to the attachment process. Two types of sorption, reversible and irreversible have been identified in the literature depending on whether the application of shear force would remove the bacteria or whether the adhesion was by extracellular polymers anchoring them to the surface. The organisms in the bottom layer of river biofilms must be irreversibly bound as they can only be removed by scraping with a blade. The organisms have to adhere to surfaces in low nutrient environments for survival. If this is so, why should organisms under polluted conditions with excessive nutrients attach to surfaces? Wardell and Brown (134) suggested that when there is excess carbon, large amounts of polymer would be produced which would cover all available binding sites thus hindering attachment of organisms. Under low nutrient conditions, there would be plenty of binding sites available. But then, how will it be possible to find larger concentration of attached organisms under polluted conditions compared to unpolluted conditions? This may be because of the "adherent growth habit"

suggested by Costerton (24) which would require the organisms to adsorb to surfaces before metabolizing the substrates. Besides many organisms would divide only if they are attached to a surface.

Various factors affecting the attachment, growth and nutrient removal capabilities of the organisms have been discussed. Surface properties of surfaces may dictate the formation of the conditioning film initially. In this respect a rougher surface typical of natural environments should promote better initial colonization due to several zones of contact in forming a conditioning film. However, the surface texture and wettability should not interfere with the subsequent attachment process. The role of the flow velocity is in transporting the organisms food molecules and nutrients to the surfaces besides shearing the attached biomass. Higher velocities would promote the transfer process and increase scour rates while maintaining the kinetics in a reaction controlled region. This would reduce or eliminate the liquid phase diffusional limitations. Shear forces prevent biofilms from building up excessively so that a condition of steady state is possible, exhibiting an oscillatory behavior corresponding to growth and scour. The prevention of excessive build up may help maintain aerobic conditions in the film, more so in a mixed film with algae and bacteria. In thick films, the bottom may become anaerobic causing product formation. However, the role of these products in changing the substrate concentration in the overlying water is unclear. Two different theories are hypothesized to explain the nutrient removal capabilities of the films. According to the first theory, the nutrient removal rates decreased after limiting thicknesses were reached whereas they reached a maximum and remained constant based on the second theory. The decrease was found to be only temporary and a constant nutrient removal rate has been observed in many studies.

It was evident from the literature that a considerable amount of theoretical work backed by experimental investigations were carried out on microbial film reactors in the sixties and seventies. In most cases, however, rate limitation was restricted to only one reactant and assumed to occur due to mass transfer in the liquid, solid phase diffusion in the slime or biochemical reaction. Subsequently, studies were done to define conditions under which an organic substrate (electron donor) or the oxygen (electron acceptor) may become limiting within a microbial film. This information could be used in the design of several biofilm reactors including trickling filters. It may be pointed out, however, that most of these studies did not consider oxygen limitations or active film depth. This may be very important in reactors treating wastewaters having high concentration of organics with thick microbial films. A few studies in the past few years have considered the concept of active film depth in their formulations. Simple material balances have been used recently in establishing the kinetics of substrate removal and biofilm growth. This is very useful considering the ease with which the model parameters can be determined. In all cases, the assumptions used in the models should be studied carefully before application.

Due to the complexity in functioning of the slime layers, the low concentration of organics and the fact of continuous flow, reaction schemes in streams have not been fully explained. Several investigators reported the successful application of continuous flow reactors in the study of mixed populations. The reasons for using a CSTR reactor were that the flow characteristics are well known and simple material balance expressions can be formulated. By a rigorous control of the experimental systems, less variable and replicable data may be obtained, but extrapolating these results to natural environments may be misleading. Artificial stream designs in this respect are more helpful because of its versatil-

ity. They can be designed as systems enclosed in environmentally controlled chambers to semi-controlled open out-of-doors systems. Artificially seeded organisms or naturally colonized films may be used. These designs can be tailored to fit the required experimental needs of the investigator concerned. Therefore, artificial streams are, as reported recently very useful in studying natural populations. Problems of using laboratory mixed cultures growing on non-selective mutli-substrate media in simulating natural populations have been discussed in the literature. Probably, an easier and cheaper method of obtaining a natural population would be to grow the organisms in the natural environment to be simulated and transfer them to an artificial stream system. In this case however, there will be several autotrophic and heterotrophic organisms making it a fairly complex microbial community presenting problems of isolation and identification.

Several water quality models have been developed in different situations based on first order or saturation kinetics. In most modeling efforts, kinetics had to be assumed since information defining the rates of reactions was lacking. Recently, first order models have been criticized based on new developments on bacterial kinetics. Numerous theoretical refinements have been made to the saturation kinetic expressions to account for various factors. Incorporating these refined expressions would theoretically give rise to more realistic models. However, validation of such models require determination of many parameters which would make it necessary to use some kind of curve fitting procedure. While this procedure is an accepted method in practice, making the parameters easily adjustable invalidates to some extent their fundamental generality. The critical problem is to determine which microbial population, suspended, attached or both play the dominant function in substrate assimilation and its kinetics. This may not be possible, admittedly, in

every situation. Once the kinetic expression is determined, it may be utilized in the stream model.

THEORETICAL CONSIDERATIONS

This section deals with all the theoretical considerations and formulations involved in the subsequent chapters on mathematical modeling of streams.

Mathematical Models

Any natural system can be looked upon as a mathematical system with complex interacting subsystems. Natural background water quality is determined by a number of external inputs to the system such as rainfall and solar radiation. In addition, the system may also be subjected to various man-made stresses caused by, for example, wastewater discharges. The response of such a system can be evaluated by studying the spatial and temporal distribution of the concentration of various substances affecting the water quality. In this study, the system is a river and the substance is organic carbon.

Conceptual Description

The system definition and the processes involved in a substrate-biomass model can best be illustrated in a diagrametic form as shown in Fig. 1. The importance and relevance of these processes have already been discussed in detail under the previous section on the review of literature.

