

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
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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.

The goal of this research is to determine substrate utilization and growth kinetics of heterogeneous river biofilms in multi-substrate environments. The East Gallatin River in Bozeman, Montana was chosen for the study because of its proximity, and the dense biofilm growth below the sewage outfall. A preliminary study was conducted in 1979 to evaluate the status of the river below the sewage outfall and formulate hypotheses. The detailed investigation carried out subsequently looks at two important aspects of organic carbon degradation in a shallow stream: (1)It determines the kinetics of organic carbon utilization by river biofilms using a pilot plant channel and compares the effectiveness of suspended microbial population in removing organic carbon with the biofilm community. (2) It verifies the mathematical models formulated for application to river water quality under steady state conditions for the substrate and biomass. River data collection included hydraulic, water quality and biofilm parameters over the summers and fall of 1979, 1980 and 1981.

The preliminary study results showed that all the water quality parameters measured returned to background levels within seven miles below the outfall and that biofilm growth controlled the organic degradation below the sewage outfall. The results of the kinetic studies done established first order kinetics for soluble organic carbon utilization by river biofilms in a specified range of substrate concentrations, flow velocities and temperature. The measured and predicted values of the proposed models for describing organic carbon degradation and biomass changes showed good agreement. Sensitivity analyses of hydraulic and biofilm parameters were also carried out to determine the impact of the variability of the parameters on the substrate decay.

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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.

The goal of this research is to determine substrate utilization and growth kinetics of heterogeneous river biofilms in multi-substrate environments. The East Gallatin River in Bozeman, Montana was chosen for the study because of its proximity, and the dense biofilm growth below the sewage outfall. A preliminary study was conducted in 1979 to evaluate the status of the river below the sewage outfall and formulate hypotheses. The detailed investigation carried out subsequently looks at two important aspects of organic carbon degradation in a shallow stream: (1) It determines the kinetics of organic carbon utilization by river biofilms using a pilot plant channel and compares the effectiveness of suspended microbial population in removing organic carbon with the biofilm community. (2) It verifies the mathematical models formulated for application to river water quality under steady state conditions for the substrate and biomass. River data collection included hydraulic, water quality and biofilm parameters over the summers and fall of 1979, 1980 and 1981.

The preliminary study results showed that all the water quality parameters measured returned to background levels within seven miles below the outfall and that biofilm growth controlled the organic degradation below the sewage outfall. The results of the kinetic studies done established first order kinetics for soluble organic carbon utilization by river biofilms in a specified range of substrate concentrations, flow velocities and temperature. The measured and predicted values of the proposed models for describing organic carbon degradation and biomass changes showed good agreement. Sensitivity analyses of hydraulic and biofilm parameters were also carried out to determine the impact of the variability of the parameters on the substrate decay.

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

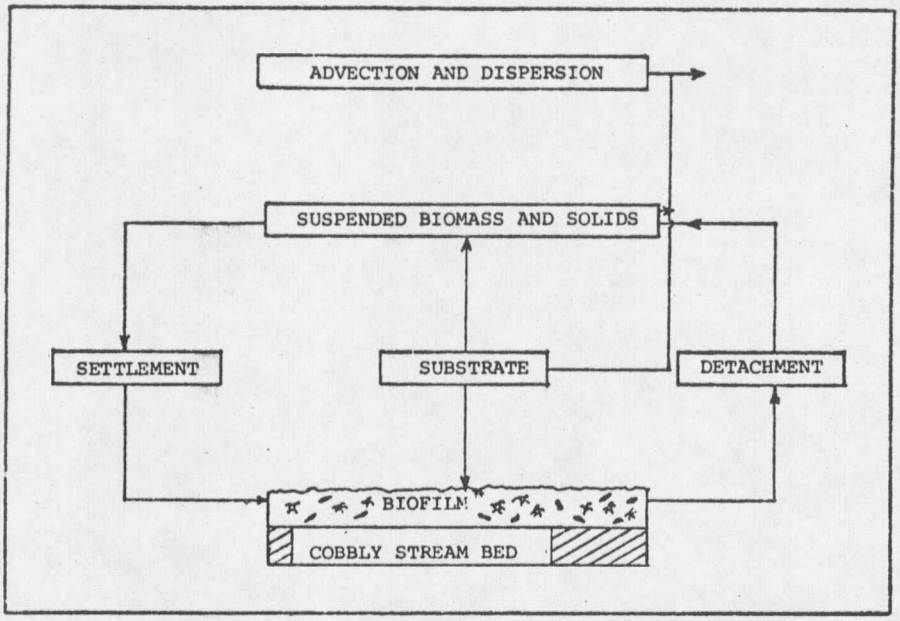


Fig. 1. Schematic Diagram of the Model Frame Work.

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.

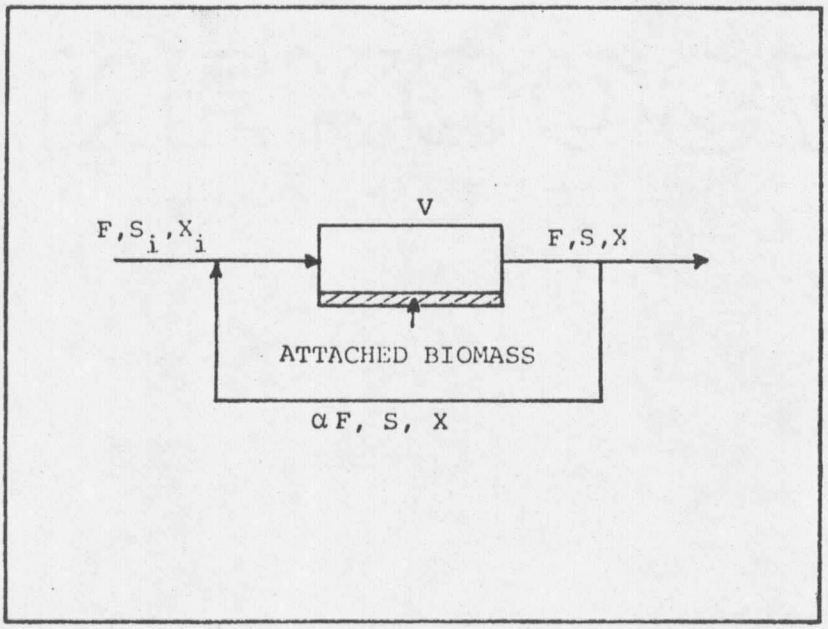


Fig. 2. CMF Reactor With Recirculation.

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

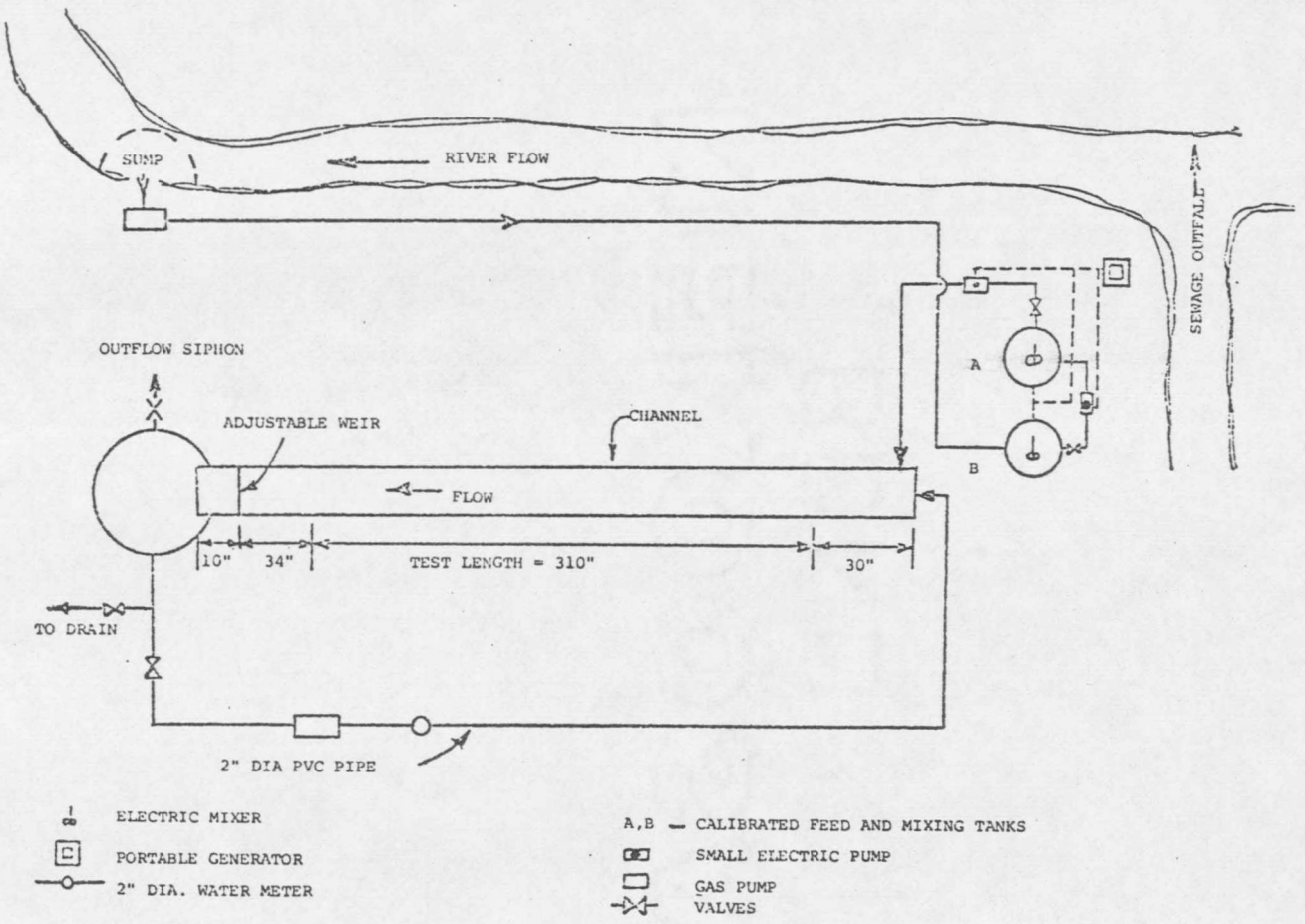
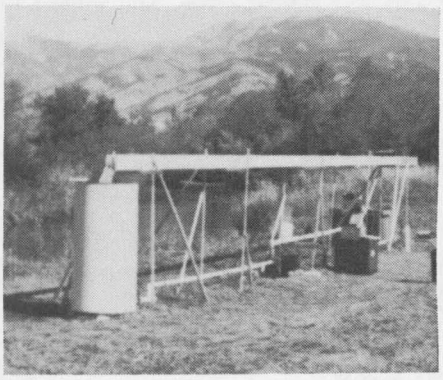
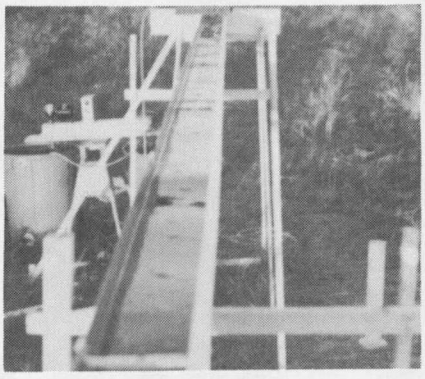


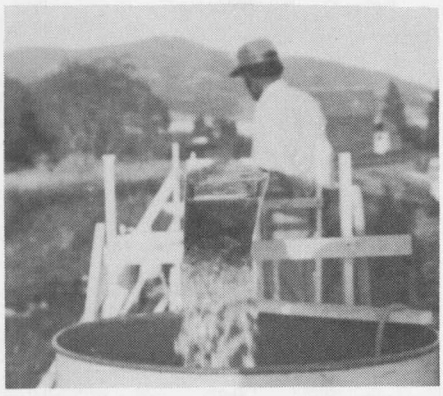
Fig. 3. Schematic Diagram of the Field Channel Experimental Set Up.



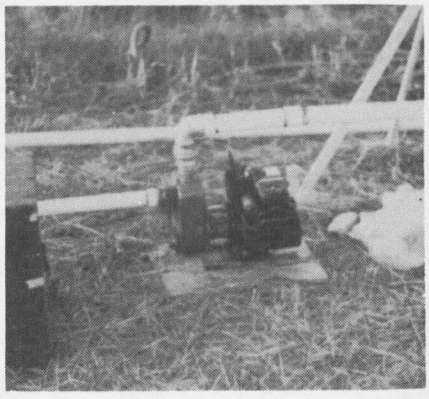
(a)



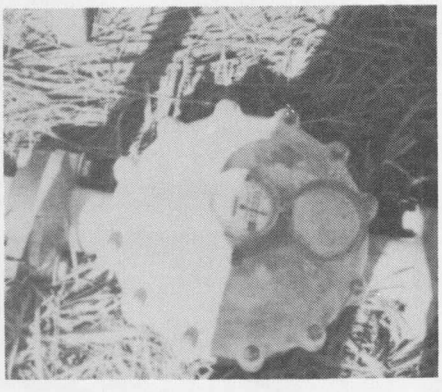
(b)



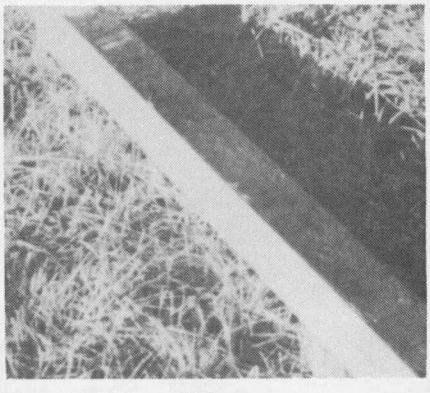
(c)



(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-

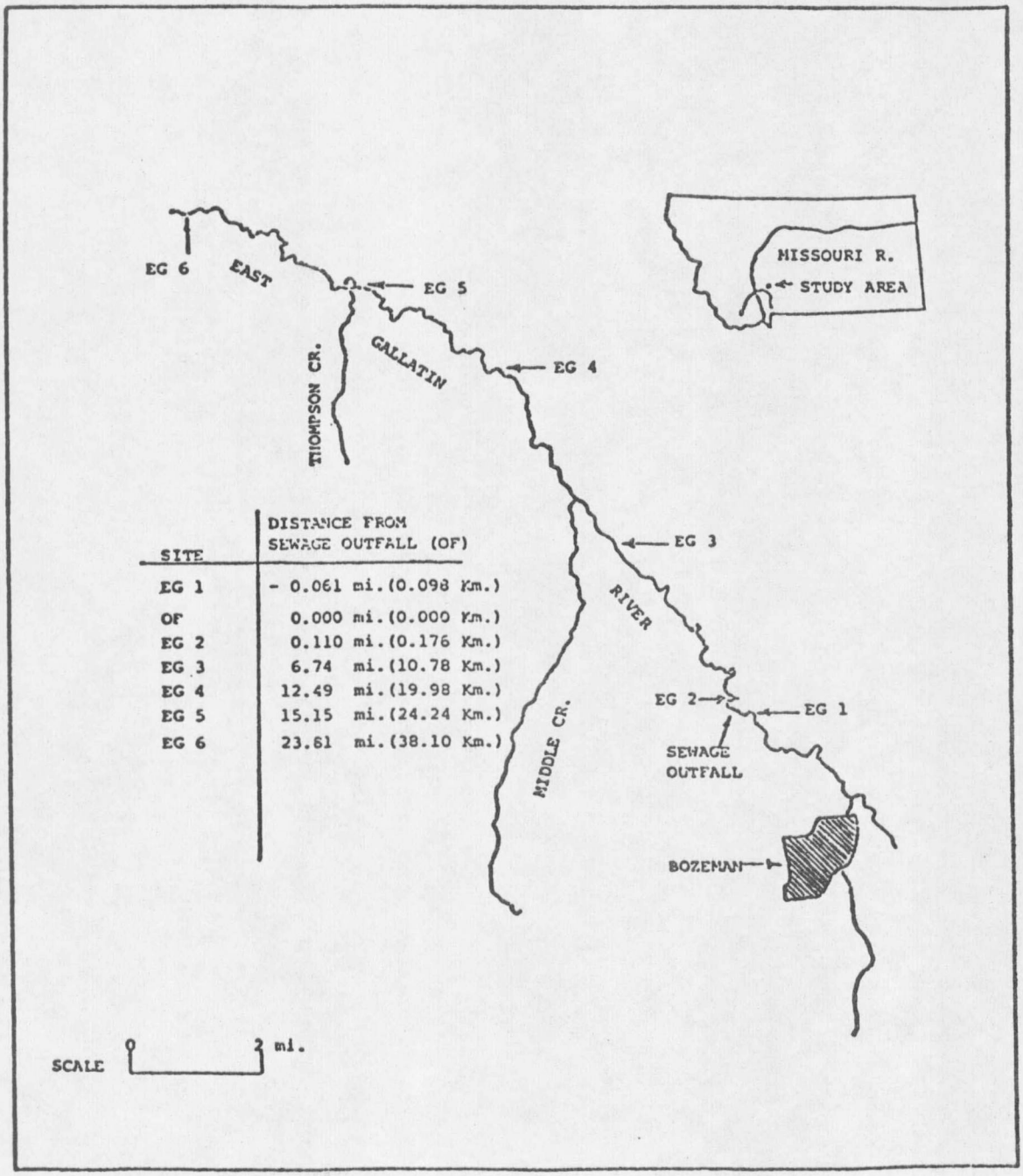


Fig. 6. East Gallatin Study Area and Sampling Sites, 1979.

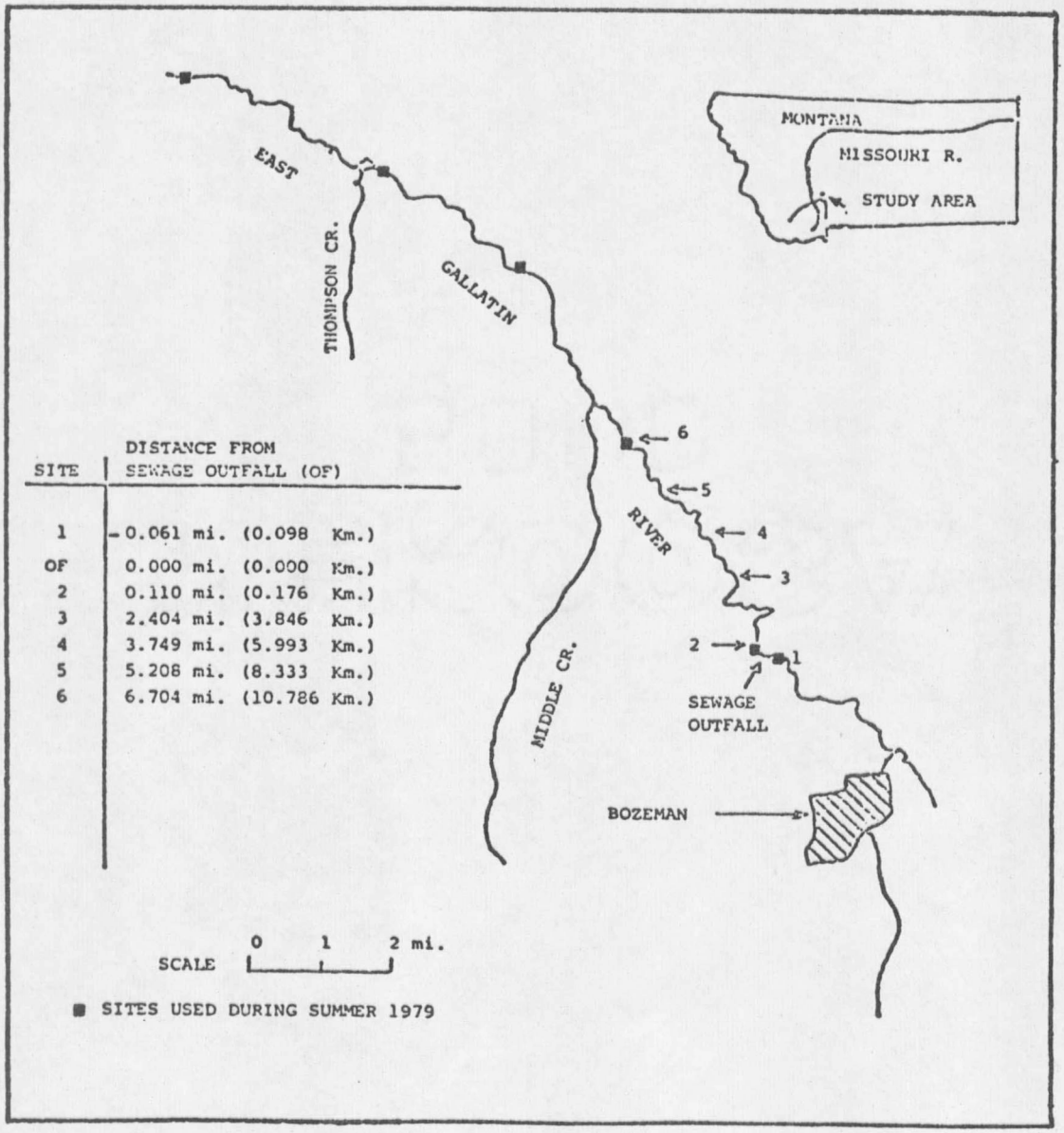


Fig. 7. East Gallatin Study Area and Sampling Sites, 1980 and 1981.

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

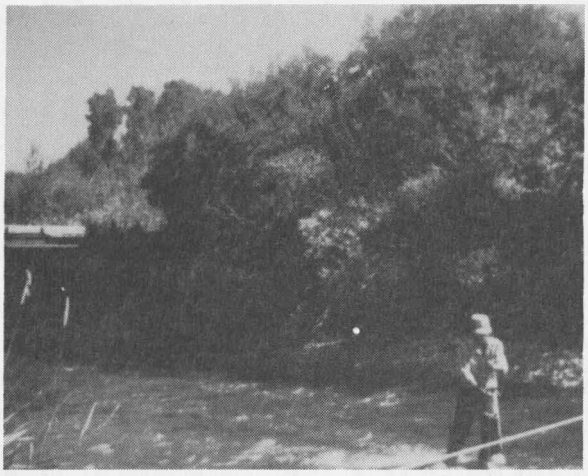


Fig. 8. Solids Sampling with a Depth-Integrating Sampler at Site 2.



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

stand in a position at least 18 in. or more from the wading rod. This procedure would least affect the velocity measurement. Width measurements were made by a 100 ft. steel tape tied across the stream. Fig. 9 shows the picture taken during a discharge measurement at Site 2.

Measurements of Biofilm Parameters

Measurements of velocity and depth were made at all six sites for the biofilm studies during 1980 and 1981 to provide constant hydraulic conditions so that changes in biofilm growth on glass slides would correspond to the water quality.

Thickness and mass measurements. Two to three glass microscope slides were removed from the bricks per site at a time and transferred to labelled petri dishes containing small volumes of river water. After collection of slides at all six sites was completed, they were transported to the laboratory for immediate analysis on morphology and film thickness. During the preliminary study, slides with films were transported to the laboratory in a slide tray immersed in river water. In order to reduce disturbances in the films caused during transport by a large amount of water in a tray, it was preferred subsequently to use petri dishes which offered minimal disturbances during transport. The biofilm thicknesses were measured using the Optical Microscope technique which has been found to be accurate up to at least 300 μm in past studies (108,128). Slides with films were kept vertically to drain the excess water for about two minutes before placing them on the Nikon Microscope Stage. Average film depth on each slide was made by taking 3 to 4 random depth measurements across the biofilm excluding the blank areas. Using the Optical Microscope technique, the film depth was measured first by lowering the 10X

objective until the film surface was in focus and noting down the fine adjustment dial setting and subsequently further lowering the objective until the slide surface was in focus. The difference of the measurements gave the film depth in microns. This procedure was repeated across the slide. The method has its own limitations. When measuring thicknesses above 300 μm, a portion of the film must be removed to expose the glass surface. This might introduce some errors. Secondly, care must be exercised in focusing and keeping track of the fine adjustment dial setting of the stage micrometer which is difficult. Errors can also be introduced into the measurements by different individual perceptions of focus points. In spite of these problems and possible errors, this technique has been found to be fairly accurate and reproducible with sufficient care.

While making the thickness measurements, biofilm morphology was also observed microscopically.

Mass Measurement: The dry weight (DW) and Ashfree weight or the volatile mass (AFW) were determined on the biofilm using the procedures recommended for periphyton in Standard Methods (118). The slide surfaces or known areas of cobble surfaces were scraped with a blade after finishing the thickness measurements and the mass transferred into tared porcelain crucibles. The scrapings were dried at 100°C to a constant weight, cooled in dessicators and weighed. The crucibles with the dry mass were ignited in a muffle furnace and volatilized at 500°C for an hour. The mass was wet with distilled water to compensate for any water lost from the minerals, oven dried, cooled and reweighed. The ash free weight (AFW) used generally as a measure of biomass was determined from the difference between dry and ash weight.

Chlorophyll measurements. The field sampling and laboratory analysis procedures used are described completely in Slack et al. (115) and Standard Methods (118). The step by step procedure therefore will not be given except for the main points. Natural biofilm samples were collected by scraping known areas from cobbles across the river section from each site. Biofilm samples grown on microscope slides were also transported to the laboratory and scraped for analysis. Samples were kept in dark enroute to the laboratory. After the homogenization was completed in a glass pestle-type tissue homogenizer with 90% acetone, the extractions were steeped in dark below 0°C for 24 hours to obtain complete extraction. In the final step, the samples were analyzed on a spectrophotometer. Initially the spectronic 20 was used but subsequently with the use of a narrow band Series 634 Varian Techtron UV-visible Spectrophotometer, measurements were taken on this instrument for better accuracy. The absorbance could be read to ± 0.001 units. Cells used were with a light path of 1 cm. Concentrated samples reading 0.5 and above were diluted with acetone for spectrophotometric measurements. At the beginning, after one trial and personal communication with Dr. Lawrence Bahls of the Water Quality Bureau in Helena, who worked on diatoms in the East Gallatin River for his Dissertation, some refinements were made in the sampling procedure. When scraping natural samples from cobbles, several cobbles at least three in number across the river section were scraped instead of cobbles from the same point. Secondly biomass and chlorophyll measurements were done on the same samples instead of sampling separately for the determination of Autotrophic Index. After extracting the chlorophyll in 90% acetone and centrifuging at 3000 to 4000 rpm for 10 minutes, the chlorophyll extractions were carefully transferred from centrifuge tubes for spectrophotometric measurements leaving the solid matter in the

bottom to be used for mass measurements in crucibles as given earlier. In this procedure, the same sample is used for both chlorophyll and mass measurements increasing the accuracy of the method.

Adenosine Triphosphate Analysis (ATP). The measurements for ATP were made only in the fall of 1981. The reason for doing this analysis will be explained in the results section. The ATP extraction procedure is outlined in Clark (21) and Standard Methods (119). ATP measurements in this investigation were analyzed on a Luminescence Biometer (DuPont Instruments) in the Microbiology Laboratory using chemicals from Packard Co. The procedure used therefore would be discussed in greater detail. Except for one sampling run, the others were carried out on natural biofilm samples scraped from cobbles. The field sampling procedure was the same as for chlorophyll. The procedure developed for this investigation was based on Standard Methods (119) and Clark et al. (21). In essence, in this procedure, ATP was extracted initially using an extracting agent (Tris Buffer) and in the presence of excess firefly luciferase enzyme (PICOZYME F from Packard) ATP produced light in a certain reaction which was measured by the Luminescence Biometer. The calibration of the Biometer was done with buffered ATP calibrator (PICOCHEC from Packard) and measurements taken were according to the step by step procedure given in the Instrument Manual. Even though Standard Methods (119) recommends ATP analyses on films grown on artificial substrates, there were insufficient slides with growths available for several ATP analyses by the time it was carried out in October 1981. Therefore natural biofilm samples in three different sets of sampling and only one set of artificial substrate samples were used in the investigation. One of the difficulties in

making the sample suspension was to use the appropriate amount of filtered water in making the suspension at the beginning so that the ATP readings could be measured on the Biometer. The instrument is capable of measuring a range of 10^5 to 10^9 fg/ml ATP. Thus a trial and error procedure had to be followed.

Electron microscopy. This was done only at specific sites on films grown on artificial substrates because of costs. The first trial was on films scraped from the slides and filtered through a 0.2 μm nuclepore filter. Subsequently the procedure was refined as the first trial did not provide good electron micrographs. The refinement was to cut very thin glass strips (3mm × 10mm) from slides with growth with minimal disturbance to the structure. This was accomplished in the work shop. The samples were immediately fixed by glutaraldehyde followed by dipping into 10%, 20%, 40%, 60%, 80% and 100% acetone solutions. In the case of the filter paper, thin strips had to be cut before this was done. A critical point drying procedure was used to completely dry the samples. The samples were taken to the Veterinary Research Laboratory for use of the Electron Microscope. Copper strips were used to fit the samples which were powdered with gold (conductor) before placing on the Electron Microscope. Pictures were taken at several magnifications ranging from 500 to 20,000 depending on the important details to be seen.

RESULTS AND ANALYSIS

Preliminary Study

Some of the results of the preliminary study have been presented elsewhere (104, 105).

Hydraulic Parameters

The overall period for preliminary phase extended from July to November 1979. The steady state conditions were based on the steady stream flow measurements made during that period and was determined to be from August to November. It is clear from Fig. 10 that during the steady state period, the stream flow measurements at sites EG 1 and EG 3 show much smaller variation than the rest of the sites and an almost constant discharge in that short reach of about 6 to 7 miles below the sewage outfall. The higher discharges below EG 3 are due to the flow contributions from the major tributaries to the East Gallatin River. The Coefficient of Variation (C.V.) defined by the (Standard Deviation/ Mean) for steady state flows varied from 8 to 10% at the first 3 sites in contrast to about 22% at the sites below them. Fig. 10 shows the distribution of mean stream flows during the overall study period in relation to the steady state period. However, if the overall study period is considered, a C.V. of about 20% was obtained at the sites EG 1 and EG 3 even though the variation at sites below EG 3 was not significantly different from that obtained for the steady state period. In other words, major changes in flow occurred during July in the stretch between EG 1 and EG 3 in a transition to a low steady state flow regime. However, such a change was not seen at the sites below EG 3. The mean flow velocities in

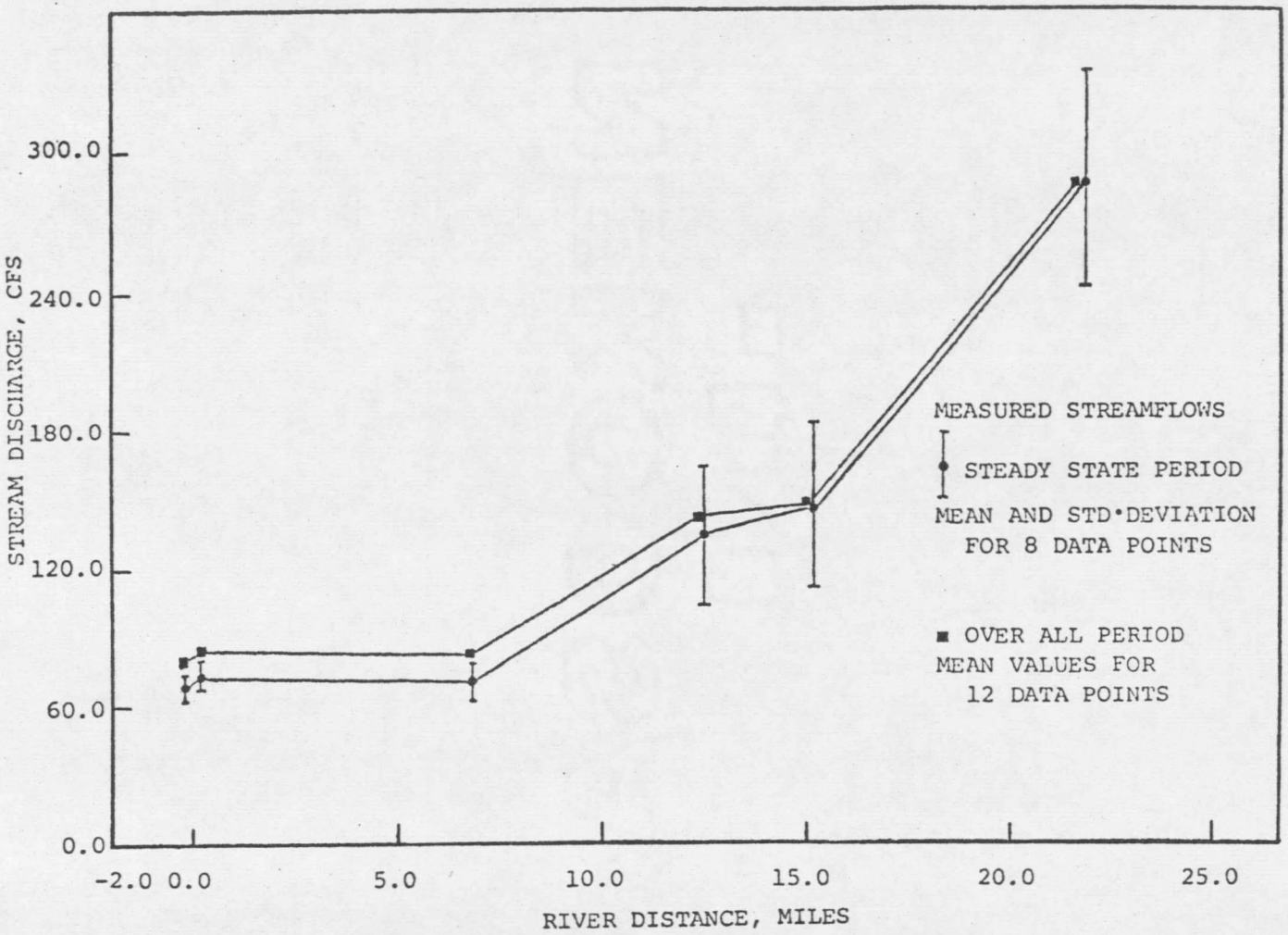


Fig. 10. Stream Flow vs. Distance, Preliminary Study, 1979.

the study reach varied from 1.50 to 2.13 ft/s with an average 2 ft/s at EG 2. Similarly depth measurements indicated a variation from 0.9 to 2.8 ft with an average 0.9 ft at EG 2. The bed elevations established at all six sites are given in the Appendix.

Water Quality and Biofilm Parameters

The sewage effluent characteristics given in Table 2 for the study period show the inadequate treatment in the treatment plant resulting in a low quality effluent.

Table 2. Mean Values for Sewage Effluent Characteristics.

BOD_5	77 mg/ℓ
NH_3-N	7.15 mg/ℓ
NO_3-N	0.14 mg/ℓ
PO_4-P	5.08 mg/ℓ
Flow	4.49 cfs

The distributions of the measured water quality parameters at the respective sites are shown in Figures 11 to 14. COD measurements provided inconsistent, inconclusive data for the river water and therefore are not reported. Turbidity measurements indicated a slight increase below the outfall and then dropped back to a nearly constant value. Unless storm runoff was present, average turbidity values ranged from 3 to 6 turbidity units. The pH distribution shown in Fig. 11 established a consistently lower value below the sewage outfall compared to EG 1 caused by the effluent discharge. The stream, however, remained slightly alkaline throughout its reach during daytime possibly due to photosynthetic activity. The water temperature distribution shown in Fig. 11 using measurements during the sampling from July to November shows a large variation due to the change of seasons.

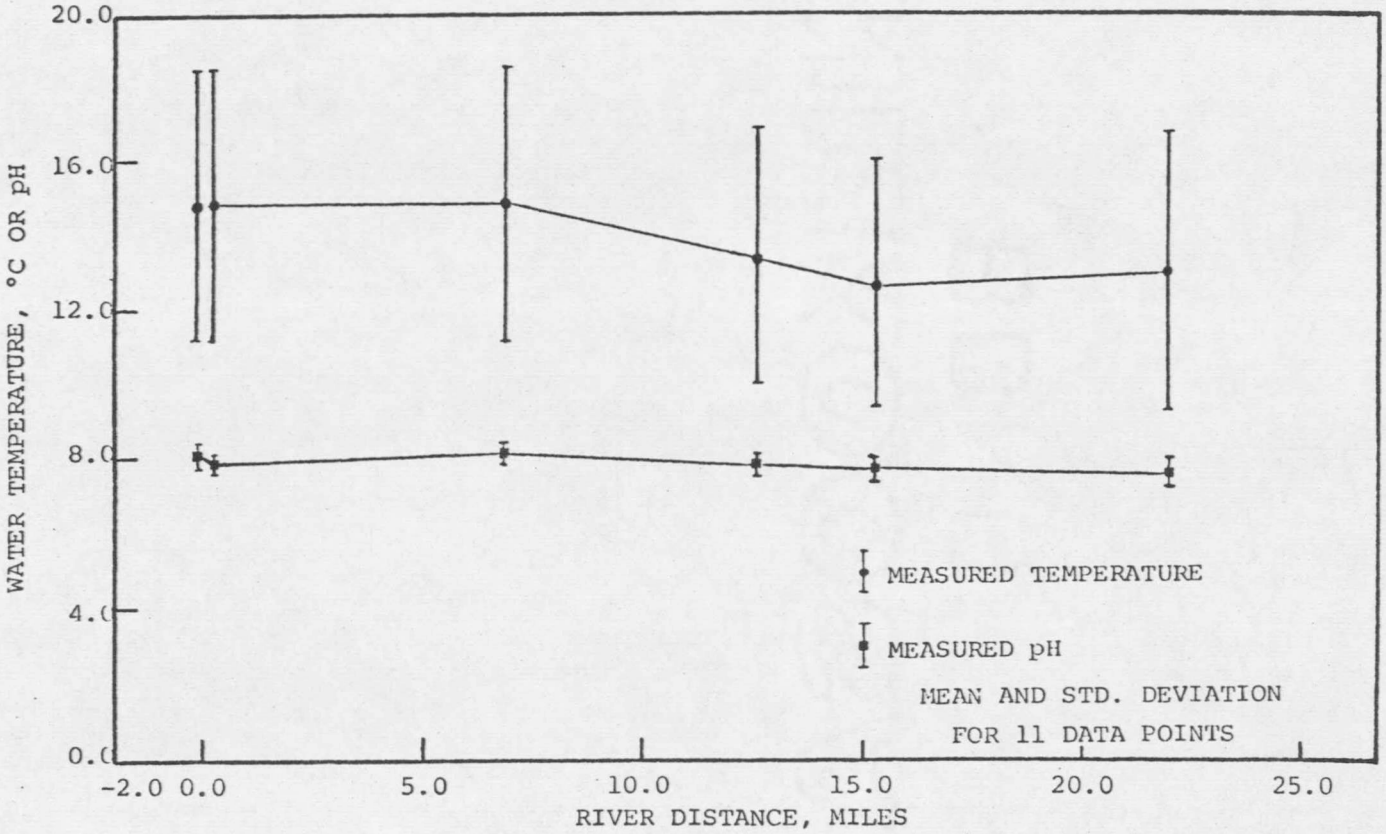


Fig. 11. Water Temperature and pH Variation with Distance, Preliminary Study, 1979.