Specification of Interactions

The mechanisms involved in affecting the model parameters have to be specified in the construction of the model. The lack of knowledge and understanding of a significant mechanism will affect the realistic modeling of some of the water quality parameters. On

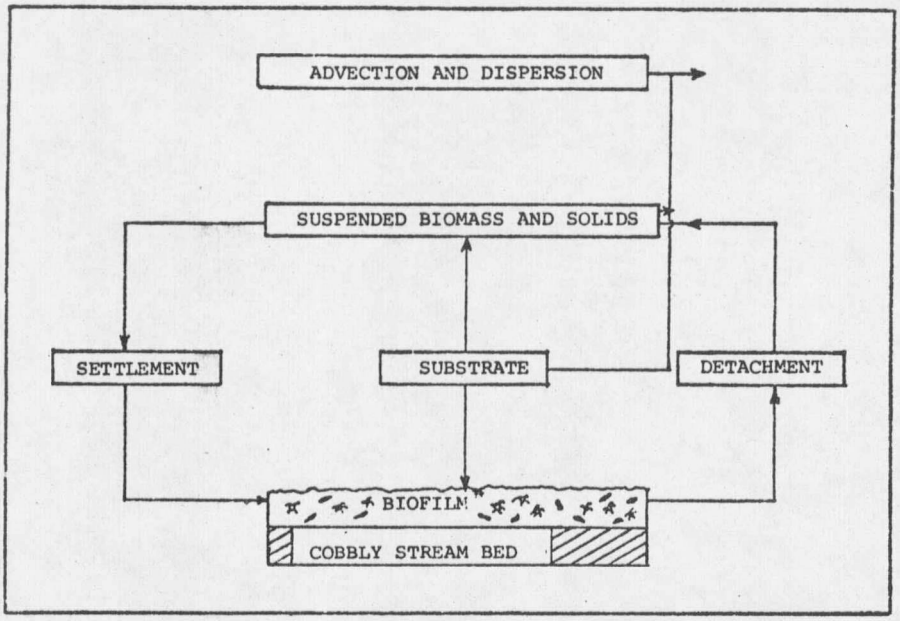


Fig. 1. Schematic Diagram of the Model Frame Work.

the other hand, the inclusion of all possible interactions will substantially complicate the model. Therefore in a complex system, it is only realistic to consider the pre-dominant mechanisms and elements.

The processes involved in the substrate biomass model are:

Transfer Processes

(1) Advection or bulk fluid flow

(2) Dispersion

Transformation Processes

(1) Substrate–Uptake by Biofilm and Suspended Biomass

(2) Suspended Biomass–Growth, Settlement and Decay

(3) Biofilm–Attachment, Growth, Decay and Detachment.

In a small stream or river, the mixing characteristics are such that the dispersion of the mass of material may be very small and it may be neglected in comparison to the flow. The computational complexity can be reduced significantly by such an assumption.

Fundamental to the analysis of the model to be described subsequently is the point form of continuity equation which describes the relationship between the flux and the sources and sinks of mass. In general,

$$\frac{\partial S_j}{\partial t} = - \nabla \cdot j + r_j \qquad (15)$$

in which,

S_j = concentration of a water quality variable

t = time

j = flux = $D_L \, \partial S/\partial Z$ - U.S = transfer processes for a one dimensional system

∇ = Del operator

U = mean stream velocity

Z = distance

D_L = Longitudinal Dispersion Coefficient

r_j = sources and sinks of S_j given by the transformation processes.

The transformation processes may be described by several reactions in natural waters with different kinetic order. However, the limiting step may be represented by a simple first or second order kinetic expression.

Assumptions and Conditions

In the analysis that follows, several assumptions are made which are given below:

(1) The substrate removal by suspended biomass is negligible in shallow streams compared to that of the pre-dominantly heterotrophic biofilm.

(2) Organic carbon is the rate limiting substrate for the heterotrophs lumped together.

(3) The spatial system parameters for the river flow such as mean velocity and depth are assumed constant.

(4) Major Dissolved Oxygen limitations are not present in the film.

(5) Temporal steady state values for all system parameters and inputs are assumed.

The consequences of these assumptions are considered under the section on discussion. Based on the last assumption, the steady state model will not be able to describe diurnal variations in water quality. However, the applicability of a predictive model in many problem situations is important under critical short term conditions. The investigator may use the steady state model and input the maximum daily waste load for the worst conditions, recognizing that the water quality response will improve at the lower steady state daily waste load levels. Unsteady state models involve complex solutions and require extensive data for validation and hence a simpler steady state model is a reasonable compromise between complexity and practicality.

Modeling Organic Carbon in Streams

Formulation of Models

The main variables identified in the model framework are substrate S, suspended biomass X and heterotrophic fraction of attached biomass, M_A. Using the general continuity Equation (15) the following material balance expressions can be written based on assumptions 2, 3, 4 and (5):

$$\frac{\partial S}{\partial t} = D_L \frac{\partial^2 S}{\partial Z^2} - U \frac{\partial S}{\partial Z} - \frac{R_s}{Y_s} - \frac{R_B}{Y_A H} \tag{16}$$

$$\frac{\partial X}{\partial t} = - U \frac{\partial X}{\partial Z} + R_s + \frac{R_D}{H} \tag{17}$$

$$\frac{\partial M_A}{\partial t} = R_B - R_D \tag{18}$$

where

D_L = longitudinal disperson coefficient ($L^2 T^{-1}$)

U = mean stream velocity (LT^{-1})

H = average flow depth (L)

Z = length along the stream with Z = 0 corresponding to the site below the sewage outfall (L)

t = time (T)

Y_A = biofilm yield coefficient

R_B = attached biomass production rate ($ML^{-2} T^{-1}$)

R_D = Net detachment rate = (detachment-settlement) of biomass per unit biofilm area ($ML^{-2} T^{-1}$)

R_S = suspended biomass production rate ($ML^{-3} T^{-1}$)

Y_s = suspended biomass yield coefficient

For shallow streams, using the first assumption and fitting an expression of the form $R_D = \alpha_D M^{\beta}$ where scour rate increases with the total biomass M including the mass of autotrophs, the equations for the complex river system can be reduced to,

$$\frac{\partial S}{\partial t} = D_L \frac{\partial^2 S}{\partial Z^2} - U \frac{\partial S}{\partial Z} - \frac{R_B}{Y_A H} \tag{19}$$

$$\frac{\partial X}{\partial t} = - U \frac{\partial X}{\partial Z} + \alpha_D \frac{(\rho Th)^{\beta}}{H} \tag{20}$$

$$\frac{\partial M_A}{\partial t} = R_B - \alpha_D (\rho Th)^{\beta} \tag{21}$$

The unique feature of these equations forming the substrate-biomass models is in not assuming the kinetic order for the substrate uptake by the biofilms. If the kinetics of organic carbon uptake rate by biofilms is determined in addition to the other defined parameters, the system can be solved using assumption 5. Thus, Equations 19, 20 and 21 can be used for one dimensional modeling of organic carbon. Under steady state transport and ecological conditions, a Lagrangian instead of a Eulerian frame of reference may be used. The Lagrangian frame of reference involves a moving frame where incremental volumes of water move as "plugs" within which mixing and reactions take place. Only one plug unit has to be modeled to generate the entire model since the water quality of each unit passing a given point is the same as the one that preceded it under steady state conditions. The equations can be solved using numerical methods employing a finite difference scheme.

Pilot-Plant Channel Study

Determination of the Kinetics of Organic Carbon Uptake Rate by Biofilms in an Artificial Channel

Consider the reactor configuration shown in Fig. 2 with an attached film.