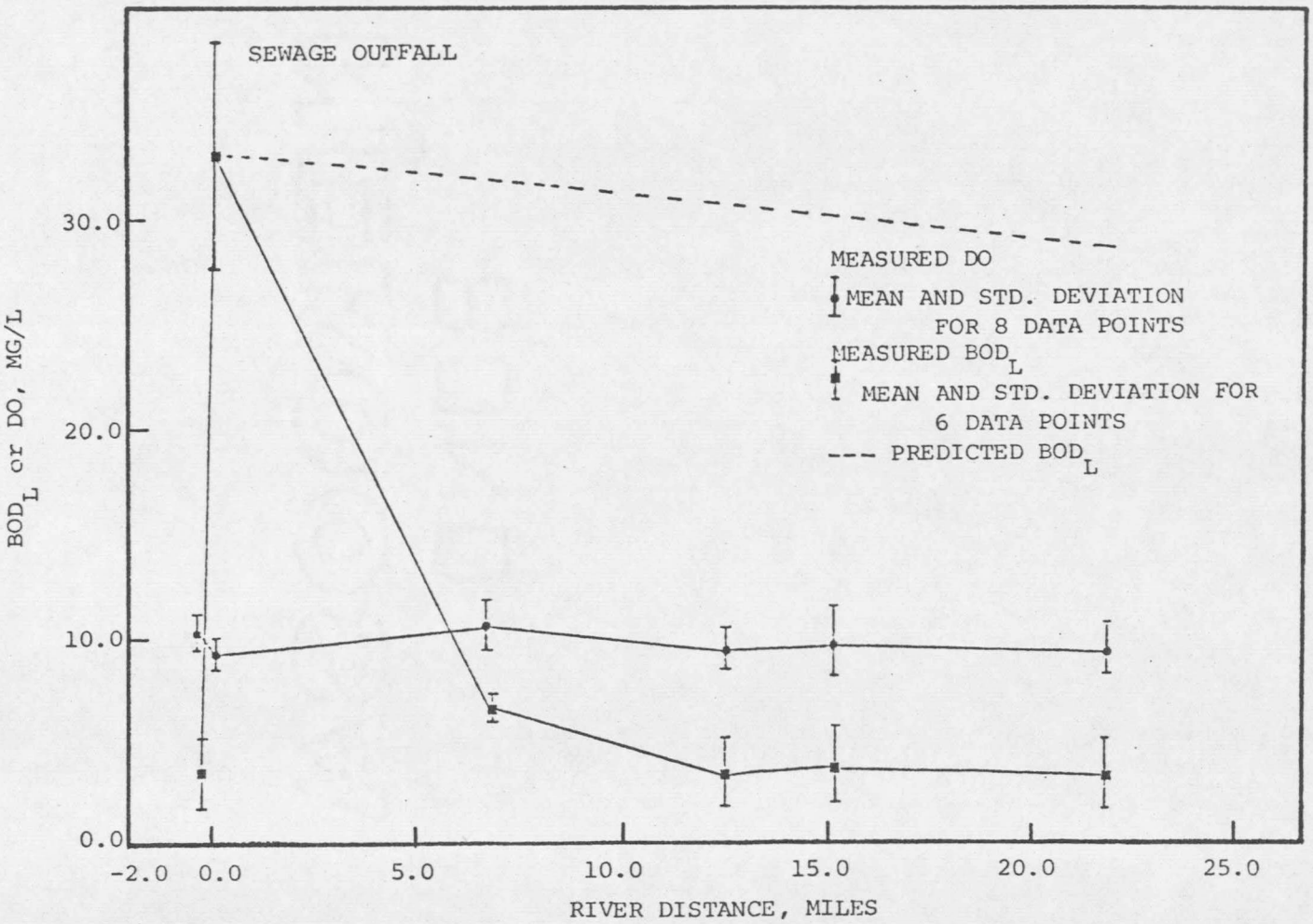


Fig. 12. Stream DO and BOD Profiles During Steady State Flow Regime, Preliminary Study, 1979.

Unless otherwise referred to, BOD in this section would refer to the total BOD_L including particulates. Fig. 12 shows that self-purification in streams occurs very rapidly below the sewage outfall in terms of BOD_L and DO. Drastic removal of BOD_L is seen within 7 miles below the sewage outfall with high levels of DO at 9 to 10 mg/l at all sites. Only a small drop in DO occurred below the sewage outfall. Using a factor of 0.6 determined from 1980 data for soluble to total BOD_L at EG 2, a drastic reduction in soluble and particulate BOD_L can be readily seen in the stretch between EG 2 and EG 3. While the particulate BOD_L changes can be attributed mainly to physical mechanisms such as settlement and scour, soluble BOD_L removal can be justifiably assumed to have been caused by biological mechanisms. Since the flow time between EG 2 and EG 3 based on the mean flow velocity at EG 2 is approximately 5 hours, the particulate BOD_L removal could have been accomplished mainly by settlement. For the same reason, the soluble BOD_L removal in the first 7 miles could be attributed to the biofilms. The effect of suspended biomass would have been smaller in this respect considering the short travel time between EG 2 and EG 3. BOD_L removal using suspended biomass based on the BOD rate constant (k_1') = 0.137 day^{-1} was used to predict values below EG 2 and is shown in Fig. 12 in contrast to the measured BOD_L at sites below the outfall. This may indicate:

1. A laboratory determined k_1' value cannot be used to realistically describe in-stream BOD_L values, which has been indicated (148).
2. Suspended biomass has a negligible effect in comparison to the biofilms growing on the steam bed in the removal of BOD.

Ammonia as NH_3-N removed or converted in the river within the 7 miles below the outfall can be seen from Fig. 13. The reduction of ammonia could have occurred in several

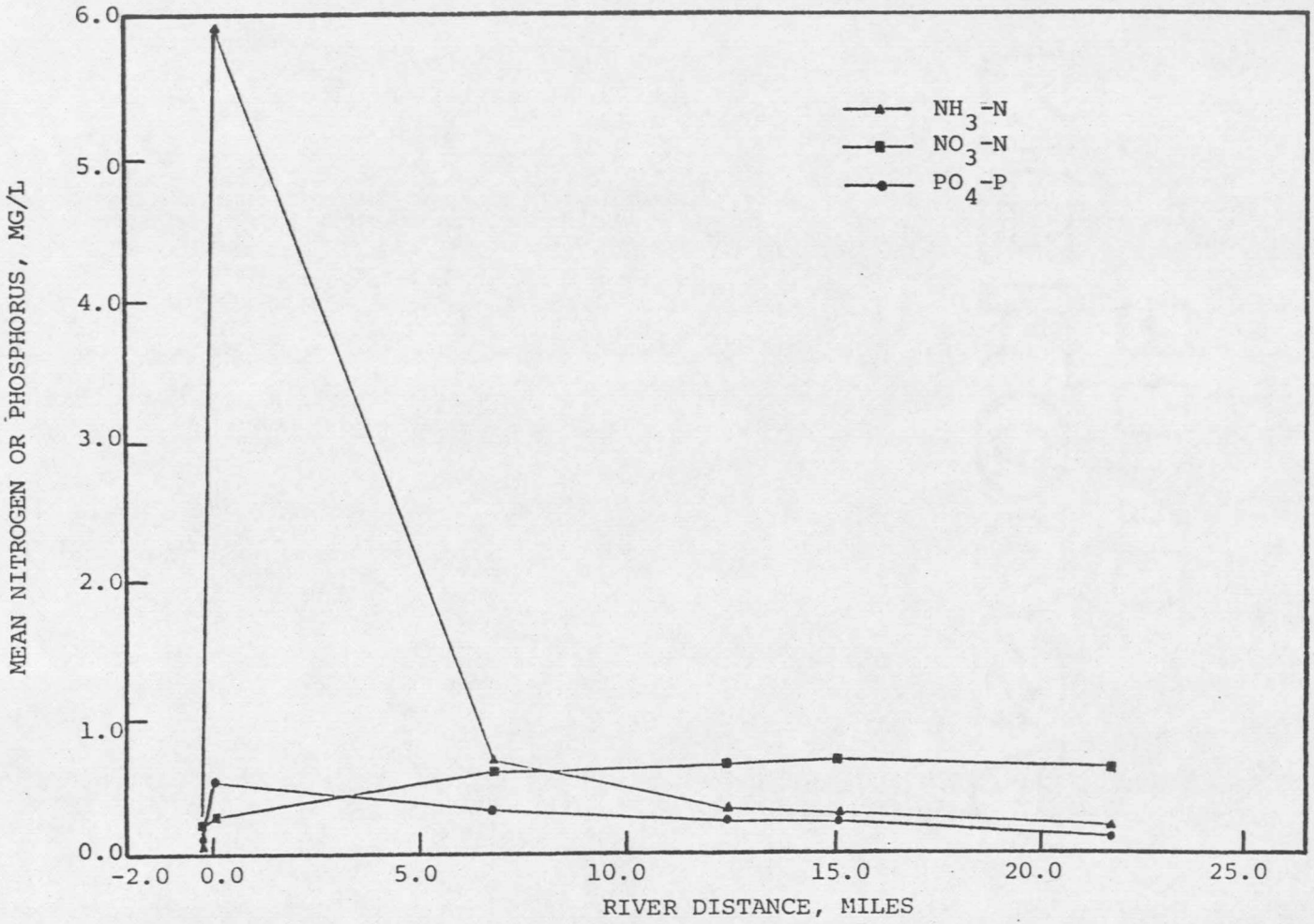


Fig. 13. Mean Chemical Parameter vs. Distance During Steady State Flow Regime, Preliminary Study, 1979.

ways. Fig. 13 shows the reduction in ammonia accompanied by a slight rise in nitrate. This could have been mainly due to nitrification in biofilms in the short stretch between EG 2 and EG 3. Nitrification occurring in biofilms has been documented in the literature (130). The contribution of organics and ammonia from tributaries should be considered below EG 3. There are other mechanisms that can be considered for the reduction in ammonia. Some residents in the area have reported ammonia smells during certain periods. Since the river is alkaline, a certain amount of ammonia stripping is possible. But considering the pH values measured during daytime in the range of pH 7 to 9, ammonia stripping can only be a small percentage of the total reduction. Another important mode of ammonia removal could be the direct uptake by algae. It is possible to have all of these mechanisms occurring simultaneously. Fig. 13 also shows the orthophosphate variation at an almost constant level with a slight increase below the outfall.

An increase of SS and VSS occurs below the outfall, even with a near constant discharge in the first 7 miles as shown in Fig. 14. The increase may be attributed to the scour of the streambed or the banks. Larger variations in SS occurred below site EG 3 and the standard deviations are therefore not shown for sites EG 5 and EG 6 in Fig. 14. This could be possibly due to the larger variations in stream flow seen below EG 3 as shown in Fig. 10. The higher flows, however did not increase the volatile solids.

Results of the biofilm characteristics during the preliminary study are given in Table 3 as reported by Rubich (104). The depth and biomass in Table 3 are considered to be equilibrium values computed by averaging the observed values after the slides had been exposed for at least 20 days in the river. The equilibrium depth is an average of 40 to 55 individual measurements whereas the biomass averages are based on 10 to 19 measure-

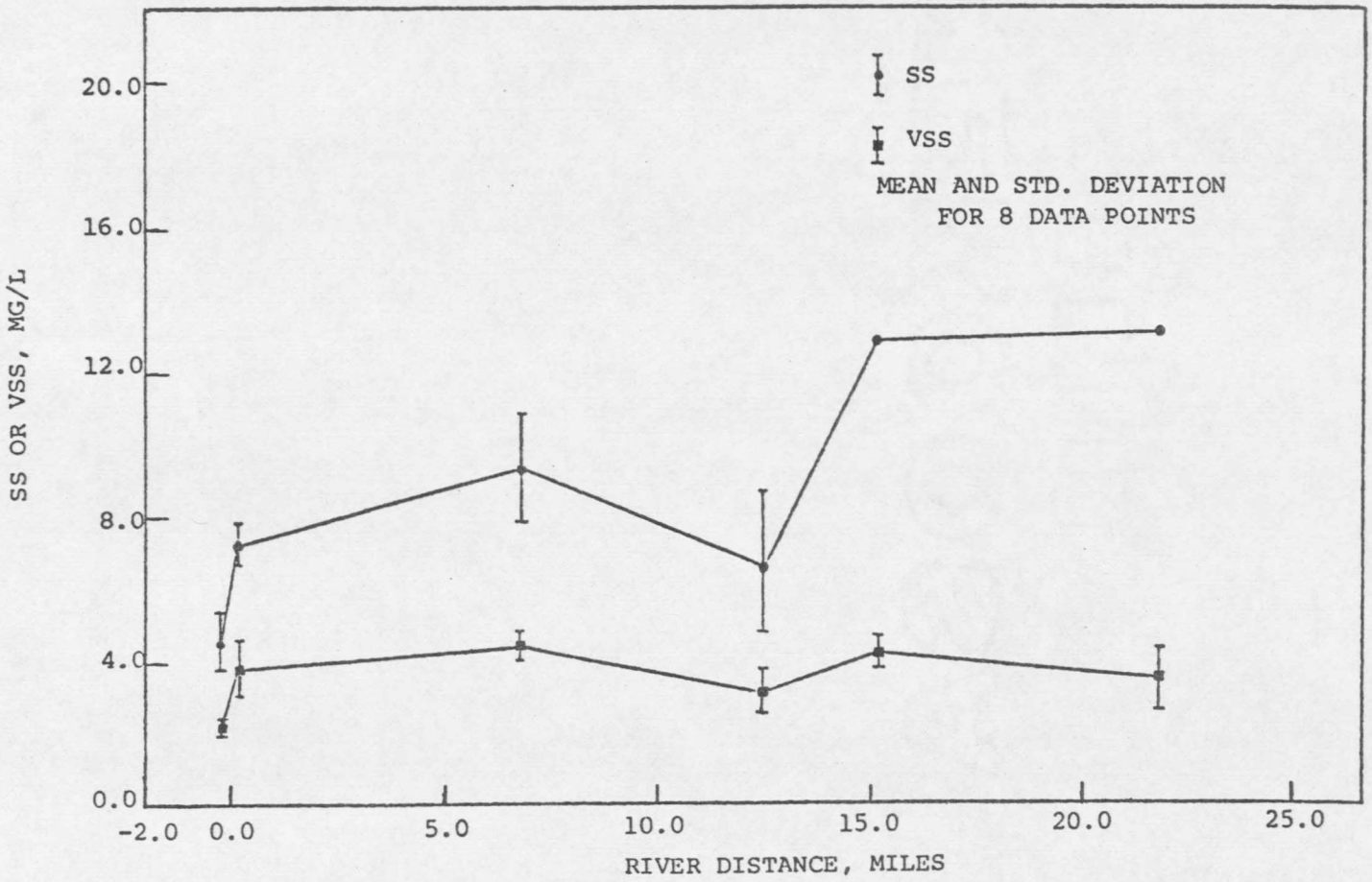


Fig. 14. Steady State Distribution of Solids with Distance, Preliminary Study, 1979.

Table 3. Biofilm Characteristics During the Preliminary Study.

Site	Film Depth μm	Biomass mg/slide	Biodensity kg/m^3	Production g/m^2-day
EG 1	364	29.2	42.8	1.2
EG 2	587	29.4	26.7	1.7
EG 3	226	14.5	34.2	0.83
EG 4	365	31.0	45.3	1.1
EG 5	255	20.9	43.7	1.1
EG 6	274	24.0	46.7	1.0

ments. Biodensity was determined using the equilibrium biomass and depth. The lowest biodensity was determined to be below the outfall while the production rate and equilibrium depth increase at the same location. The significant changes below the outfall could be attributed to higher organic loading. Organic enrichment causing massive slime outbreaks have already been discussed under the review of literature.

Fig. 15 compares the growth pattern of river biofilms growing under polluted and normal conditions. The normal growth curve is produced by combining data from EG 1, EG 4 and EG 6. EG 3 and EG 5 were excluded because of the difference in stream bottom environment. EG 2 was used to represent polluted conditions with higher organic loading. The 90% confidence limits on the normal growth curve indicate similar growth patterns at EG 1, EG 4 and EG 6. EG 2 shows a wider divergence in the confidence limits indicating a significant variability in the growths up to 30 days using slides placed at certain intervals during the study period. Each data point is an average of 10 to 60 individual depth measurements. The curves in Fig. 15 follow a general growth curve with the initial lag followed by log growth and a plateau phase. The study has to be extended more than 30

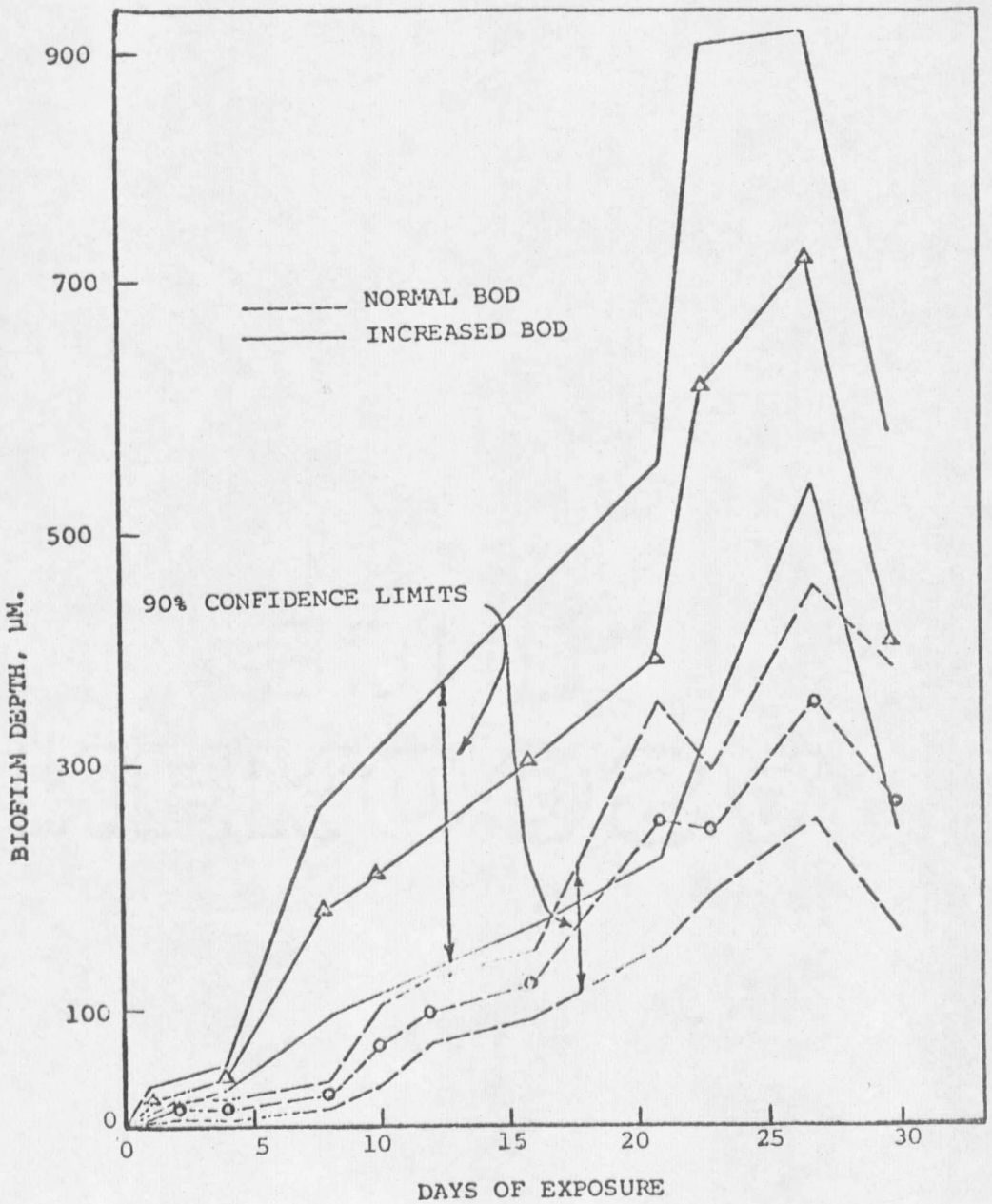


Fig. 15. Biofilm Depth vs. Time, Preliminary Study, 1979.

days to determine the plateau phase. Even though the study period of 30 days is not long enough to determine the equilibrium depth, both curves begin to drop before the end of 30 days indicating the beginning of a plateau phase. A longer lag period is also seen for the normal growth curve compared to that of the polluted conditions.

A plot of mean biofilm depth with distance gives the river biofilm profile as shown in Fig. 16 indicating a direct relationship between the biofilm depth and organic load. If similar hydraulic and meteorological conditions are present at all sites, the variation in the biofilm profile below the outfall can be attributed to the changes in water quality. The principal water quality parameter that controlled the film profile in Fig. 16 was organic carbon.