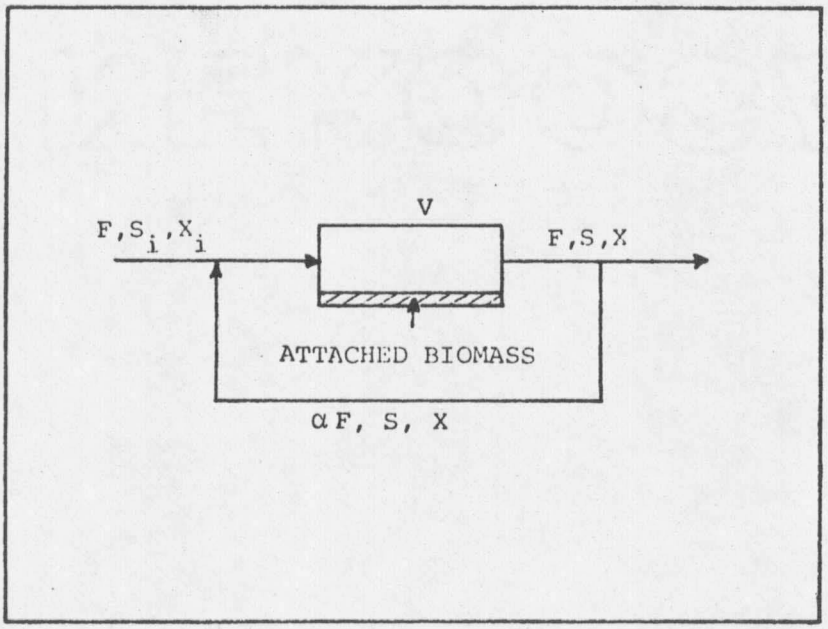


Fig. 2. CMF Reactor With Recirculation.

Material balances for substrate as soluble organic carbon S, suspended biomass X and heterotrophic fraction of attached biomass M_A similar to those shown in Trulear and Characklis (129) result in,

$$V \frac{dS}{dt} = F(S_i - S) - \frac{R_B A}{Y_A} \tag{22}$$

$$V \frac{dX}{dt} = F(X_i - X) + R_D A \tag{23}$$

$$\frac{dM_A}{dt} = R_B - R_D \qquad (24)$$

where

V = volume of reactor (L^3)

F = flow rate ($L^3 T^{-1}$)

A = area of biofilm (L^2)

All the other parameters have already been defined.

Under steady state conditions, R_B, R_D and Y_A could be determined from the three equations. By varying S, the kinetics of substrate uptake could be studied. A relationship between R_D and M would provide a scour coefficient α_D and a constant β for the system where $R_D = \alpha_D M^{\beta}$ as already defined.

EXPERIMENTAL INVESTIGATION

Experimental Apparatus: Pilot Plant Channel Study

General Layout

A field experiment was designed in an artificial channel on the river bank, the schematic view of which is shown in Fig. 3. The photographic details of the reactor system are given in Figs. 4a to f. The Bozeman Wastewater Treatment Plant facilities provided an optimal location to conduct an experimental study of this nature. The system consisted mainly of: a long channel, recirculating tank connected to a high capacity pump, flow meter, mixing and feeding tanks, variable speed mixers, small electric pumps and a generator for the influent feeding system. The influent feed was controlled by flow regulating valve while the effluent was siphoned off from the recirculating tank. A sump location was chosen close to the experimental channel system and river water was pumped into the mixing tank where it was mixed with sewage effluent during selected runs. The influent was then pumped from the feeding tank into the channel. The entire channel system was assembled and constructed in an open area on the stream bank, below the sewage outfall.

Details of the Reactor Channel and Appurtenances

Channel description. A long aluminum trough, 32 ft. X 4 in. X 6 in., coated on the inside with an inert paint served as the artificial stream channel as it was a cost-effective method of conducting this study. A weir at the end of the channel as shown in Fig. 3 permitted adjustments in depth. The recirculation line was connected to a small box at the influent end of the channel which helped to dissipate some energy and hence provide

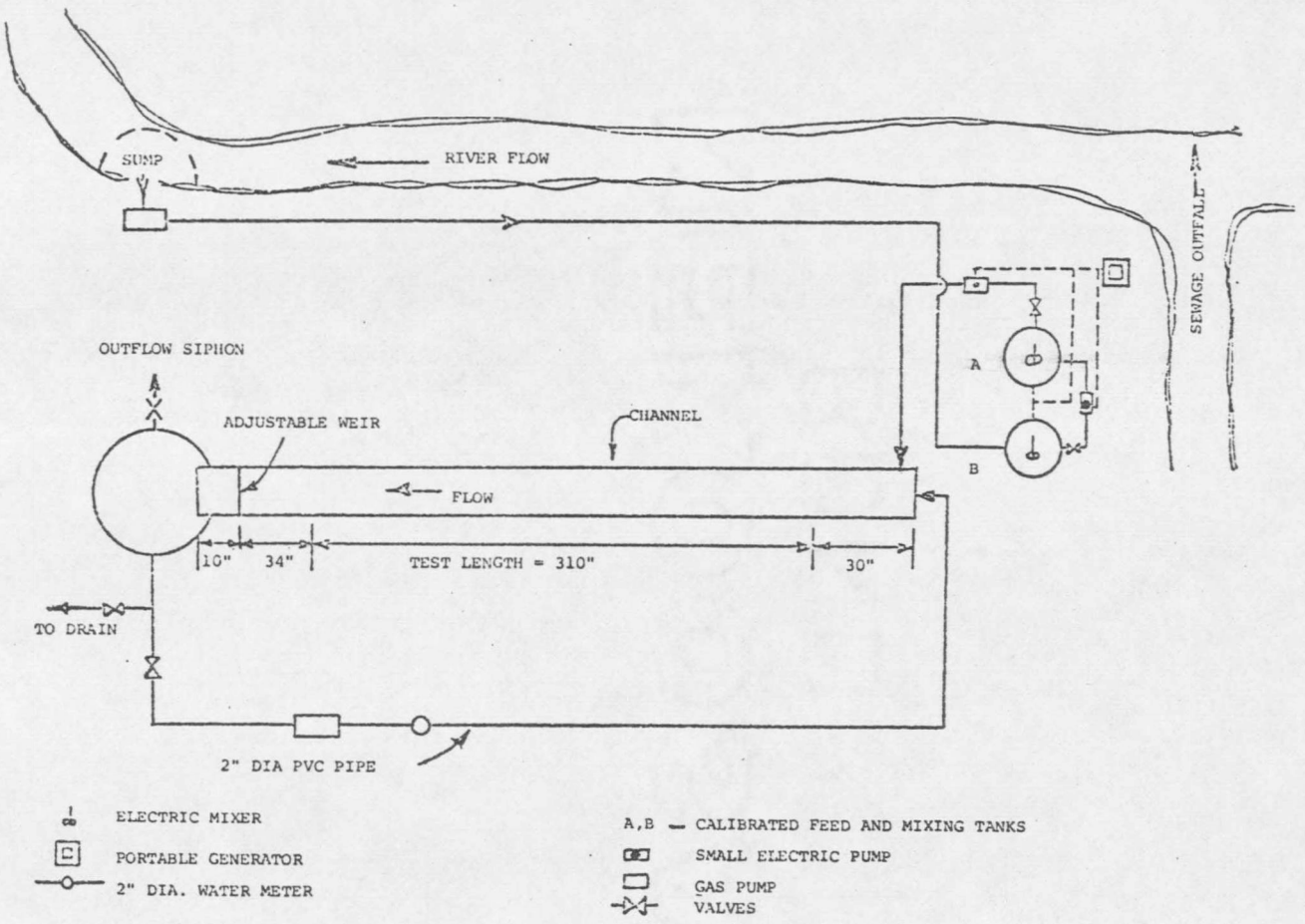
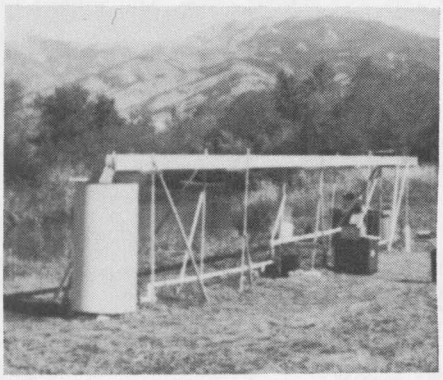
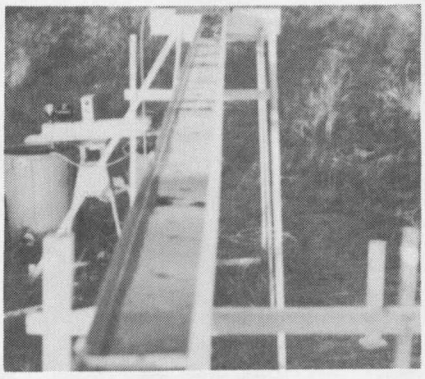


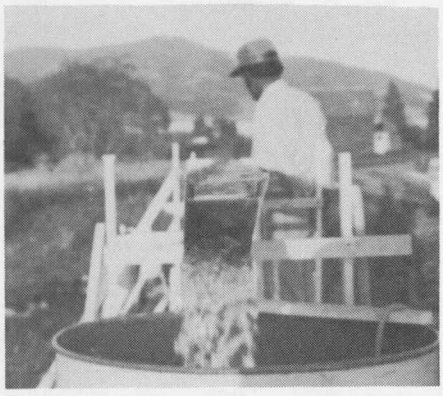
Fig. 3. Schematic Diagram of the Field Channel Experimental Set Up.



(a)



(b)



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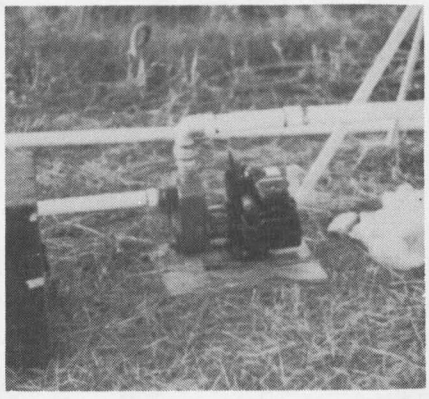
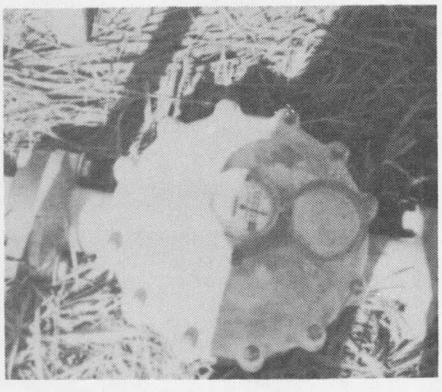
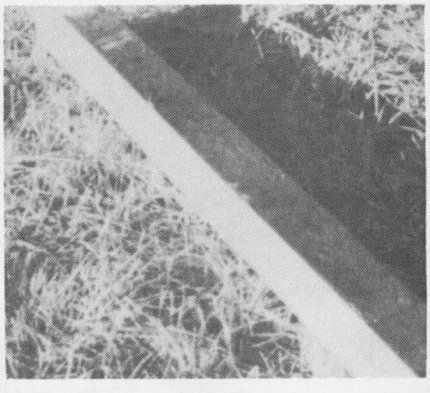


(d)



(e)



(f)

Fig. 4. (a) A View of Channel Set Up, (b) Plan View of Channel, (c) End View of Channel, (d) Recirculation Pump, (e) 2 in. Water Meter, (f) Plexiglass Plate with Biofilm used in the Channel.

a steady flow in the channel. A plastic strainer was placed at the beginning of the channel to reduce the turbulence created by the high recirculation flow.

Channel design considerations. It was obvious that with the channel system used the average flow depth of 0.75 ft in the river could not be simulated. A design depth of 0.25 ft was thus selected. The average velocity of 2 fps in the river below the sewage outfall was an important variable in studying biofilm kinetics in the channel. This velocity could be maintained in the channel by providing the appropriate recirculation. The maintenance of hydraulic similarity between the prototype river and model channel would require the same Froude number. This would mean a reduction in the average velocity in the channel. However, it was decided to provide the same average velocity in the channel as in the river, as sloughing was an important consideration in the biomass model. Moreover, the flow regime can be categorized as turbulent, as in the temperature range of 18 to 26°C, an average Reynolds number (Re) of 4.92×10^4 was estimated for the channel design velocity and depth of flow. This would ensure a reaction controlled region in the channel with negligible external diffusional limitations as established under the literature review section. Another major consideration was to obtain a balance between a measurable change in substrate concentration between the influent and effluent and at the same time complete a run in the channel in the field within the number of hours available for field work per day. The unsteady state equation (22) was solved and reasonable values were used for parameters for this purpose. For example, it was determined that with an hour of detention time, an organic carbon removal of 9 to 4.5 mg/l is effected in about three hours. The maximum number of hours that were available for field work per day was determined to be eight hours. Allowing for start-up and finishing procedures, the channel system was

operated for about five hours. The five hour channel operation, which is five times the detention time, was the trade off found to satisfy the requirements for attaining steady state conditions and available field time. The adequacy of this design operation time was subsequently checked by actual field measurements. Assuming a Mannings coefficient of 0.01 (20) and for a velocity of 2 fps, a mild slope of 3.74×10^{-3} was provided for uniform flow in the channel. Provision of this exact slope was not necessary as long as uniform flow was maintained. The depth of flow at several points in the channel were measured to ensure uniform flow.