Major Conclusions from the Preliminary Study

1. Based on the water quality parameter measurements done upstream and downstream of the sewage outfall, the impact of the sewage effluent in the East Gallatin River can be said to be felt only within seven miles of the outfall.
2. Biofilms are predominant in degrading the organics within 7 miles below the sewage outfall. They grow to an equilibrium depth before significant sloughing occurs. The river biofilm profile appears to correspond to the water quality changes below the outfall.
3. Even though near constant stream flow conditions exist in the first 7 miles below the outfall, an increase in SS and VSS is seen over the same distance.

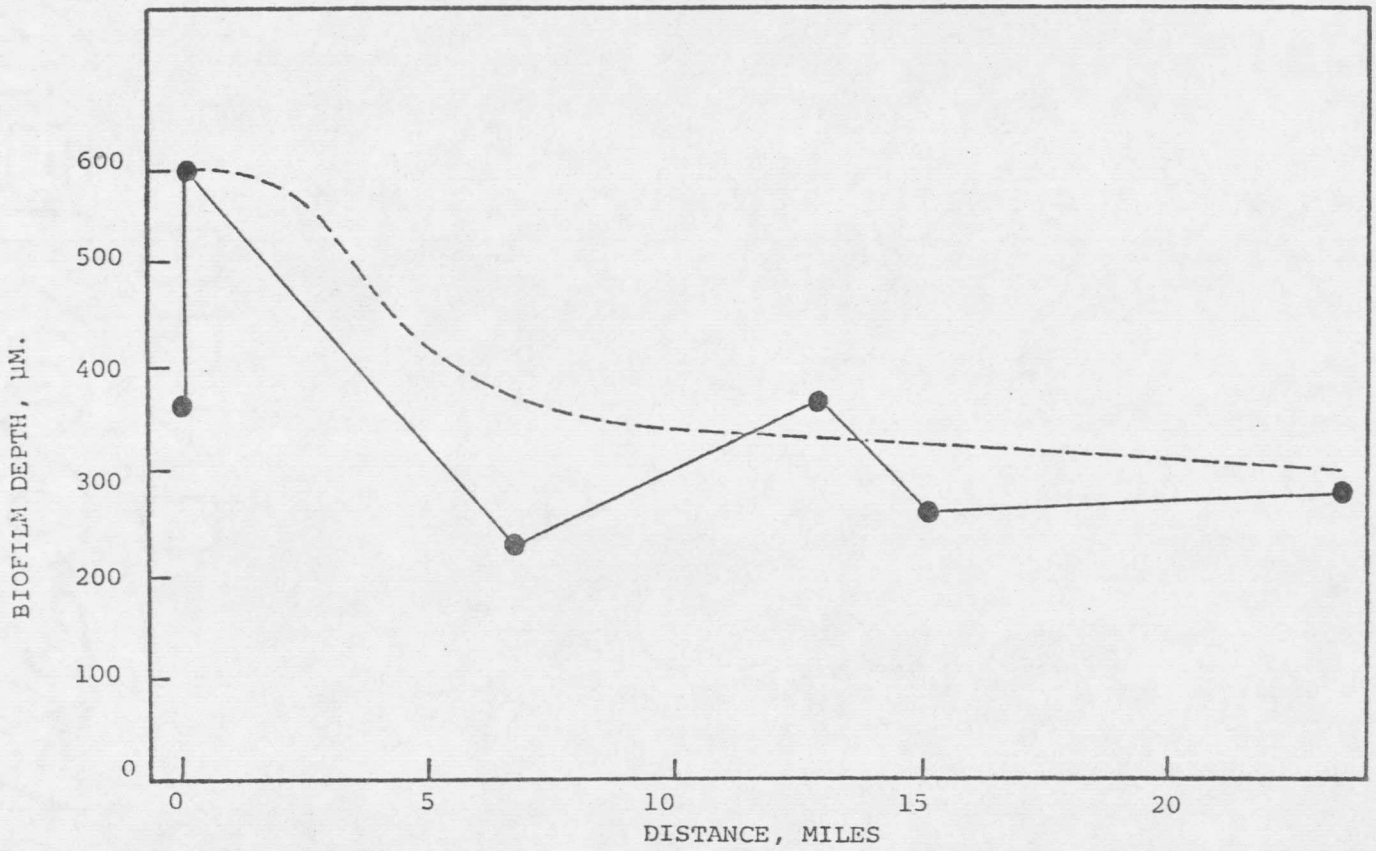


Fig. 16. River Biofilm Profile, Preliminary Study, 1979.

Kinetic Studies

Pilot Plant Channel Study

Unless otherwise mentioned, substrate suspended and attached biomass are measured in this study as soluble organic carbon (SOC), volatile solids (VSS) and Ash-free dry weight (AFW). Twenty experimental runs including three control runs and a run with tap water were made in this phase of the study. Five runs considered questionable because the influent organic carbon concentration varied significantly during the run have been omitted for purposes of analysis. They are, however, attached to the Appendix. Figs. 17 to 21 and 23(b) show the variation of SOC, VSS and DO for the regular runs with attached biomass in contrast to the variation shown for the control runs in Figs. 22 and 23(a) without the attached biomass. Fig. 23 illustrates the direct comparison of a regular run and a control run. The variations shown for control runs in Figs. 22 and 23(a) establish the negligible contributions of the suspended biomass in the utilization of soluble organic carbon at the concentration levels studied. These concentrations cover the range of levels existing in the river. The range of influent SOC values used in the study varied from 5 to 20 mg/l and the control runs were also made at different concentrations.

A large increase in VSS was seen with tap water possibly as a result of endogenous decay of the biomass contributing to sloughing as shown in Fig. 24. The DO also dropped for this run but not significantly. Fig. 25 shows typical variation of channel water temperature, influent and effluent pH for regular and control runs which consistently indicated steady state conditions after 3 hours of the experimental runs. This was also evident from Figs. 17 to 21 for DO, SOC and VSS. The effluent pH also increased compared to the influent generally in all the runs, the increase being the smallest for the control runs and

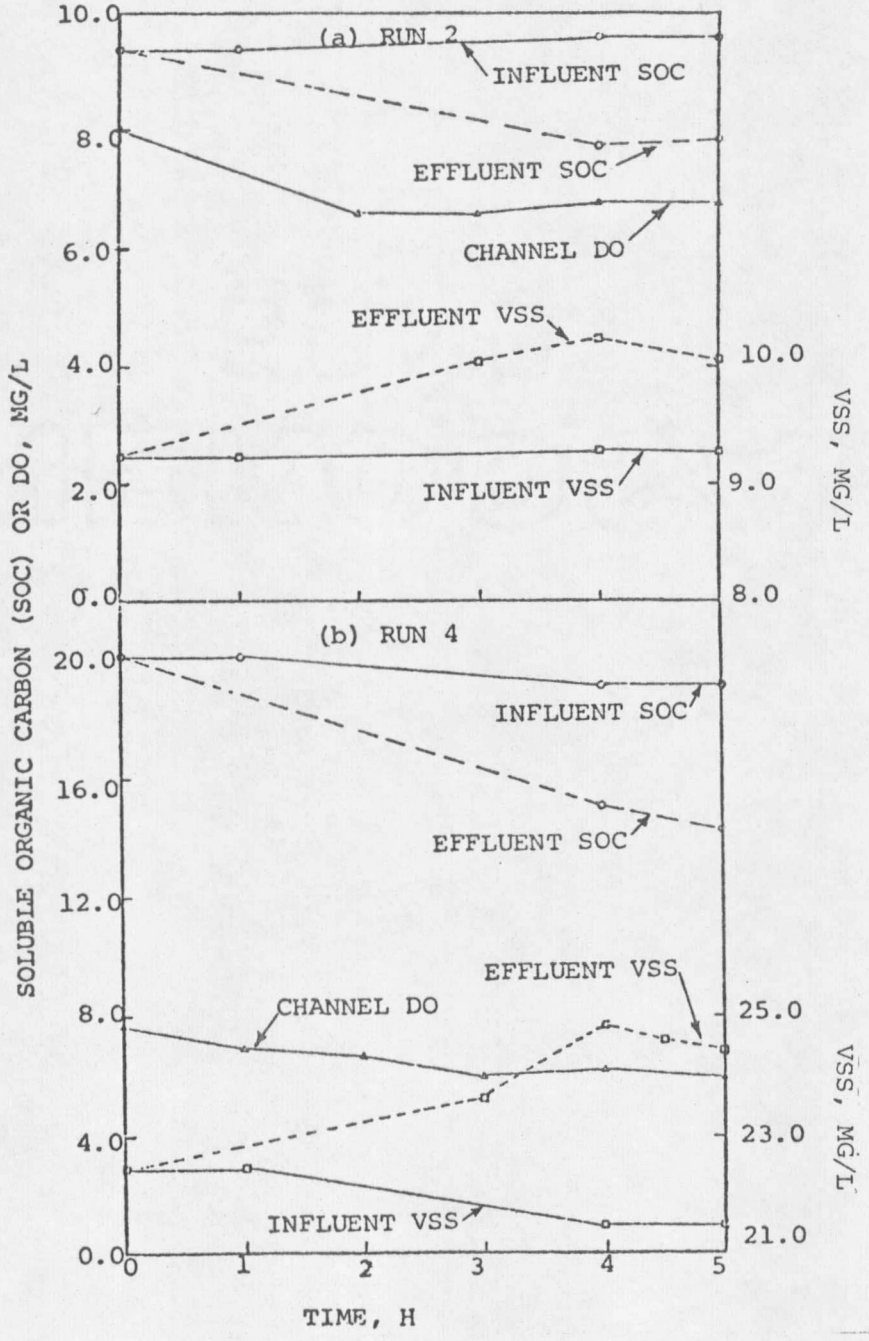


Fig. 17. Organic Carbon, Channel DO and VSS Variation with Time for (a) Run 2 and (b) Run 4.

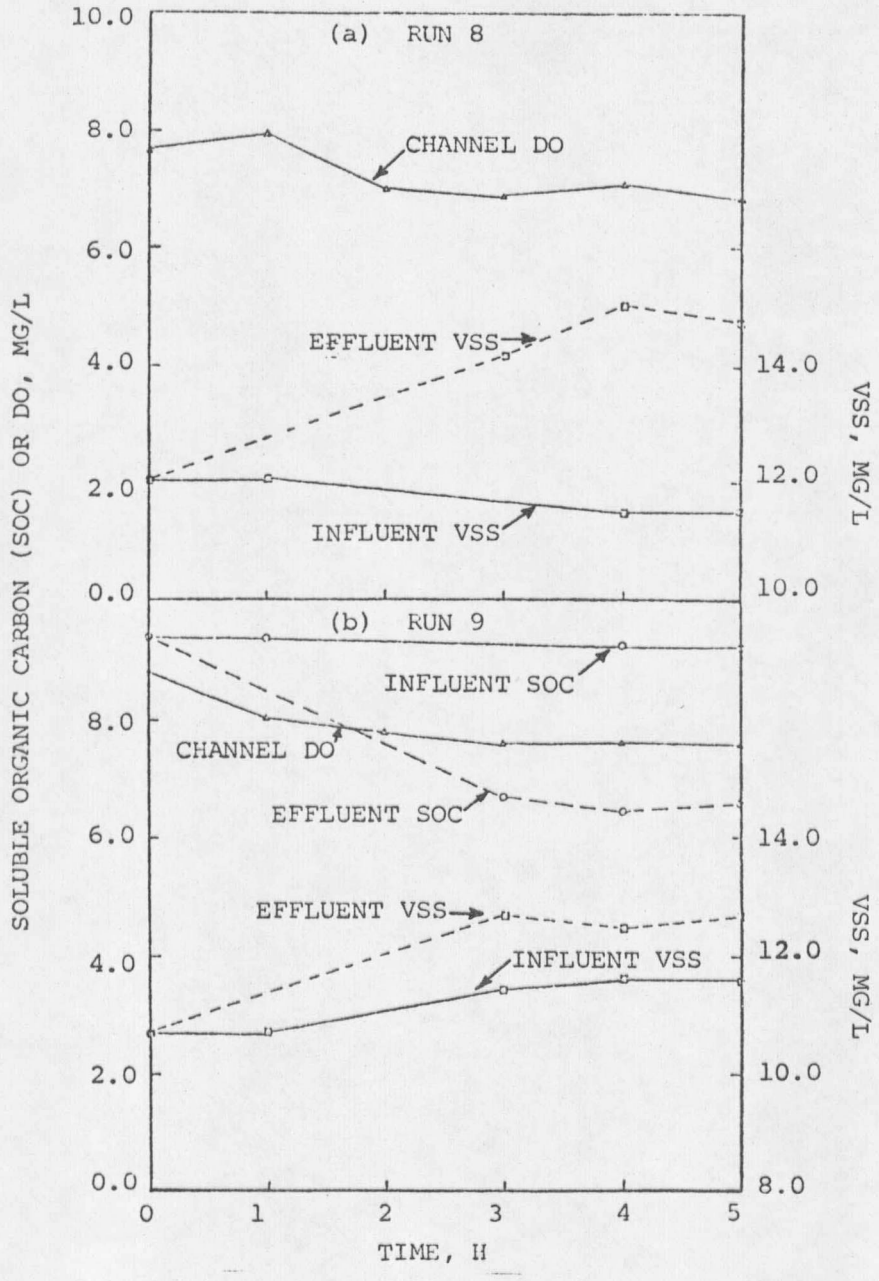


Fig. 18. Organic Carbon, Channel DO and VSS Variation with Time for (a) Run 8 and (b) Run 9.

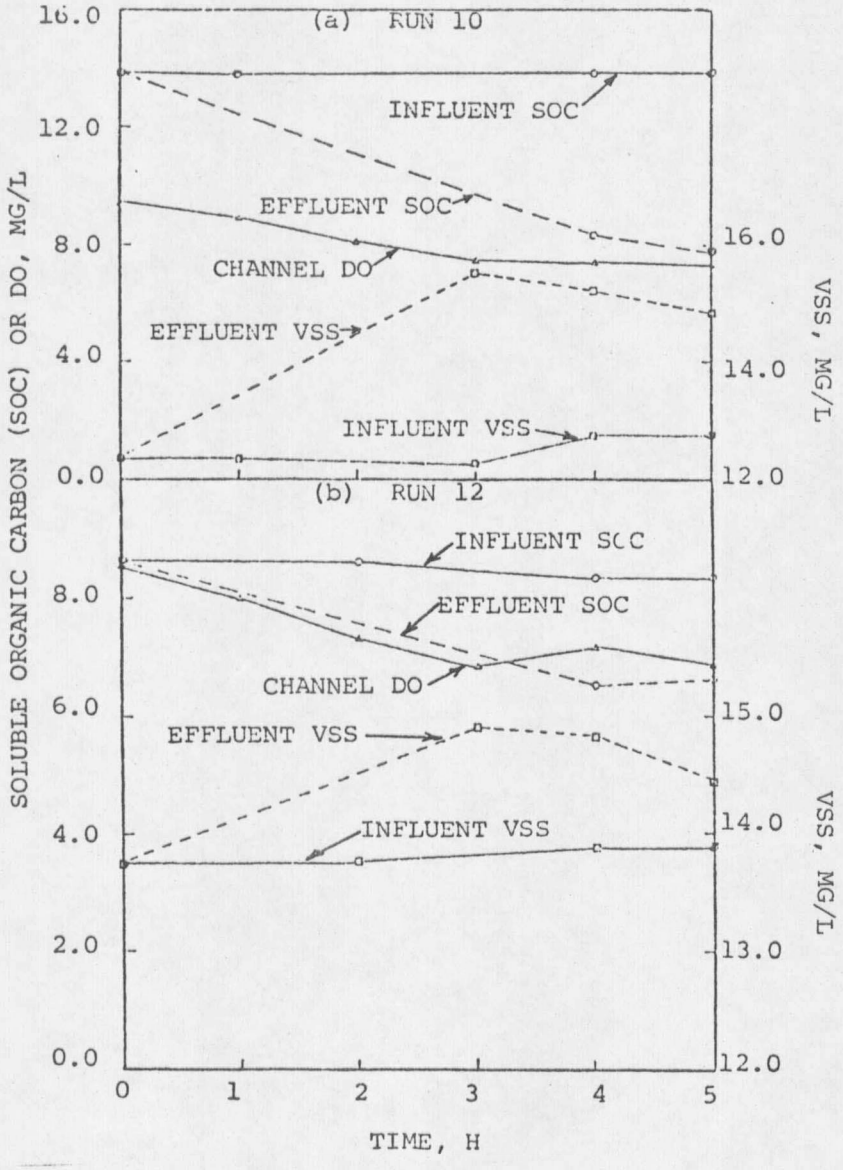


Fig. 19. Organic Carbon, Channel DO and VSS Variation with Time for (a) Run 10 and (b) Run 12.

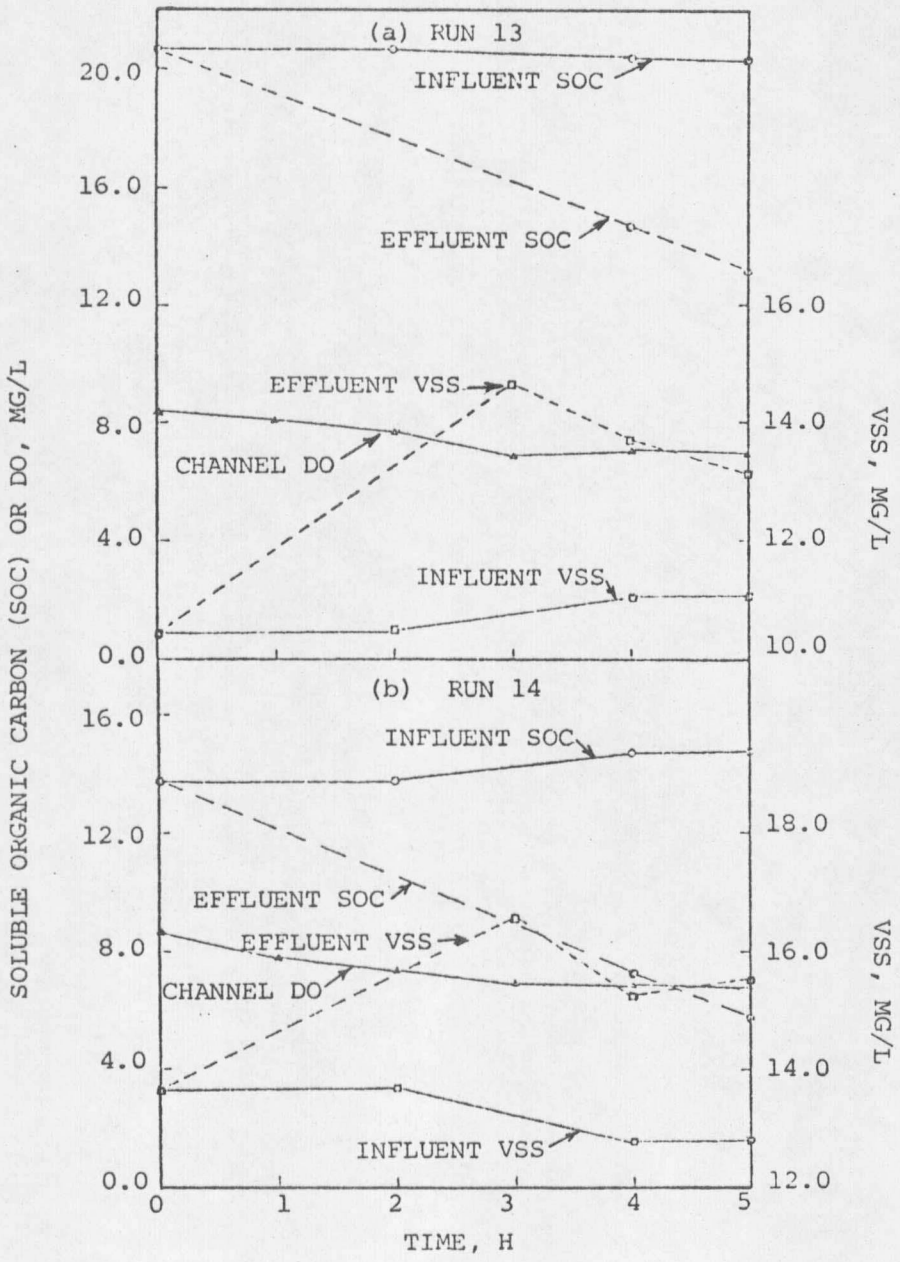


Fig. 20. Organic Carbon, Channel DO and VSS Variation with Time for (a) Run 13 and (b) Run 14.