The design parameters are summarized as follows: Design velocity = 2 fps, depth = 0.25 ft, detention time = 1 hour, duration of experiment = 5 hours, channel slope = 3.74×10^{-3}.

Influent feeding system. Two calibrated plastic tanks A and B as shown in Fig. 3 of 90ℓ capacities were used as feeding and mixing tanks with provision for maintaining a constant head with an overflow pipe which enabled a constant pumping rate for the feed pump. The feed rate was continuously monitored every 15 minutes with a measuring cylinder. Another pump was used to fill tank A from the mixing tank B. River water was supplied to the mixing tank by pumping from the river which was used as the substrate after mixing with or without the treatment plant effluent brought from the sewage outfall. This was done to vary the concentration of substrate fed into the channel. The proportion of sewage effluent used was not more that 25% so that the range of concentrations used in this experimental study would not be too far off the measured substrate concentrations in the river. Two electric paddle mixers provided the mixing which was further supplemented by hand mixing in the mixing tank.

Effluent recycling system. A tank 1 ft 10 in. diameter and 3 ft. high was used as a sump for the recirculation pump, with a provision for controlling the recirculation flow by a valve. The drainage valve in the recirculation line made of PVC pipes permitted cleaning of the tank and draining water at the end of experimental runs. A siphoning system consisting of ½ in. diameter rubber tube with a valve was used to discharge the effluent at an outflow rate of 6.33×10^{-4} cfs. The effluent flow was monitored continuously every fifteen minutes to ensure a constant outflow.

Pumps, motor, generator and watermeter. Two pumps 60 gpm and 100 gpm capacities were used for pumping water from the East Gallatin River and for recirculating the water in the channel. The recirculation pump was a 3 hp Jacuzzi Model 80200 with 1½ in. and 2 in. suction and delivery lines with the base mounted. Two centrifugal polyethylene electric pumps with 5/8 in. suction and discharge openings (VWR Cat. No. 54902-000) were used as feed pump providing an influent flow of 6.33×10^{-4} cfs and to fill the feeding tank from tank B. The 1/20 hp motor for the mixers used in the two tanks was manufactured by Bodine Electric Company, Type NSH 33R which had a speed control (Minarick Electric Co.). This electrical system in the influent feeding unit was run by a portable army generator. The recirculation flow was monitored by a 2 in. water meter manufactured by Hersey-Sparling, Dedham, Mass. (U.S.A.) to provide a flow of 79.6 gpm in order to have a flow velocity of 2 fps in the channel. The high ratio of recirculation to feed (284:1) provided a complete mix condition in the channel.

Reactor Start-Up

The recirculation tank, feed and mixing tanks were filled, first with river water pumped directly from the river. In cases where treatment plant effluent was mixed with river water, the mixing tank was used to mix known proportions and the recirculation tank was filled up to a specified mark to provide the volume needed for the recirculation pump to maintain the required flow. The recirculation pump was primed by opening the valve on its suction side. The pump was then started and the system was allowed to recirculate the flow. The influent feed was started and the siphoning of the effluent was put in operation. The system was allowed to function steadily for a few minutes. Both inflow and outflow were calibrated to provide the design flow. The attached biomass used in the channel was grown on several plexiglass plates in the river for more than 30 days. When the system was functioning under steady conditions, the plexiglass plates with biofilms were retrieved from the river and brought under water in a small tank to be placed in the channel bed over the 310 in. test length. A known area of biomass was scraped at the beginning and end of the runs. Once the plates were placed on the channel bed, the time was set as $t = 0$. For the control runs, the plexiglass plates were not placed on the channel bed. The flow depths at several points in the channel were measured by a scale and the flow depth adjusted to the design depth. The flow meter, inflow and outflow rates were continuously monitored every fifteen minutes to provide the required design velocity and detention time in the reactor. At the end of each run, with the system running, the plexiglass plates containing biofilms were placed back on the river bed for the future runs. Thus an economic method of conducting this study was achieved.

Experimental Observations

General description of runs. In the year 1980, a total of twenty runs were made in 2 series, first of which contained sixteen regular runs and the second series had three control runs and one with tapwater. The regular runs with the attached biomass were made over a soluble organic carbon concentration range of 5 to 20 mg/l. The design was to get at least duplicate runs of five different concentrations. Since the substrate used was river water, the concentration of which was varied by mixing with sewage effluent, the approach was to make several runs at different combinations of river water to sewage effluent. River water pumped from below the outfall was the main source of substrate and nutrients for the predominantly heterotrophic biofilm. The microscope slides attached to the plexiglass plates permitted continuous monitoring of biofilm growth throughout the study. The control runs in the second series were made without using the plexiglass plates containing the biofilm but with inflow and outflow as in the regular runs. These runs were also made at high and low substrate concentrations in order to assess the importance of the suspended microbial population. During some runs, the weather was cool at the beginning but became warmer during the last two to three hours. Development of some foam in the channel and recirculating tank was also observed during most runs. The tapwater used in the final run was obtained from the backyard of the plant supervisor's house, the source of which was ground water with negligible organic carbon and solid concentrations. This water had to be transported to the channel site like the sewage effluent for the study. Difficulties were experienced in the operation and maintenance of the generator which had to be repaired several times before use. During the final runs, several hundred feet of electrical cord was used to get power from the nearest electrical outlet at the Treatment

Plant. This eased the whole operation of the influent feeding system and the uncertainties of a generator breakdown.

Observations during a run. For each run pH, TOC, SS and VSS were measured for influent and effluent. DO, pH and temperature at three locations in the channel were measured hourly while TOC, SS and VSS were measured at selected hours at the beginning and end of the runs. DO and temperature were measured by a calibrated DO meter with a field probe. Influent DO was also checked for any drastic variations which never occurred during the runs. Samples taken for the measurement of pH, TOC and solids were transported on ice to the laboratory where the TOC samples were stored below 0°C until the analysis was completed. The sample preservation techniques used were according to Standard Methods (118). pH and solids were measured as described under the section on Analytical Procedures.

During several runs a known area of the biofilm was scraped at the beginning and end, for biomass determination. Microscope slides attached to the plates were used for film thickness measurements.

Field Studies—River Measurements

Study Area Description

The East Gallatin River used in the study originates east of Bozeman, Montana, and flows northwesterly through the Gallatin Valley for about 45 miles before joining the Gallatin River, north of Manhattan. This is one of the three tributaries to the Missouri River. The drainage area of the East Gallatin is estimated at 640 square miles and is agricultural in

nature. This river was chosen for the study because it receives a partially treated sewage effluent, high in suspended solids (SS) and Organic Carbon (OC) from the Bozeman Waste Water Treatment Plant, the single major point source of pollution. The confluence of the sewage outfall and the river can be seen in Fig. 5.

Fig. 5. Confluence of Sewage Outfall and East Gallatin River.

River Flows and Morphology

The maximum reported discharge has been reported to be 1230 cfs in June 1953 and the minimum 12 cfs in December 1944 and March 1955 (121). Spring high flows measured during the study period indicated a range of 300 to 600 cfs compared to the low summer flows of 55 to 80 cfs. The average discharge of the sewage plant was approximately 5 to 7 cfs during summer. The highly meandering river has a cobbly bed covered with dense bio-film growths below the outfall during summer and fall. Measured river widths during summer flows at the selected sites indicated a variation from 30 to 60 ft. and average water depths in the riffles from 0.7 to 1.30 ft. in the pool areas. Based on field inspection, it may

be termed a shallow, swift mountain river. In the study reach used for the detailed investigations during the summers of 1980 and 1981, there are no major tributaries, but there are small irrigation canals withdrawing water for irrigation.

Description of Sites

Preliminary study. Six sites were established, one above and five below the sewage outfall over a distance of 24 miles during the summer of 1979 as shown in Fig. 6. These sites were surveyed to determine the bed configuration and elevations with respect to permanent bench marks available in the study area. Municipal sewage effluent enters the river between the first and second sites.

Detailed investigations. These investigations, carried out during the summer and fall of 1980 and 1981 covered only a river distance of 6.74 miles below the sewage outfall based on the preliminary study. Six sites were established one above and five below the outfall within this distance. Fig. 7 shows these sites 1 to 6 in relation to the previous sites selected for the preliminary study EG1 to EG6. Sites 1, 2, 6 of 1980, 1981 and EG1, EG2, EG3 are the same.

Field Measurements

For the preliminary study which extended from mid July to November 1979, data was collected on the stream bed characteristics, discharge, sediment transport, several water quality parameters and biofilm growth parameters. The water quality parameters measured on grab samples included BOD, COD, DO, pH, SS, VSS, Orthophosphates, ammonia and nitrate. The water quality parameters were measured on 12 different oc-

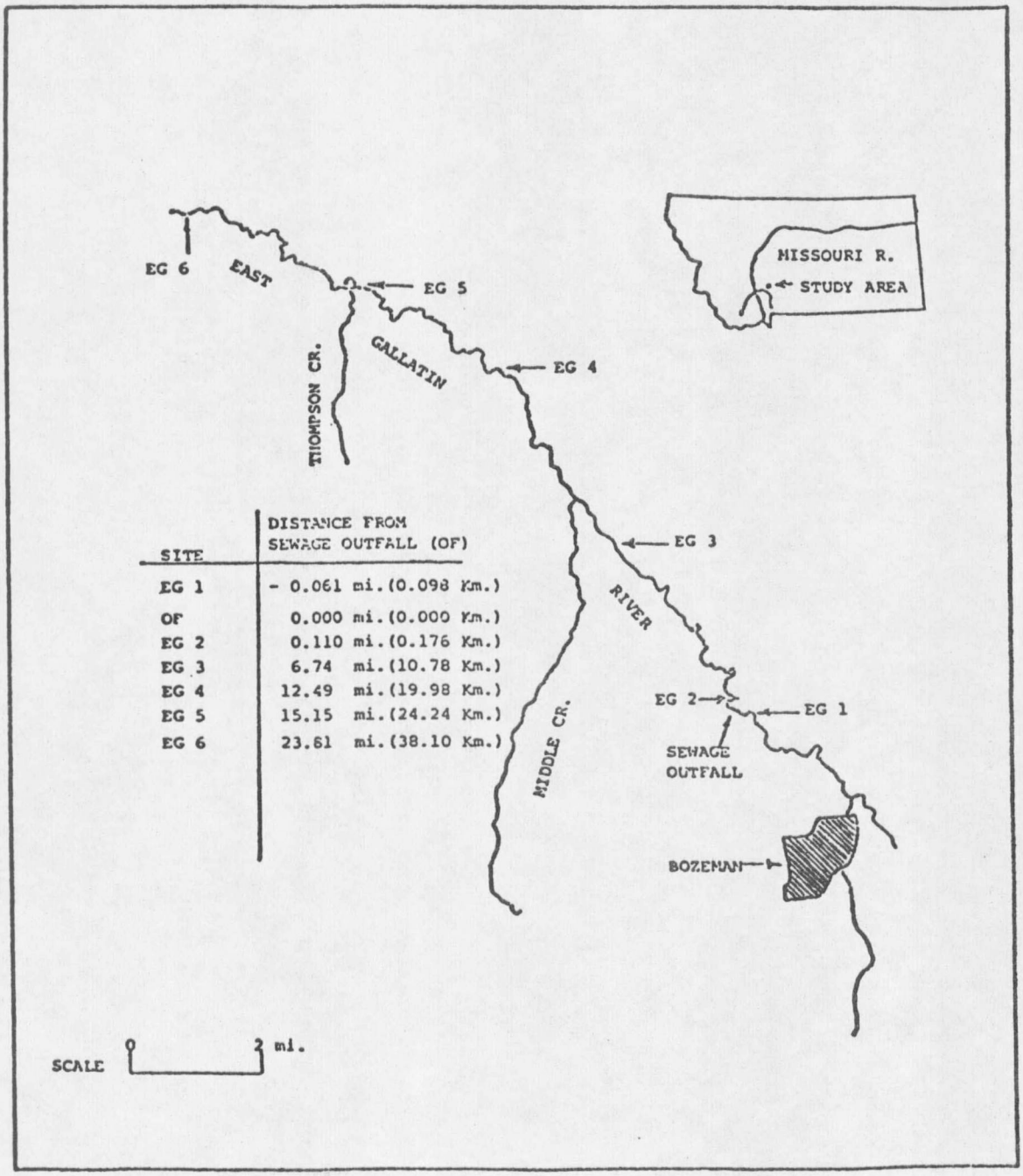


Fig. 6. East Gallatin Study Area and Sampling Sites, 1979.