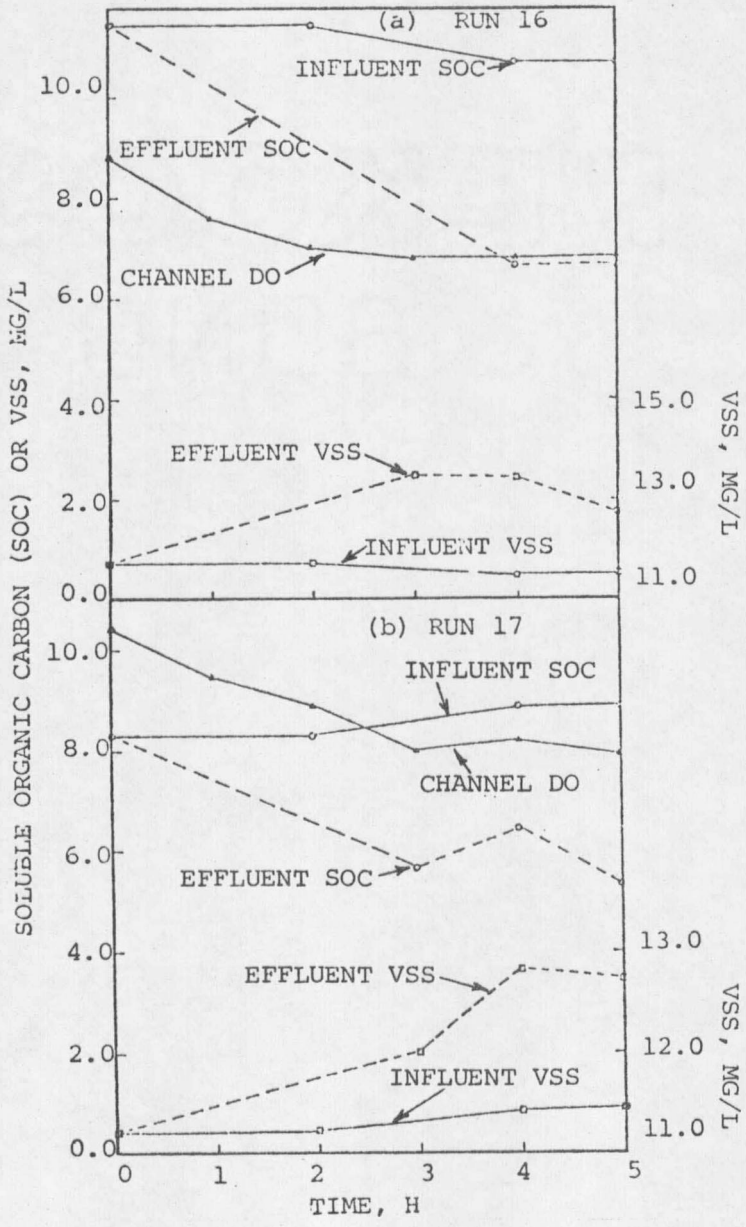


Fig. 21. Organic Carbon, Channel DO and VSS Variation with Time for (a) Run 16 and (b) Run 17.

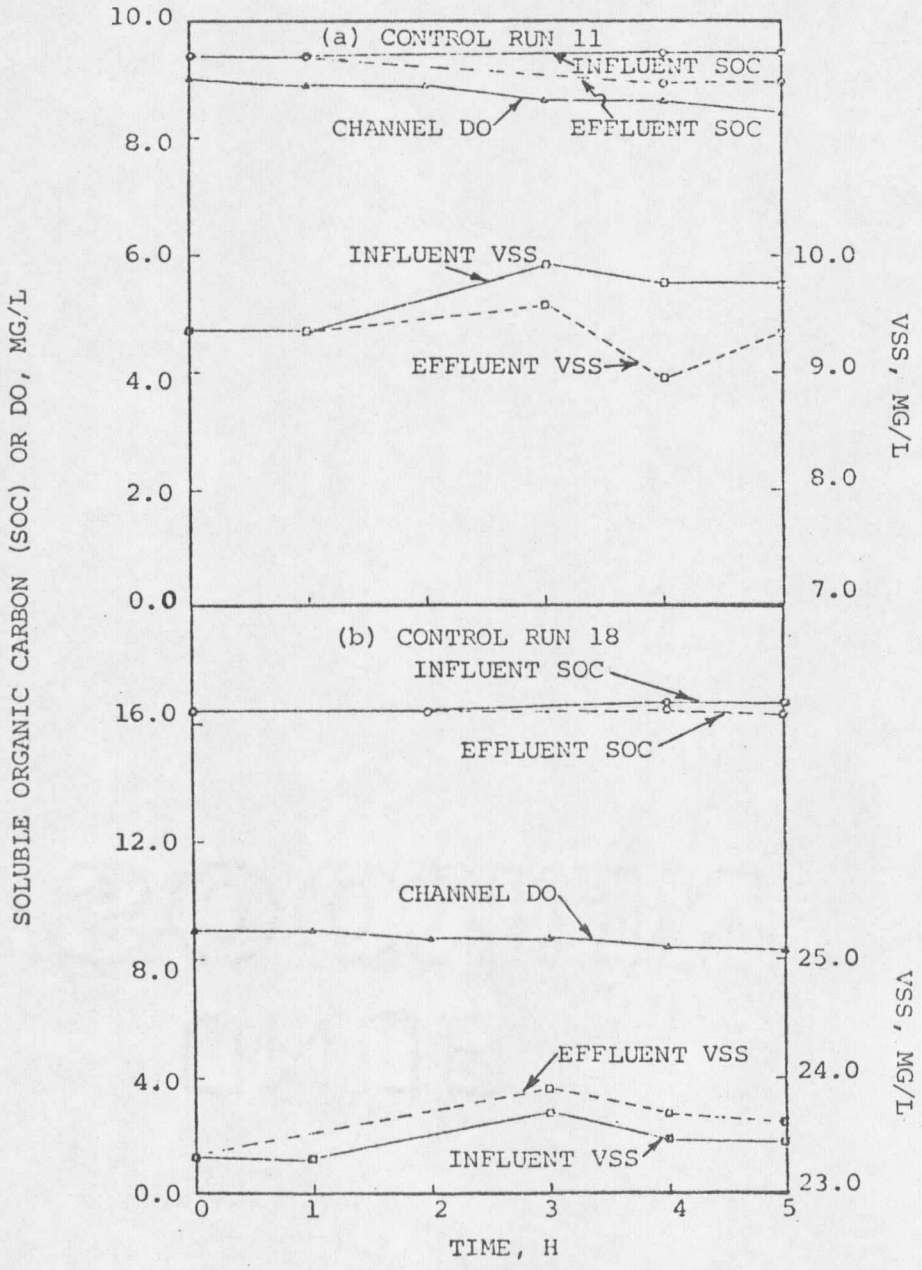


Fig. 22. Organic Carbon, Channel DO and VSS Variation with Time for (a) Control Run 11 and (b) Control Run 18.

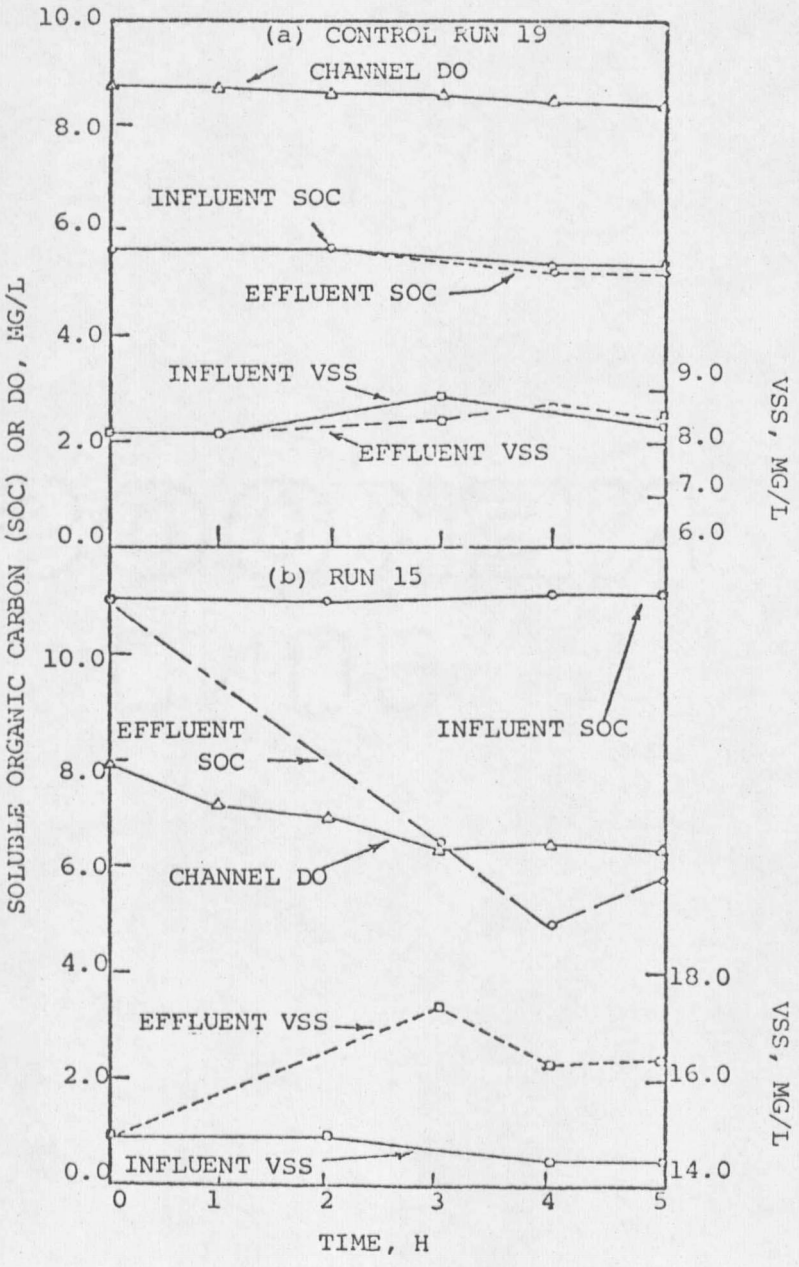


Fig. 23. Typical Variations of Organic Carbon, Channel DO and VSS Variation with Time for (a) a Control Run and (b) a Regular Run.

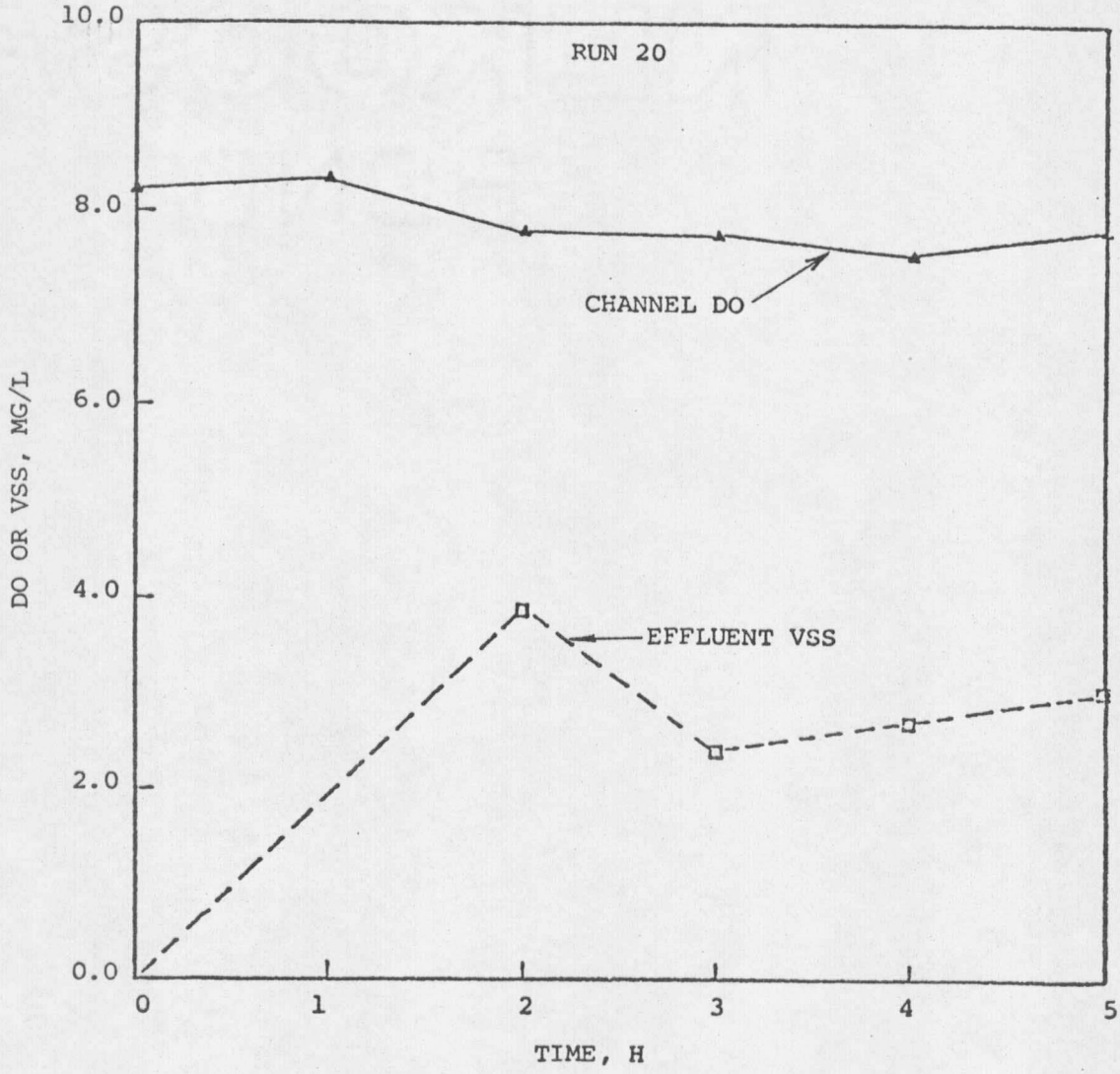


Fig. 24. Variation of Channel DO and VSS with Time Using Tap Water for Run 20.

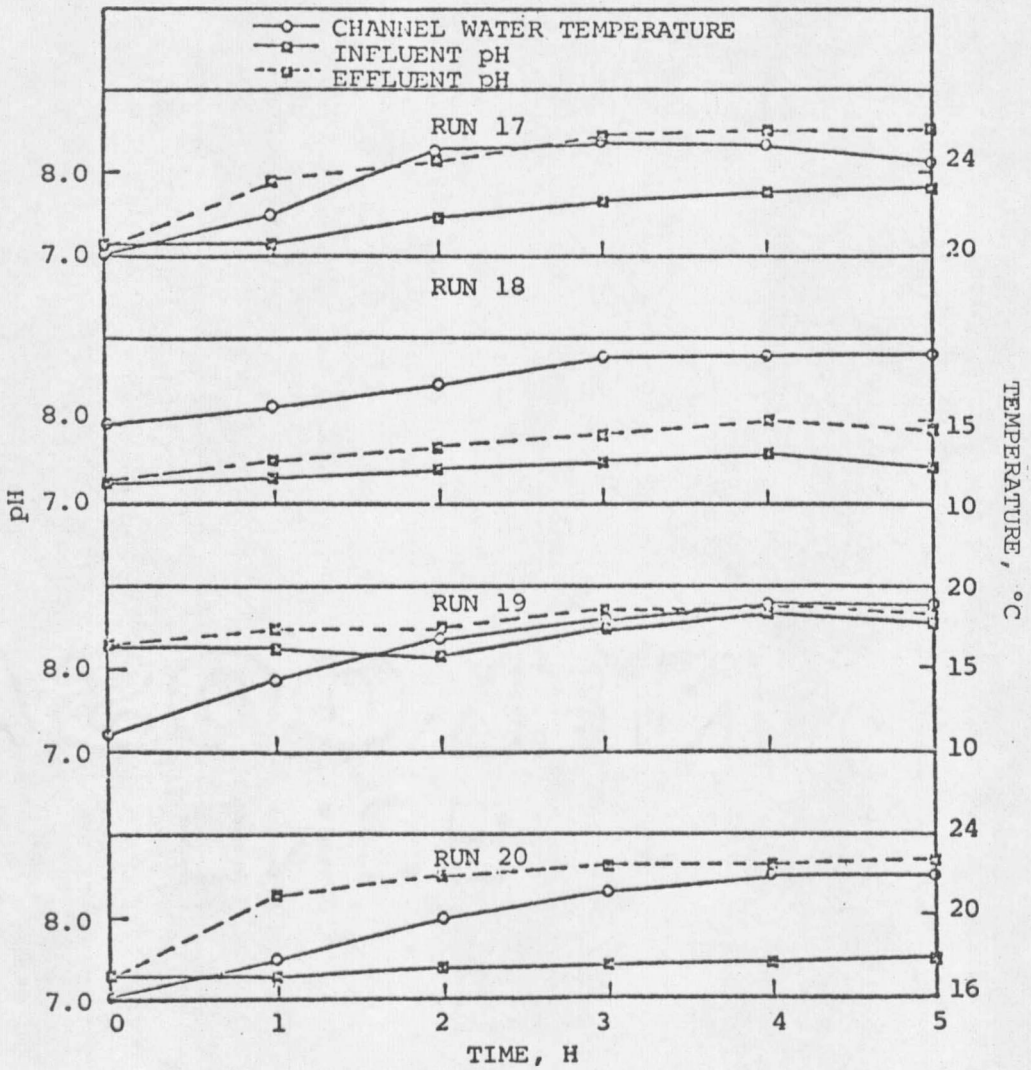


Fig. 25. Plots of pH and Temperature with Time for Runs 17 to 20.

highest in the tapwater run. The pH increase could be attributed to photosynthetic activity. Measurements of DO and pH at three different locations in the test length also indicated complete mixed conditions which can be expected because of the high velocity maintained in the channel.

Determination of and Relationships for R_B, R_D and Y_A

Determination of R_B, R_D and Y_A. The calculations involved in determining R_B, R_D and Y_A are given in this section. The equations developed under the theoretical considerations are given below:

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

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

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

The notation has already been defined. It has been preferred in these set of equations to use biofilm yield coefficient in the substrate material balance. It may be reiterated that S used for substrate in this equation will be measured as soluble organic carbon (SOC) and X will be volatile solids (VSS). Table 4 summarizes the values for the 3 parameters determined by solving the simultaneous algebraic equations at steady state. The derivatives given in equations 22 to 24 are much smaller compared to the total changes in the system for substrate, suspended and attached biomass. The steady state conditions are therefore justifiable. The mean values reported in the table are based on the measurements taken during the last 2 to 3 hours of a run. The variation of S and X for influent and effluent are

Table 4. Determination of R_B, R_D and Y_A Under Steady State Conditions.

$$R_B = R_D = \frac{F}{A}(X-X_i) \quad (26)$$

$$Y_A = \frac{(X-X_i)}{(S_i-S)} \quad (27)$$

Run	Average Channel Water Temperature (T) °C	Mean SOC S_i mg/ℓ	S mg/ℓ	Mean VSS X_i mg/ℓ	X mg/ℓ	Feed Rate F m^3/h	Bed Area A m^2	R_B g/m^2-h	R_D g/m^2-h	Y_A g.VSS/g.SOC
2	18.0	9.61	7.81	9.26	10.18	0.065	0.80	0.076	0.076	0.51
4	23.0	19.89	14.80	21.99	24.67	0.065	0.80	0.217	0.217	0.53
8	18.8	+	+	11.50	14.70	0.065	0.80	0.259	0.259	+
9	18.9	9.27	6.67	11.70	12.66	0.065	0.80	0.078	0.078	0.37
10	25.2	13.96	8.09	12.84	15.25	0.065	0.80	0.195	0.195	0.41
11	22.5	9.44	8.95	9.96	9.34	0.065	0.80	*	*	*
12	20.3	8.39	6.67	13.82	14.65	0.065	0.80	0.067	0.067	0.48
13	22.5	20.45	13.99	10.35	13.41	0.065	0.80	0.248	0.248	0.47
14	26.3	14.30	6.60	13.25	15.42	0.065	0.80	0.176	0.176	0.28
15	26.8	11.14	5.77	14.77	16.66	0.065	0.80	0.153	0.153	0.35
16	26.3	11.10	6.69	11.67	13.24	0.065	0.80	0.127	0.127	0.36
17	25.2	8.67	5.89	11.34	12.57	0.065	0.80	0.099	0.099	0.44
18	19.0	16.60	16.41	23.60	23.88	0.065	0.80	*	*	*
19	18.8	5.35	5.32	8.58	8.48	0.065	0.80	*	*	*
20	22.0	**	**	0.00	3.04	0.065	0.80	**	**	**

*Control Runs
**Tap Water Run
+ OC samples were inadvertently thrown out before completion of the analysis.

much smaller during the last two to three hours compared to the total changes in the system. The biofilm used in the study was grown in the river for at least 30 days to ensure steady state growth conditions. The variation of biomass during the whole study period is given subsequently in Table 5 with a mean of 35.75 g/m^2 and a Standard Error (S.E) of 1.59 g/m^2. Assumptions of steady state are therefore considered justifiable in this study.

Relationships of Y_A with S and $(S_i - S)$. The relationships of the calculated yield coefficients with the reactor substrate concentrations measured as SOC and the substrate removed indicated no significant correlation as shown in Fig. 26. The mean Y_A was determined as 0.42 with a Standard Error of 0.02. The Y_A vs (S_i-S) plot could also be interpreted as showing very little oxygen limitation as otherwise Y_A would have decreased with increasing (S_i-S). An effort was made to compare these calculated steady state yield coefficients with measured yield coefficients which were determined using the formula,

$$Y_A = \frac{(M_t - M_o) + F\int_o^t (X - X_i)dt}{F\int_o^t (S_i - S)dt} \qquad (28)$$

Where M_t = biomass after time t, M_o = biomass at t = 0 and the other symbols have already been defined. In showing this comparison, it is important to make a clear distinction between the methods used in the determination of the calculated and measured yield coefficients. Calculated Y_A values, as already shown previously, were determined using steady state values in $Y_A = (X - X_i)/(S_i - S)$. Measured Y_A values were determined using equation (28) where M_o and M_t were measured and the integrals were evaluated from the plots for S and X for the respective runs from t = 0 to 5 hours. The measured Y_A values were cor-

Table 5. Biofilm Growth on Plexiglass Plates--Biomass Measurements.

Run	Days of Exposure	Biomass (g/m^2)
2	33	35.93
4	37	41.67
9	49	30.49
10	50	41.66
12	52	30.78
13	55	43.94
14	56	32.53
15	58	36.59
16	62	32.11
17	63	31.86
	Mean	35.75
	S.E.	1.59

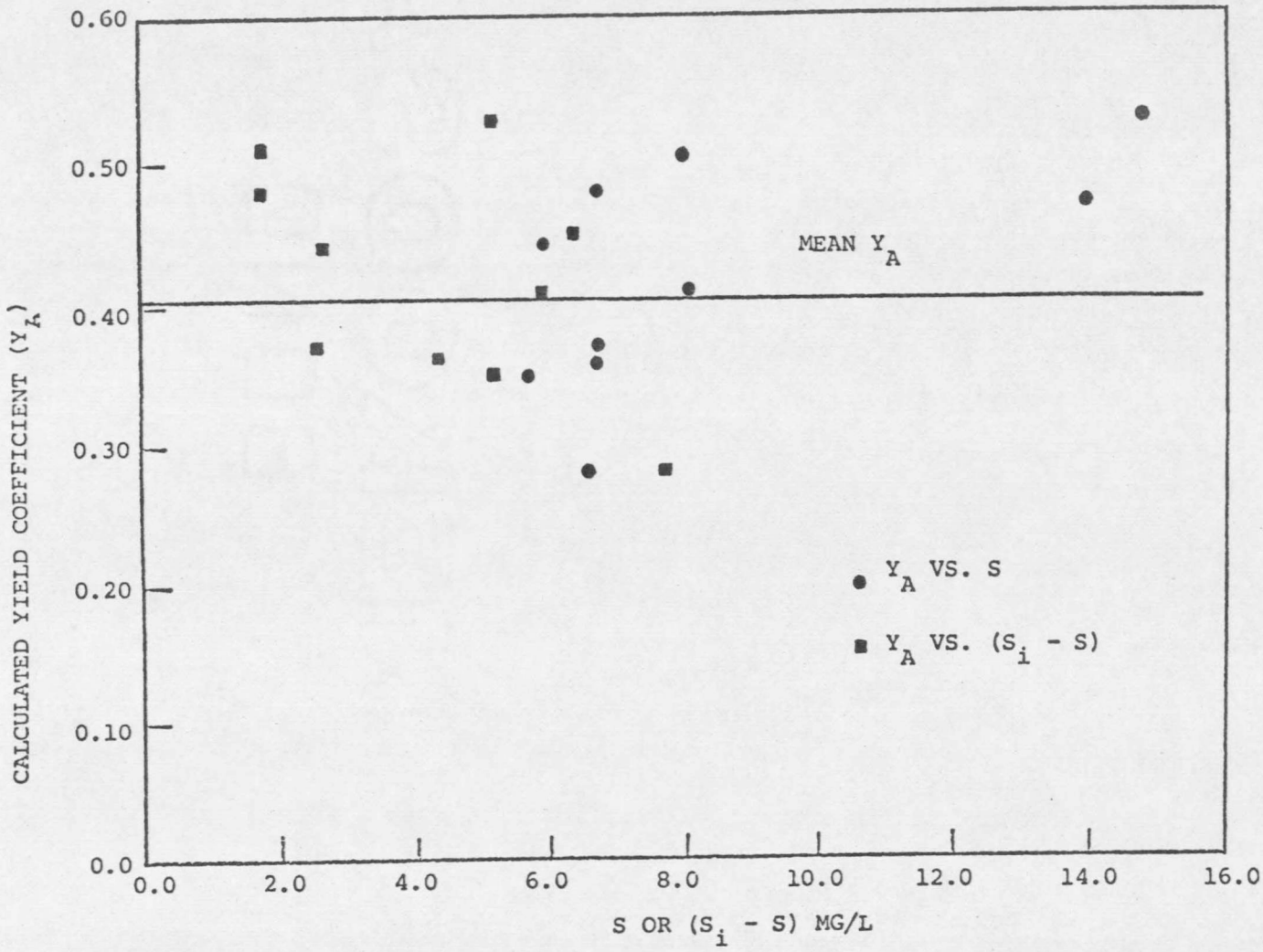


Fig. 26. Variation of Yield Coefficient with Organic Carbon Removed and Reactor Organic Carbon Concentrations.

rected for the contribution to organic accrual by autotrophs using two different methods. The objective of the two methods described below is to determine this correction factor (CF).

Method 1: A method similar to this has been used by Weber and McFarland (135). The correction factor to account for organic accrual by autotrophs

$$CF = \frac{67}{AI} \tag{29}$$

in which

$$AI = \text{Autotrophic Index}$$

$$= \frac{\text{Organic Biomass } (g/m^2) \text{ as Ash free weight}}{\text{Chlorophyll a } (g/m^2)}$$

Weber (136) employed and used the AI to indicate changes in the ratio of autotrophic to heterotrophic community structures. An AI of 67 has been used as an average value for pure algal cultures.

Method 2: This method is based on conversion factors found in the literature. The ratio of Chl. a to cellular organic carbon is first used to estimate algal biomass as organic carbon. Similarly converting biomass measured as Ash free weight (AFW) to cellular organic carbon,

$$C.F = \frac{\text{Algal biomass as organic carbon}}{\text{Total biomass measured as AFW converted to organic carbon}} \tag{30}$$

Even though published conversion factors for mg cellular carbon/mg Chlorophyll a vary from 25 to 100 (21,42), the commonly used ratios range from 30 to 60 (21,42,140). In this method the range of 30 to 60 will be considered and the ratio that yields compatible

results with Method 1 will be used in the analysis. Researchers have also estimated Aufwuchs organic carbon by taking half the value of AFW.

It can be readily seen that there are problems in choosing conversion factors in these methods because of variations during seasons and changes in community structures. However, for the purposes intended here, it is sufficient to use the conversion factors available in the literature. The mean equilibrium biomass grown on plexiglass plates during the experimental study period is 35.75 g/m^2 with a Standard Error (S.E) of 1.59 g/m^2 as shown in Table 5. Using the mean chlorophyll a of 100.3 mg/m^2 as given in Table 6 and the mean equilibrium biomass of 35.75 g/m^2, the Autotrophic Index can be determined as AI = 356. The biofilm growth study done on glass slides at Site 2 as shown subsequently under the section of river modeling yielded an average biomass of 31.55 g/m^2 and a chlorophyll a of 95 mg/m^2. This shows the close relationship of plexiglass and glass slide growths.

Table 6. Chlorophyll a Measurements on Plexiglass Plate Growths.

Run	Days	Chlorophyll a (mg/m^2)	Comment
9	49	96.4	Shaded area of channel
9	49	84.0	Unshaded area of channel
16	62	112.8	Shaded area of channel
16	62	108.0	Unshaded area of channel
	Mean	100.3	
	S.E.	6.4	

During some experimental runs, on days with clear skies, about one third of the plexiglass plate on the channel bed was shaded by the channel sides. The chlorophyll measurements in Table 6 are not sufficient to make any definitive statement about the difference in shaded and unshaded areas and attribute them to the shade. Based on the measurements and conversion factors given, the correction factors can be determined as follows:

$$\text{Biomass} = 35.75\ g/m^2, \text{Chl. a} = 100.3\ g/m^2, \text{AI} = 356$$

Method 1: $$\text{C.F.} = \frac{67}{\text{AI}} \times 100 \quad \text{using Eq. 29.}$$

$$= 18.8\%$$

Method 2: Algal biomass as cellular carbon = 100.3×30 to $100.3 \times 60\ mg/m^2$

$$= 3009 \text{ to } 6018\ mg/m^2$$

$$\text{C.F.} = \frac{3009 \times 100}{0.5 \times 35.75 \times 1000} \text{ to } \frac{6018 \times 100}{0.5 \times 35.75 \times 1000} \quad \text{using Eq. 30}$$

$$= 16.8\% \text{ to } 33.6\%$$

Considering both methods and the values obtained the correction factor to account for the autotrophic contribution to organic accrual is nearly 18%. The measured yield coefficients calculated from Eq. 2 and corrected for the autotrophic contribution to organic accrual are tabulated in Table 7. The comparison and good agreement of measured Y_A given in the third column of Table 7 and the calculated Y_A is shown in Fig. 27. It must be recognized however, that after correcting for autotrophic contribution to organic accrual, the balance biomass would include the gelatinous material in the film in addition to the heterotrophs.

Table 7. Measured Yield Coefficients.

Run	† Measured Y_A $\frac{g \cdot VSS}{g \cdot SOC}$	†† Measured Y_A Corrected for Autotrophs $\frac{g \cdot VSS}{g.SOC}$	* Calculated Y_A $\frac{g.VSS}{g.SOC}$
(1)	(2)	(3)	(4)
10	0.38	0.31	0.41
12	0.69	0.57	0.48
13	0.70	0.57	0.47
14	0.49	0.40	0.28
15	0.54	0.44	0.35
16	0.60	0.49	0.36
17	0.54	0.44	0.44
Mean	0.56	0.46	0.40
S.D.	0.11	0.09	0.07
S.E.	0.04	0.03	0.02

† Determined from $Y_A = \dfrac{(M_t - M_o) + F \int_o^t (X - X_i)dt}{F \int_o^t (S_i - S)dt}$

†† Column 3 = Column 2 x 0.82 based on the correction factor = 18%

* From Table 4

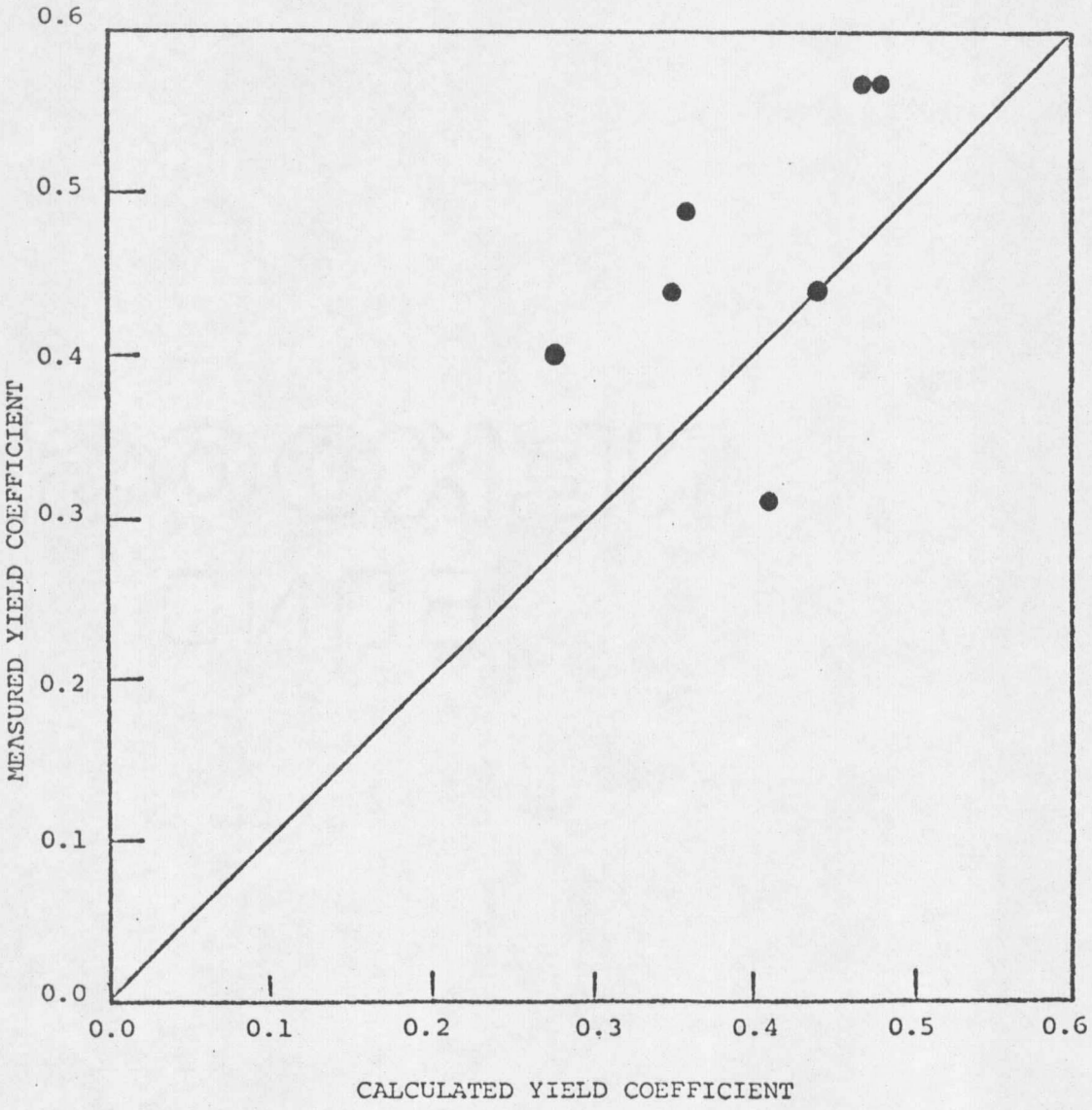


Fig. 27. A Plot of Measured and Calculated Yield Coefficients.

Temperature Relationships for R_B

Temperature effects on biological reactions have been discussed in the literature review. Temperature correction coefficients θ of the form $k_T = k_{20}\theta^{T-20}$ have been used where k_T and k_{20} are reaction rates at T°C and 20°C. The value of θ has been found to vary from 1.056 to 1.135 in different temperature ranges and a common value of θ = 1.047 has been widely used in biological reactions (85). Instead of using values from the literature, an effort was made to determine θ from data available in this study.

Let
$$R_B = R_{B_{20}}\,\theta^{T-20} \tag{31}$$

$$[\ln R_B] = \ln\theta\,[T - 20] + \ln R_{B_{20}} \tag{32}$$

The plot for this equation is shown in Fig. 28. Two outliers in the plot have been excluded because of a much lower correlation coefficient. From the computer output of the 'MREGRESS' subroutine of the statistical programs available in the computer library at Montana State University, the regression statistics are summarized in Table 8. The results of the t-test indicates that a significant correlation exists at a level of significance of 0.01. The correlation coefficient is 0.84. From the slope given in the fit, θ = 1.098. R_D values can also be corrected for temperature using the same relationship since $R_B = R_D$ under steady state conditions.

Table 8. Regression Statistics in the Determination of θ.

Slope	Std. Error (S.E.) of Slope	Intercept	Correlation Coefficient (R)	T-value	p-value †
0.094	0.025	- 2.50	0.84	3.75	0.0096

† Descriptive level of significance.

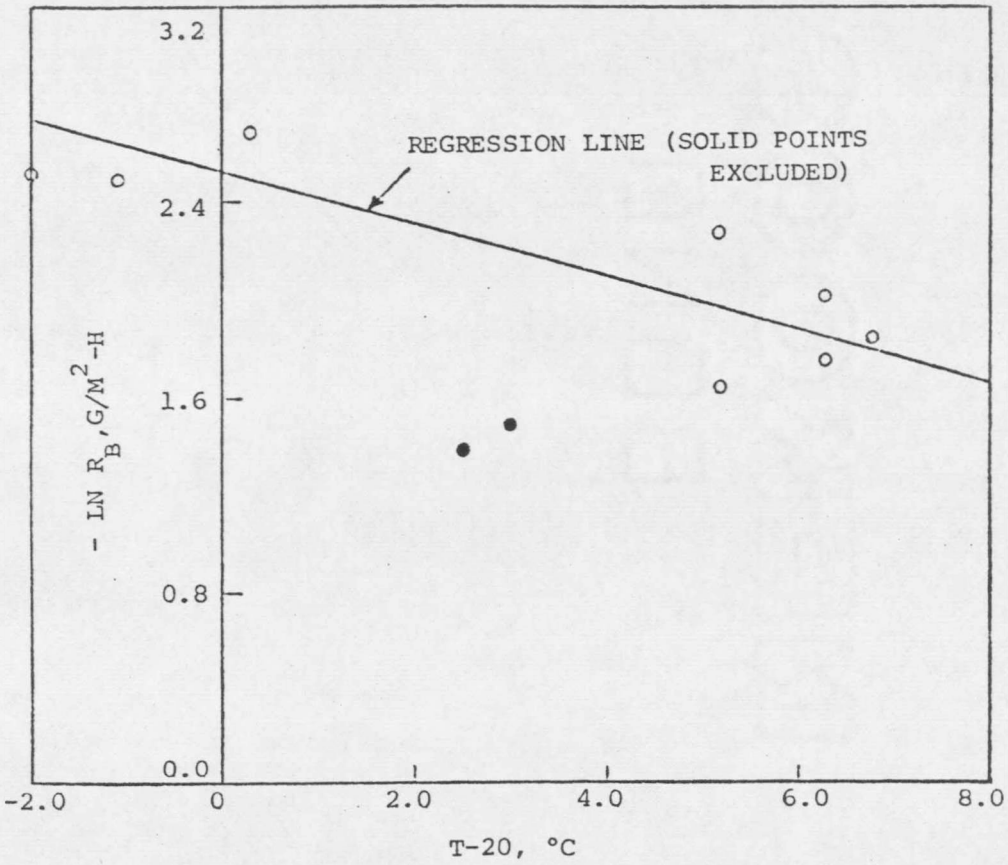


Fig. 28. Plot of ln R_B vs. Temperature.