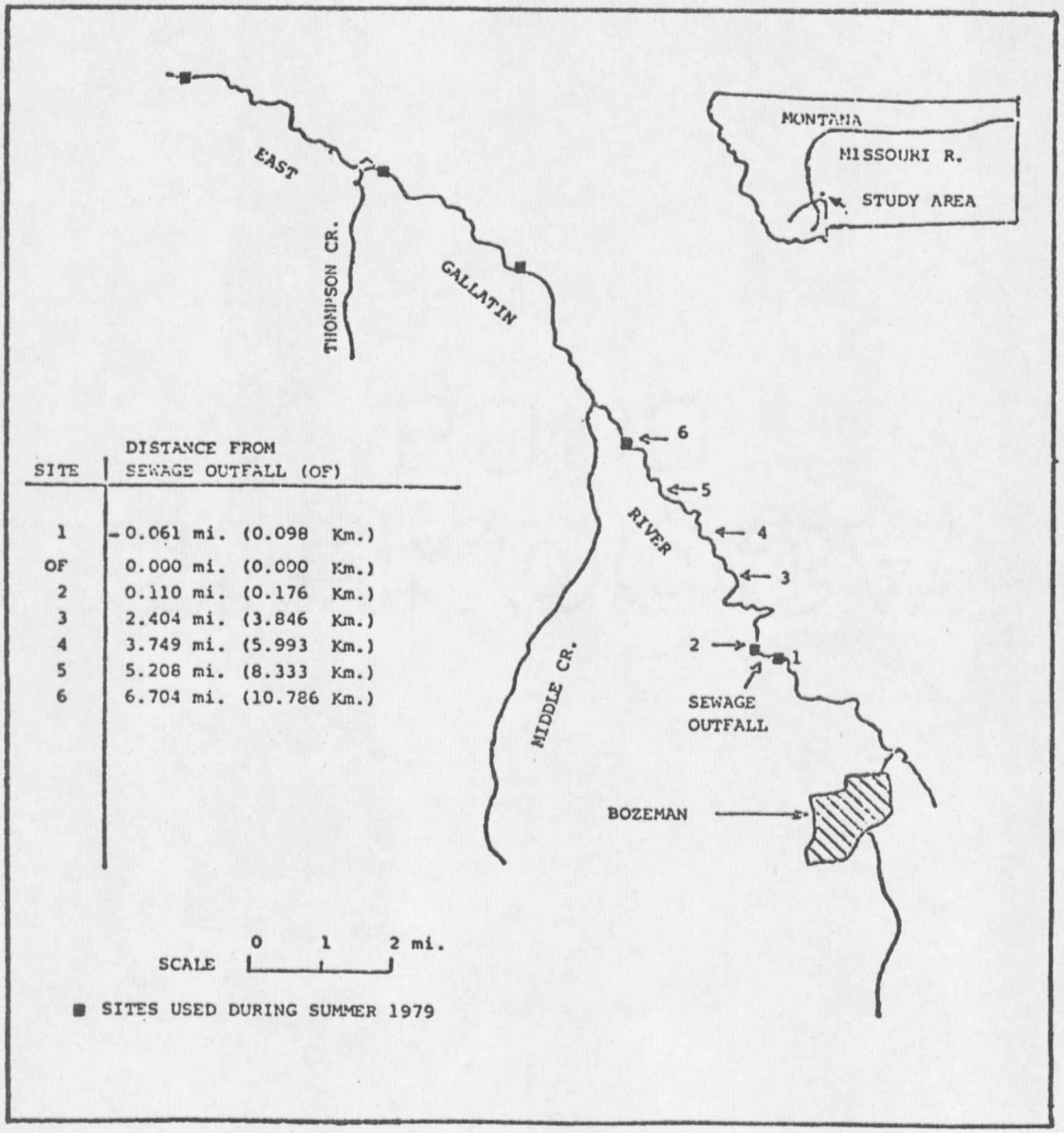


Fig. 7. East Gallatin Study Area and Sampling Sites, 1980 and 1981.

casions during the study period whereas the biofilm samples were analyzed on an average of every three days for average film depth, dry and ash free dry weight and gross appearance. A 30 day growth study period was used during the preliminary study. Discharge measurements were taken weekly on an average but twice a week occasionally.

The detailed investigations of 1980 and 1981 extending from July to November in 1980 and July to October in 1981 concentrated upon selected water quality parameters. TOC measurements were introduced to be used in quantitative analyses during 1980 and 1981. BOD was also measured during 1980 to make comparisons with the preliminary study results. Selected water quality parameters were TOC, DO, pH, SS and VSS even though NH_3-N and NO_3-N measurements were continued during 1980. Water quality parameter and water discharge measurements were taken weekly during the period July to August and in October. Two diurnal measurements were done on TOC, pH, DO and temperature during October 1980 at six hourly intervals. The field channel study was conducted during Sept. to Oct. 1980. Biofilm samples were collected on several bricks with slides placed at different times, every five to ten days up to about 70 days during 1980 and 60 days during 1981. During this period, in addition to the measurements made during the preliminary study, Chlorophyll, ATP measurements and Electron Microscopy studies were done on the biofilms. ATP measurements were mainly done on biofilm growths on natural substrates and only during 1981. A cobble to slide, growth comparison study was also done during the same period by monitoring growth on clean cobbles placed next to the microscope slides and measuring chlorophyll and volatile mass accretions.

Field Sampling and Laboratory Analytical Procedures

Measurement of Water Quality Parameters

Unless otherwise mentioned, grab samples were used for water quality analysis. Samples were collected below the surface in the middle of the stream. The parameters that could not be evaluated in the field were transported on ice in a cooler to the laboratory in acid washed plastic bottles. Sample preservation techniques were done according to Standard Methods (118). The methodology used in the determination of each water quality parameter is tabulated in Table 1.

Measurements of COD, TOC, BOD_L. The rapid method of determining COD was used to minimize the time for laboratory analyses considering the number of samples. Several COD samples were stored in the refrigerator at 0°C and analyzed at one time for convenience. This was also done for the TOC samples which were obtained from the experimental channel study. Eight samples could be analyzed at a time, both filtered and unfiltered. Of the 100 ml samples collected for COD or TOC, about 50 ml were passed through a Millipore vacuum filtering device fitted with 0.45 μm membrane filters. The filtered portions of the samples were used for determinations of soluble organic carbon on the Organic Carbon Analyzer or for rapid COD determinations. The balance samples were generally refrigerated until the analyses were completed. In the TOC determinations, standard solutions of organic carbon were required which were made by preparing a stock solution of an organic compound. Potassium hydrogen phthalate standard stock solution was used for this purpose and a series of standard solutions containing 3 to 20 mg C/liter were prepared by serial dilution of the concentrated stock solution. Five different standard

Table 1. Analytical Procedures for Water Quality Parameters.