Determination of Relationships Between R_B and S

Relationships between R_B values, uncorrected and corrected to 20°C and substrate measured as soluble Organic Carbon (S) are developed using statistical methods and shown in Table 9.

The results in Table 9 indicate that in developing a relationship between R_B and S, the uncorrected R_B values are significant at a level of significance of 0.02 while the

Table 9. Regression Statistics for R_B vs. S.

Run	T °C	R_B g/m^2-h	R_{B20} g/m^2-h	S g/m^3
2	18.0	0.076	0.092	7.81
4	23.0	0.217	0.164	14.80
9	18.9	0.078	0.086	6.67
10	25.2	0.195	0.120	8.09
12	20.3	0.067	0.065	6.67
13	22.5	0.248	0.196	13.99
14	26.3	0.176	0.098	6.60
15	26.8	0.153	0.081	5.77
16	26.3	0.127	0.070	6.69
17	25.2	0.099	0.061	5.89

Regression	Slope	SE of Slope	Intercept	Correlation Coefficient (R)	T-value	†p-value
R_{B20} vs S	0.0126	0.0017	-0.0014	0.93	7.396	0.00
R_B vs S	0.014	0.0047	0.027	0.72	2.97	0.018

† Descriptive level of significance.

temperature corrected R_{B20} values are significant at 0.01. The correlation coefficient increases from 0.72 to 0.93 on correcting for temperature. The best fit for the data can be given as:

$$R_{B20} = 0.0126\,S - 0.0014 \qquad (33)$$

Since the intercept is small compared to 0.0126S, Eq. (33) can be fitted with a zero intercept and tested for its significance as shown in Table 10. Table 10 shows that the intercept given in Eq. (33) is insignificant which provides a statistical justification for a zero intercept.

With a zero intercept, the relationship becomes,

$$R_{B20} = 0.0126S \qquad (34)$$

A plot of this equation is shown in Fig. 29. With a constant steady state yield coefficient, this suggests first-order kinetics for the substrate (Soluble Organic Carbon) uptake by biofilms.

Table 10. Testing the Significance of Intercept in R_{B20} vs. S.

Intercept	SE of Intercept	T - Value	†p - value
- 0.0014	0.015	- 0.094	.928

† Descriptive level of significance

Detachment Rate-Biomass Relationships

It is reasonable to assume that increase in biomass for biofilms would increase detachment rates. Therefore, it is justifiable to formulate R_D as a function of biomass in the fol-

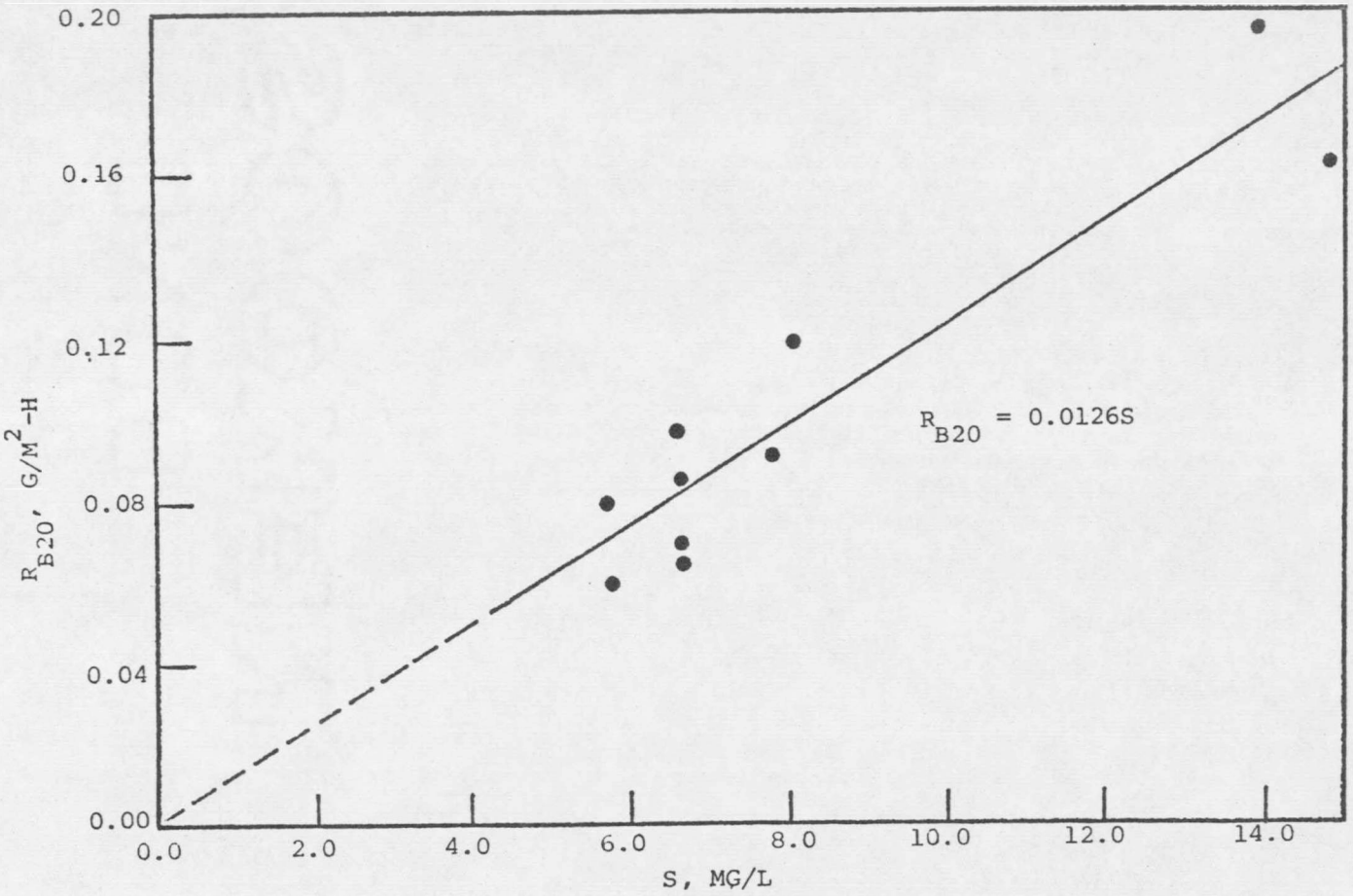


Fig. 29. Variation in Attached Biomass Production Rate with Reactor Soluble Organic Carbon Concentrations.

lowing manner:

$$R_D = \alpha_D M^{\beta} \tag{35}$$

in which the symbols have already been defined under theoretical considerations. In this section, scour coefficients α_D and β will be evaluated for the system.

Eq. (35) can be linearized as follows:

$$\ln R_D = \beta \ln M + \ln \alpha_D \tag{36}$$

Table 11 summarizes the linear regression statistics for Eq. (36).

The relationship given in Eq. (35) becomes:

$$R_D = 1.47 \times 10^{-5} M^{2.46} \tag{37}$$

The variation of R_D with M is shown in Fig. 30. A linear plot was also possible for R_D vs M with almost the same correlation coefficient. But the linear form of the equation had an intercept term. Therefore, based on theoretical considerations, Eq. (35) was preferred in establishing the R_D - M relationship. The confidence intervals for all the relevant parameters will be summarized in the subsequent section at the end of the results on channel study.

Other Possible Relationships

Specific biomass production rate vs. substrate concentrations. If R_B is converted into a specific biomass production rate, $r_s = R_B/M_A$, Fig. 31 shows the variation of r_s with S. In this ratio, R_B values corrected to 20°C and biomass corrected for the autotrophs (M_A) were used. The implications of this curve in relation to saturation kinetic expressions will be discussed subsequently under discussion.

Table 11. Relationships of R_D and M with Statistical Analysis.

Run	†R_D g/m^2-h	M g/m^2	ln R_D	ln M
2	0.092	35.93	- 2.39	3.58
4	0.164	41.67	- 1.81	3.73
9	0.086	30.49	- 2.45	3.42
10	0.120	41.66	- 2.12	3.73
12	0.065	30.78	- 2.73	3.43
13	0.196	43.94	- 1.63	3.78
14	0.098	32.53	- 2.32	3.48
15	0.081	36.59	- 2.51	3.60
16	0.070	32.11	- 2.66	3.47
17	0.061	31.86	- 2.80	3.46

Regression:

Slope	SE of Slope	Intercept	SE of Inter-cept	Correlation Coefficient (R)	T-value Slope	T-value Inter-cept	p-value Slope	p-value Inter-cept
2.46	0.48	-11.13	1.73	0.87	5.06	-6.42	0.0	0.0

† Scour coefficients α_D and β for the system are evaluated from the best possible correlation between R_D and M. For this purpose, R_D values used are corrected to 20°C which gives a correlation coefficient (R) of 0.87 compared to 0.78 without the correction. Both relationships were significant at a level of significance of 0.01.

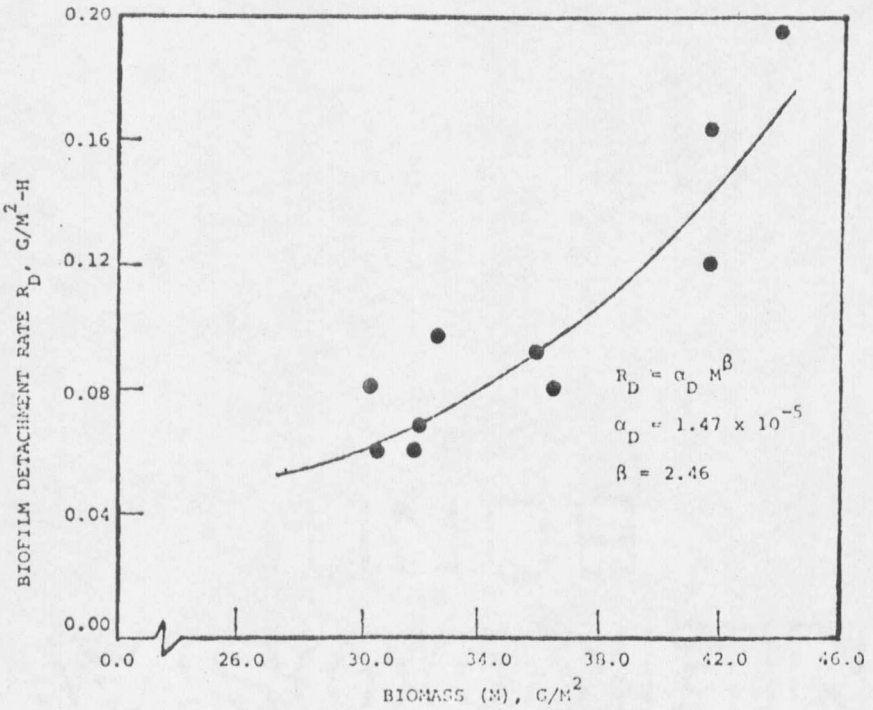


Fig. 30. Biofilm Detachment Rate Variation with Biomass.

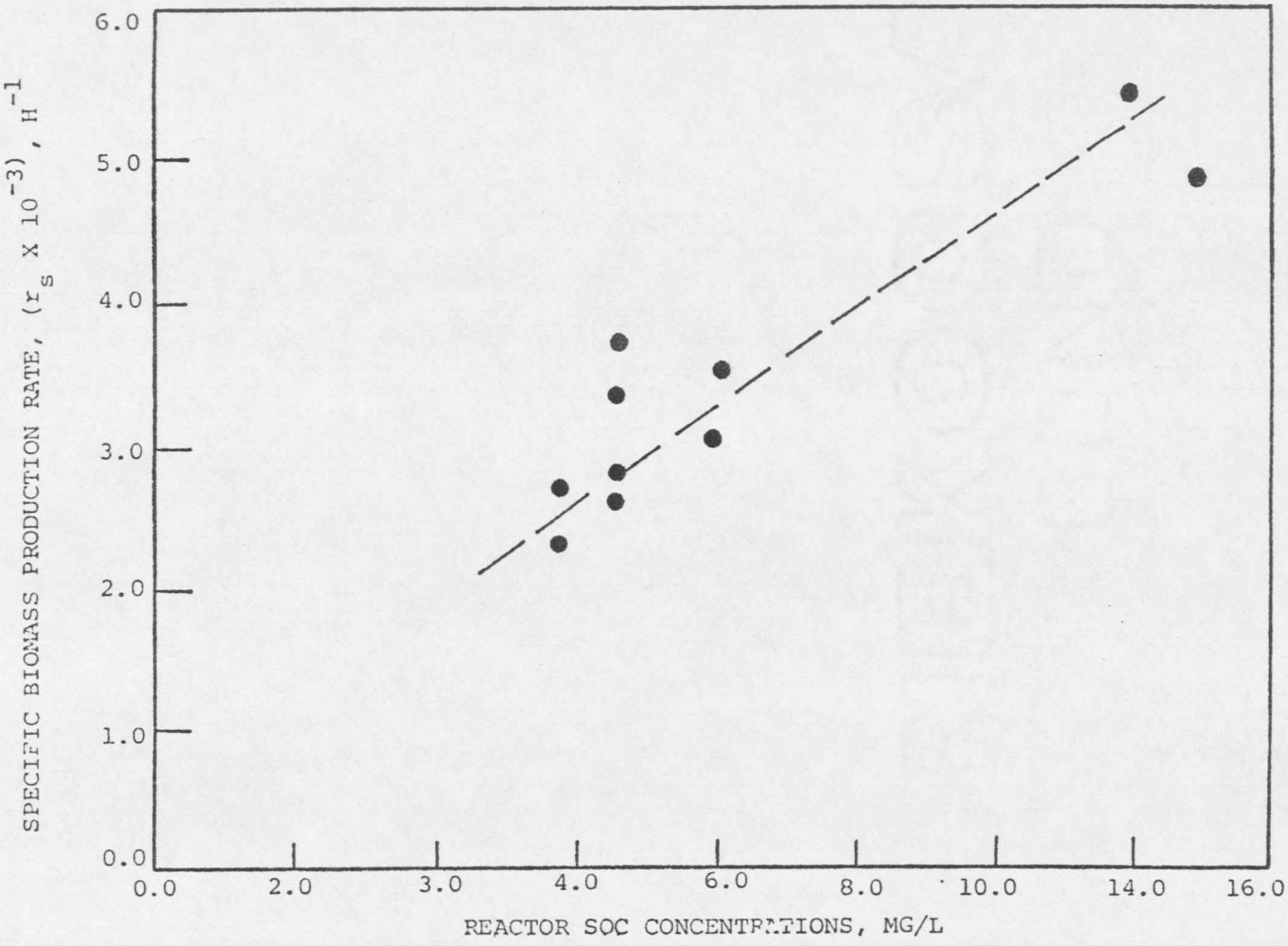


Fig. 31. Variation of Specific Biomass Production Rate with Reactor Organic Carbon Concentrations.

M_A values were obtained by multiplying values of M given in Table 5 by 0.82 based on the correction factor developed earlier.

Soluble organic carbon removal and loading rate. The values of soluble organic carbon loading rates (R_L) calculated according to, $R_L = F\ S_i/A$ are tabulated in Table 12 in which F = 0.0648 m^3/h and A = 0.8 m^2.

Fig. 32 shows a large increase in SOC removal in the range of R_L from 0.7 to 1.0 g/m^2-h and possible stabilization beyond 1.0 g/m^2-h. Increasing the substrate concentra-

Table 12. Calculation of Soluble Organic Carbon Loading Rates.

Run	S_i mg/ℓ	R_L g/m^2-h
2	9.61	0.78
4	19.89	1.61
9	9.27	0.75
10	13.96	1.13
12	8.39	0.68
13	20.45	1.66
14	14.30	1.16
15	11.14	0.90
16	11.10	0.89
17	8.67	0.70

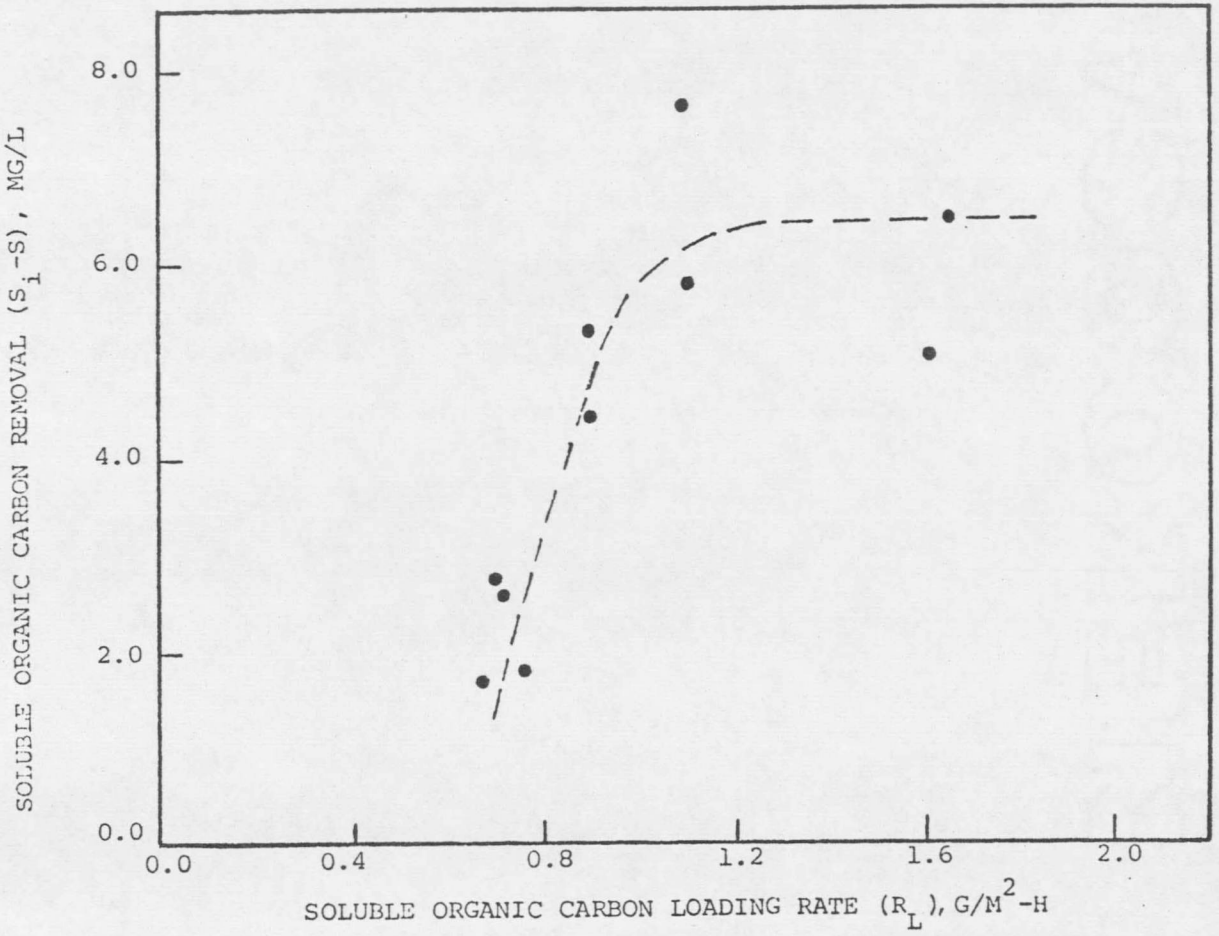


Fig. 32. Plot of Soluble Organic Carbon Removal vs. Loading Rate.

tion would enhance substrate penetration into the film thus increasing the metabolic activity. This may increase the substrate removal rate. However, with further increases in substrate concentrations, the changes of oxygen limitations in the bottom of the film are also increased. The balance between the increased metabolic activity of the film and anaerobic product formation would tend to stabilize the substrate removal rate.

Summary Statistics for Important Parameters Developed

Relationships of R_B and S. A statistically significant relationship was developed as given below:

$$R_{B20} = k_F \cdot S \tag{34}$$

where k_F = 0.0126 m/h, S.E. (k_F) = 0.0017 from Table 9.