Parameter	Methodology
BOD_5	Titrimetric - Standard Methods (118)
COD (Rapid)	Titrimetric
TOC	Persulfate digestion Organic Carbon Analyzer OIC Model 0524B
DO	Winkler-Azide Modification - Standard Methods (118) YSI Model 54BP DO Meter
pH	Orion Model 404 Portable pH Meter Orion Model 801A pH Probe
Water Temperature	YSI Model 54BP DO Meter, Thermometer
Turbidity	Hach Model 2100 Turbidimeter
Nitrate	Orion Model 801 A, Specific Ion Probe
Ammonia	Orion Model 801 A, Specific Ion Probe
Phosphate (Ortho)	Stannous Chloride Method - Standard Methods (118)
SS and VSS	Gravimetric and Volatalization Field Sampling - U.S.G.S. procedure by Guy and Norman (46) Analysis - Standard Methods (118)

solutions were used over the expected organic carbon concentrations. The samples suspected of containing more than 15 to 20 mg C/l were diluted and the proper dilution factor used for conversions. Three to four ampules giving triple to quadruple data per sample were used in the analysis. The step by step procedure to be followed is described clearly in the Instrument Manual. In essence, in this procedure organic carbon was digested with persulfate and converted to gaseous carbon dioxide. The gas was passed through an Infrared analyzer where infrared absorption by the CO_2 was determined. The inorganic carbon present in the sample was initially removed by acidification and stripping prior to the persulfate digestion which was accomplished in sealed glass ampules kept in a pressure vessel in an oven at 180°C for at least 8 hours. For convenience, this was done overnight and the pressure vessel was air cooled for several hours the following day before analysis. A strange phenomenon of ampule breakage was observed only at the beginning of the summer study in 1981. Several circular ampule bottoms were neatly blown off at the end of the digestion and cooling even though only air cooling was used. Breakage of ampules when water cooling was used with insufficient air cooling had been observed by others working on TOC. OI Corporation suggested a reduction in temperature from 180°C to 130°C based on recent experimentation (44) and experiences by others. However, a reduction in temperature did not solve the problem. Finally, the problem was solved in consultation with the Organic Chemistry division of the Department of Chemistry at Montana State University by using only 1 ml persulfate with 1 ml sample instead of 2 ml persulfate with 1 ml sample. Using too much oxidant at high temperatures can produce higher internal pressures inside the ampules with its own decomposition products especially with low organic carbon concentrations. This probably would have blown off the

ampule bottoms. Whether this was the cause or not, this problem never occurred when 1 ml persulfate was used. Two ml of oxidant could probably be used with a 2 ml sample and it probably depends on the type of sample and amount of organic carbon in it. The sample size, however, can be 1 to 2 ml. Super cleanliness and uniformity in procedure must be observed in TOC analysis at the expense of time for consistent results. Soluble BOD_5 measurements were made on filtered samples like the soluble TOC. During the preliminary study period, however, generally only total BOD_5 values were measured without filtering. Daily DO values were measured on selected samples to determine the BOD decay coefficient. Seeding was not required for the river water in the BOD analysis.

Measurements of DO, pH, water temperature. During the preliminary study DO measurements in the field at the different sites were measured by filling BOD bottles with river water and immediately fixing them with the required chemicals as prescribed in Standard Methods (118) before transporting them to the laboratory for analysis. This method however, was dispensed with in the subsequent investigations and a DO meter with a field probe was used for in-situ DO measurements. The DO meter was calibrated in the field before each measurement. The water temperature was measured before the DO measurements. Occasionally a thermometer was used to check the temperatures measured by the DO meter. The main points to note before leaving to the field with a DO meter are: checking the batteries, membrane for any damage and the barometer reading for the pressure. Sometimes it would be possible to see air bubbles inside the membrane, if the membrane was improperly placed over the KCl solution entrapping air bubbles. In such cases, the air bubbles must be removed and new KCl solution should be used to properly

seal the end with a membrane. The DO meter, with proper care and handling gave faster and accurate measurements for all purposes intended in this investigation.

pH measurements were made with a portable Orion 404 pH meter at the beginning of the study till it was out of order. Subsequently samples were transported to the laboratory for measurements on the Orion 801A. NH_3-N, NO_3-N and Orthophosphate measurements were made during 1979 and 1980. Dark sample bottles were used for orthophosphate measurements. Specific ion probes were used for nitrate and ammonia measurements on the Orion meter, Model 801A. The procedures are clearly described in the Instrument Manual.

Suspended and volatile solids. The samples for suspended solids were collected in 473 ml capacity standard 1 pint milk bottles with a depth integrating sampler DH-48 in the river as shown in Fig. 8. Samples were collected at 3 verticals across a section as prescribed in Guy and Norman (46) at each site and transported in a wire case to the laboratory where analysis of SS and VSS were carried out according to Standard Methods (118). The sampling procedure involves inserting a clean sampling bottle into the sampler, after checking the nozzle for any obstruction and then lowering to the water surface for proper orientation. If the sampler was properly oriented, when the nozzle was just above the water, the tail vane should be in the water. Depth integration at this point was accomplished by lowering the sampler to the stream bed at a "uniform transit rate." When the sampler touched the stream bed, the direction was reversed and the sampler was raised at a "uniform transit rate" until it cleared the water surface. The use of the depth integrating sampler facilitated obtaining samples representative of the water solid mixture moving in the stream in the vicinity of the sampler. The ¼ in. nozzle in the sampler allowed water

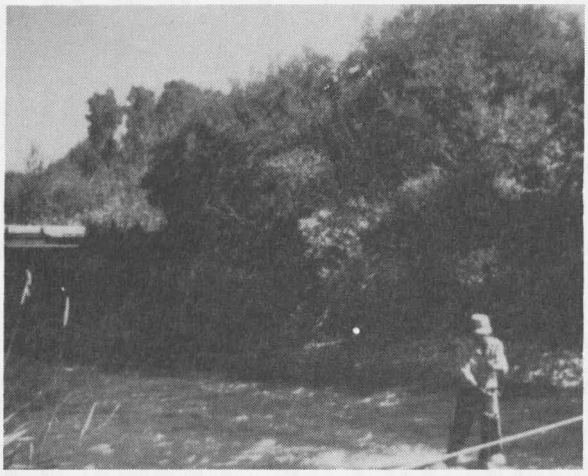


Fig. 8. Solids Sampling with a Depth-Integrating Sampler at Site 2.

Fig. 9. Current Meter Measurements at Site 2.

movement into the sample bottle at the same velocity as the surrounding water velocity. Sample concentrations obtained by this procedure at the 3 verticals were averaged out to determine the mean across a section.

Hydraulic Measurements

Field instruments and equipment: Price type AA current meter No. 622, parts for the round wading rods; 100 ft. steel tape; automatic counter and head phone; stop watch.

The current meter measurements were done according to the U.S.G.S. procedure described in Buchanan and Somers (16) using the six-tenths depth method with the field instruments and equipment given above. The discharge measurement was based on the equation,

$$Q = \Sigma (r_c \cdot u) \tag{25}$$

where

Q = total discharge across a section

r_c = an individual cross-sectional area

u = corresponding velocity for the cross-sectional area

Velocity measurements were taken every 2 ft. close to the banks and 3 to 4 ft with uniform depth in the middle of the stream. Partial discharges were obtained using the average of two mean velocities, the two depths and the distance between the locations. Current meter measurements during the summer and fall were made by wading the stream. Six-tenths-depth adjustments for velocity measurements were made manually as a top-setting rod was not available. This method is considered satisfactory when the depth in a stream is between 0.3 and 2.5 ft. The important point to remember in these measurements is to