The upper and lower limits of 95% confidence level for k_F are 0.0164 and 0.0088 m/h. The intercept obtained for Eq. (33) was found to be statistically insignificant as shown in Table 10. The effect of the variation of k_F on the model for substrate decay will be studied under sensitivity analysis.

Relationship of R_D and M. A detachment rate-biomass relationship was developed as given below:

$$R_D = \alpha_D M^{\beta} \tag{37}$$

in which $\alpha_D = 1.47 \times 10^{-5}$, $\beta = 2.46$. From Table 11, S.E. (β) = 0.48. The upper and lower limits at 95% confidence level for β are 3.54 and 1.38 which become 3.12 and 1.80 respectively at 80% confidence level. S.E. ($\ln \alpha_D$) = 1.733. The 95% confidence intervals for α_D =

7.39×10^{-4} and 2.91×10^{-7} which become 15.9×10^{-5} and 0.13×10^{-5} respectively at 80% confidence level.

The method of obtaining confidence intervals for a parameter like α_D in a non-linear expression from a linearized plot was adapted from Sokal and Rohlf (117). The 95% confidence bands for α_D and β appear to be wide and the use of lesser confidence levels have also been shown.

Biofilm yield coefficient. Mean steady state biofilm yield coefficient (Y_A) was determined to be 0.42 mg.VSS/mg.SOC. With a S.E. (Y_A) = 0.02, 95% confidence band for Y_A is 0.38 to 0.46.

Temperature correction coefficient (θ). An expression of the form $R_B = R_{B20} \theta^{T-20}$ was developed as given in Eq. (31) where $\theta = 1.098$. S.E. ($\ln \theta$) = 0.025. The upper and lower limits at 95% confidence level for θ are 1.165 and 1.035 respectively.

Field Investigations in the River

River Data Collection and Modeling

On the basis of the preliminary study results given earlier, data collection during the summer and fall of 1980 and 1981 were restricted to about 7 miles (11.27 km) below the sewage outfall, which is here-in-after referred to as the study reach, as the water quality parameters returned to the background levels within this distance. Data collection of hydraulic, water quality and biofilm parameters during 1980 and 1981 will be presented together in the subsequent sections.

Hydraulic and physical parameters in the study reach. Stream flow measurements were made at the selected sites in the study reach on several occasions. The determination of steady state values for hydraulic parameters are shown in Tables 13 and 14 which indicates mean stream flows of 72.4 cfs and 80.3 cfs for 1980 and 1981 respectively. Measured spring high flows in 1980 which are not included in this report indicated a range of 300 to 600 cfs in the study reach compared to the low summer flows given in Tables 13 and 14. Water widths tabulated in Tables 13 and 14 indicate a variation of 38 to 61 ft. More water width measurements were made than the stream flow because they were quicker. Tables 13 and 14 give average steady state flow velocities of 2.0 ft/s and 2.08 ft/s at Site 2 during 1980 and 1981 respectively. The relevance of all this information in developing model input data for the hydraulic parameters will be shown subsequently. Summary of steady state values for hydraulic parameters for 1980 and 1981 are given in Table 15.

In the short study reach, average steady state values for the flow velocity and depth have so far been established based on such measurements made at selected sites. A procedure developed using the mean steady state stream flow in the study reach to estimate the average depth and velocity for the whole stretch is shown below:

$$\text{Estimated Mean Flow depth in the study reach (H)} = \frac{Q \cdot t_f}{A_p} \qquad (40)$$

where

Q = steady state stream flow

t_f = time of flow = L/U_2

L = length of study reach

Table 13. Hydraulic Measurements, 1980.

Date	Site	Streamflow cfs	† Flow Velocity ft/s	† Flow Depth ft	Cross-Sect. area of flow sq.ft	Water width ft
7/10/80	2	154.5	2.30	1.01	58.4	48.0
7/10/80	5	139.0	2.81	0.89	45.9	56.0
7/18/80	2	122.5	2.20	0.90	42.2	50.0
7/18/80	4	105.7	1.95	0.89	47.0	60.0
7/18/80	6	100.5	1.20	1.35	59.7	45.0
8/01/80	2	90.8	2.01	0.80	43.4	46.0
8/01/80	4	82.9	1.70	0.70	45.5	64.4
8/01/80	5	-	-	-	-	42.0
8/01/80	6	74.2	*1.03	*1.21	*54.5	45.0
8/08/80	2	65.3	2.05	0.69	26.2	42.0
8/08/80	4	-	-	-	-	58.0
8/08/80	5	50.8	1.69	0.70	30.7	44.0
8/08/80	6	50.0	*0.86	*1.28	*48.4	41.0
8/19/80	2	88.7	2.00	0.72	34.5	46.0
8/19/80	4	79.5	1.79	0.74	40.8	58.5
8/19/80	5	-	-	-	-	42.0
8/19/80	6	72.9	*1.03	*1.36	*60.8	44.0
8/29/80	2	76.3	1.95	0.71	32.5	47.0
8/29/80	4	74.6	1.61	0.71	41.0	59.0
8/29/80	5	60.0	1.70	0.76	33.1	40.0
8/29/80	6	-	-	-	-	44.0
10/20/80	2	75.3	1.98	0.68	33.7	46.0
‡Mean		72.4	1.85	0.72	36.14	47.6
S.E.		3.70	0.05	0.009	1.96	1.80

† Each flow velocity and depth is an average of 15 to 20 measurements across the respective sections.

‡ Only measurements below the dotted line are used in this determination of steady state values for parameters.

* Site 6 measurements were not considered in determining the mean, to be consistent with the mean determined for 1981. Besides Site 6 measurements for average depth and velocity do not reflect those of a shallow turbulent stream as evident from personal observation and measurements at other sites in the study reach.

Table 14. Hydraulic Measurements, 1981.

Date	Site	Stream flow cfs	†Flow Velocity ft/s	†Flow depth ft	Cross-Sectional area of flow sq. ft	Waterwidth ft
7/23/81	2	124.7	2.54	1.18	45.2	39.6
7/31/81	2	120.0	2.37	1.15	43.6	39.8
7/31/81	4	115.5	2.15	0.77	43.2	61.0
7/31/81	5	96.6	1.84	1.00	44.8	45.0
8/07/81	2	91.8	2.26	1.08	40.4	38.0
8/07/81	3	-	-	-	-	45.0
8/07/81	4	-	-	-	-	60.0
8/07/81	5	81.1	1.89	0.86	36.4	45.0
8/07/81	6	-	-	-	-	43.0
8/14/81	2	84.0	2.01	0.99	36.5	38.0
8/14/81	3	-	-	-	-	45.0
8/14/81	4	86.0	1.71	0.83	39.8	60.0
8/14/81	5	66.4	1.76	0.72	33.1	46.0
8/14/81	6	-	-	-	-	44.0
8/20/81	2	88.6	2.06	1.04	38.6	38.0
8/20/81	3	-	-	-	-	45.0
8/20/81	4	87.3	1.71	0.81	39.4	60.0
8/20/81	5	76.9	1.78	0.73	33.2	48.0
8/20/81	6	-	-	-	-	44.0
8/28/81	2	78.9	2.01	1.00	36.3	38.0
8/28/81	3	-	-	-	-	44.0
8/28/81	4	-	-	-	-	60.0
8/28/81	5	62.0	1.72	0.69	31.7	48.0
8/28/81	6	-	-	-	-	43.0
‡Mean		80.3	1.89	0.88	36.5	46.6
S.E.		3.05	0.06	0.04	0.95	1.70

†Each flow velocity and depth is an average of 15 to 20 measurements across the respective sections.

‡Only measurements below the dotted line are used in this determination of steady state values for parameters.

Table 15. Summary of Measured Steady State Values for Hydraulic Parameters During the Study Period.

Year	Flow Velocity ft/s	Flow Depth ft	Cross-Sectional area of flow sq. ft	Waterwidth ft.	Stream flow cfs
1980	1.85	0.72	36.14	47.6	72.4
1981	1.89	0.88	36.50	46.6	80.3

U_2 = mean flow velocity at Site 2

A_p = estimated plan area of the study reach

Futile attempts were made to measure A_p directly from the largest available areal maps of the study area because of the small river dimension for the width. This problem was overcome by using the average measured water width (W) in the study reach during steady state as given in Tables 13 and 14. Then A_p can be estimated by $A_p = (L.W)$.

Using the measured average cross-sectional area of flow (Ac) and steady stream flow in the study reach, mean flow velocity in the study reach (U) can be estimated as,

$$U = Q/Ac \tag{41}$$

Equations (40) and (41) can be used to determine the estimated mean flow depth and velocity in the study reach during the steady state flow period.

Now using 1980 values from Table 13,

$$t_f = L/U_2 = \frac{6.74 \times 5280}{2.0 \times 3600} = 4.94 \text{ hours}$$

$$Q = 72.4 \text{ cfs}$$

$$W = 47.6 \text{ ft}$$

Therefore,

$$H = \frac{Q \cdot t_f}{A_p} = \frac{72.4 \times 4.94 \times 3600}{6.74 \times 5280 \times 47.6}$$

$$H = 0.75 \text{ ft}$$

Also,

$$U = \frac{72.4}{36.14} = 2.00 \text{ ft/s}$$

Similarly using 1981 values from Table 14,

t_f = 4.75 hours

Q = 80.3 cfs

W = 46.6 ft

H = 0.83 ft

U = 2.2 ft/s

The estimated flow depths by this procedure are H = 0.75 ft and 0.83 ft compared to H = 0.72 ft and 0.88 ft as given in Table 15 for 1980 and 1981 respectively. A similar comparison of flow velocities indicate estimated flow velocities of U = 2.0 ft/s and 2.2 ft/s in comparison to U = 1.85 ft/s and 1.89 ft/s. The chief difference between the estimated values for H and U and values given in Table 15 stem from the fact that estimated values were based on steady state stream flow considerations and morphometry in the study reach. The estimated values for H and U calculated by using steady state stream flow conditions would be used in the model input subsequently. The variations for H and U however, can be tested by a sensitivity analysis. Determination of mean water temperatures in the study reach during the sampling period are given in Table 16 for 1980 and 1981.

Table 16. Measured Average Water Temperature in the Study Reach During Sampling Period, 1980 and 1981.

Date 1980	Date 1981	†Average Temperature (°C) 1980	1981
7/10/80	7/23/81	21.4	16.3
7/18/80	7/31/81	17.5	17.3
8/01/80	8/04/81	19.6	19.8
8/08/80	8/07/81	19.3	19.2
8/19/80	8/12/81	18.5	21.5
8/29/80	8/14/81	18.8	19.2
10/20/80	8/18/81	17.8	18.7
-	8/20/81	-	20.7
-	8/25/81	-	18.8
-	8/28/81	-	21.6
‡ Mean		18.8	19.9
S.E.		0.3	0.4

† Each average temperature in the study reach is an average of 6 temperatures measured at the different sites.

‡ Mean Water temperature in the study reach to be used in model testing is an average of 30 invidual temperature measurements taken during sampling periods in 1980 and 48 in 1981.

The steady state values for hydraulic and physical parameters used as input to the model are summarized in Table 17.

Table 17. Summary of Steady State Values for Hydraulic and Physical Parameters in the Study Reach Used as Input to the Model.

Year	Study Length L mile(Km)	Stream Flow Q cfs(m^3/s)	Flow depth H ft(m)	Flow Velocity U ft/s(m/h)	Mean Water temperature T°C
1980	6.74 (10.85)	72.4 (2.05)	0.75 (0.22)	2.00 (2195)	18.8
1981	6.74 (10.85)	80.3 (2.28)	0.83 (0.25)	2.20 (2415)	19.9

Water quality parameters. Several measurements of the water quality parameters were made on different occasions during the study period. TOC measurements were introduced during 1980 and continued during 1981 for purposes of quantifying organic carbon. Figs. 33 and 34 show the relationship between soluble and total organic carbon at various sites. The points corresponding to soluble organic carbon in these figures are slightly displaced for clarity. These figures indicate that during the steady state low flow period, a major proportion of the organic carbon in the river is soluble. Also, the highest particulate carbon concentration exists at Site 2 below the outfall, and a significant reduction of the particulate carbon occurs by Site 3 or 4. Changes in organic carbon are almost complete by Site 5. Compared to the change in SOC occurring between Sites 2 and 3, Figs. 33 and 34 indicate minimal changes between Sites 3 and 4. It is important to recognize that the measured OC values in the overlying water include contributions from the sewage outfall, algal by products and streambed scour. BOD_L follows a decay pattern similar to that of organic carbon as shown in Fig. 35. As observed in the preliminary study, the

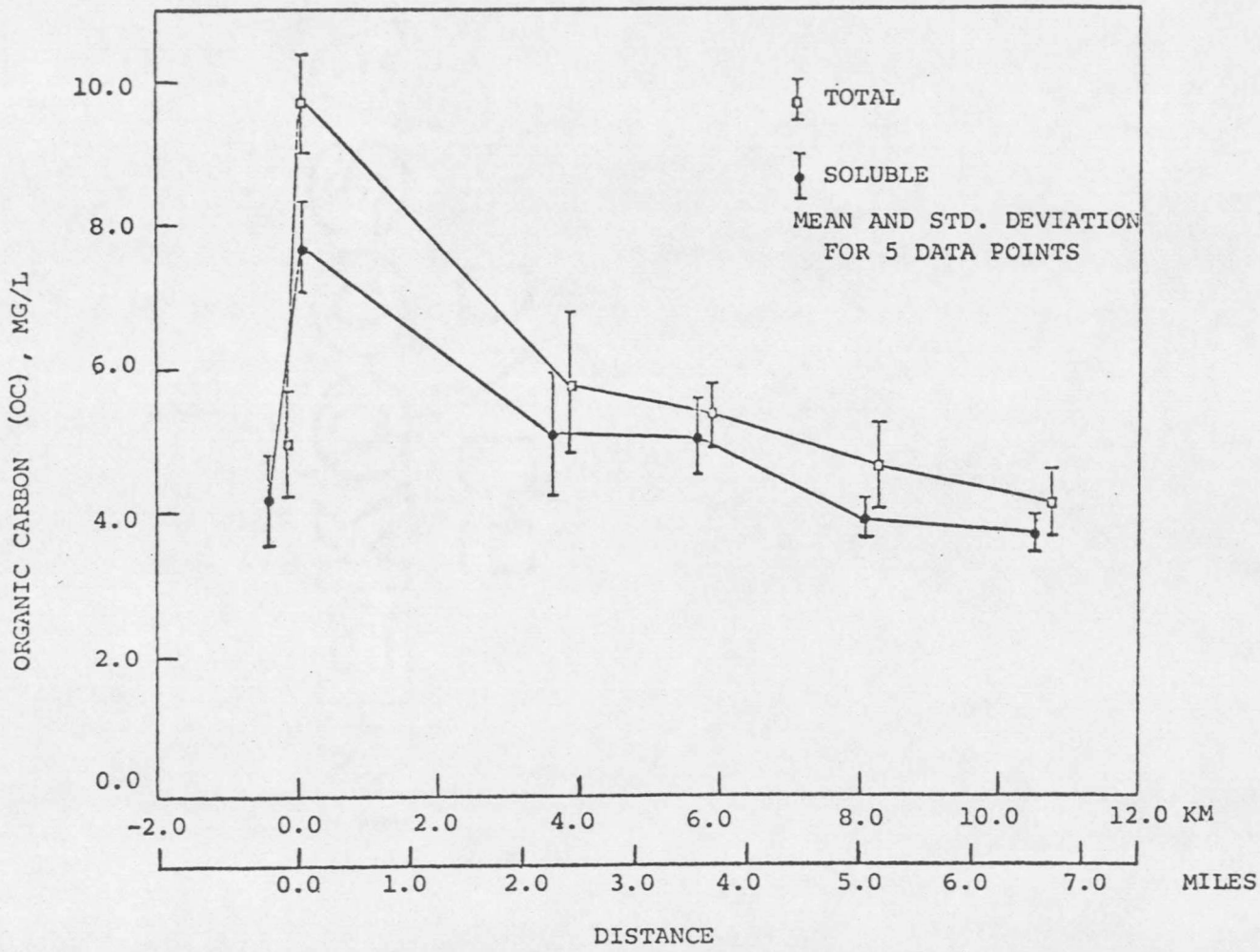


Fig. 33. Distribution of Soluble and Total Organic Carbon Under Steady State Flow Conditions, 1980.

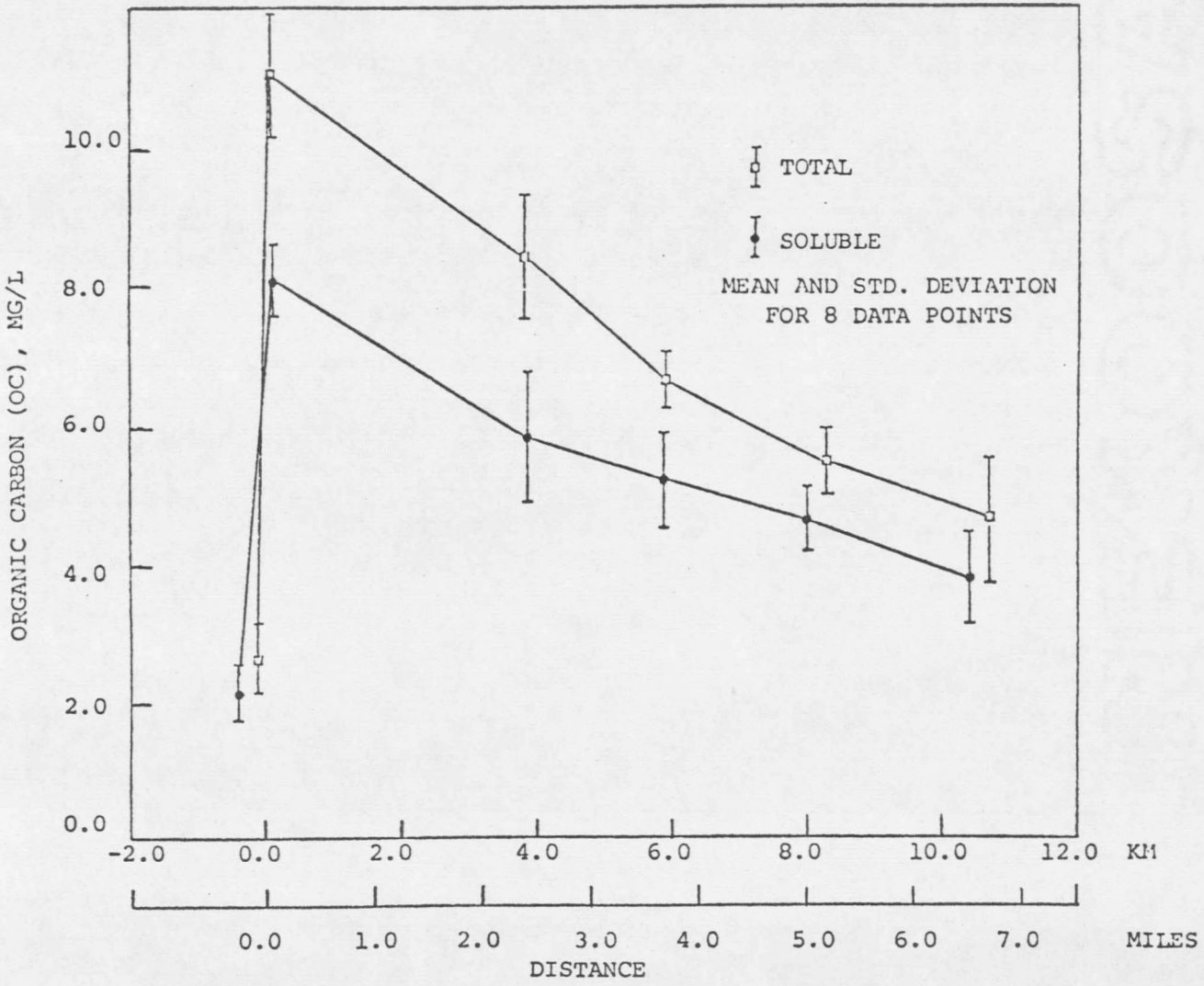


Fig. 34. Distribution of Soluble and Total Organic Carbon Under Steady State Conditions, 1981.

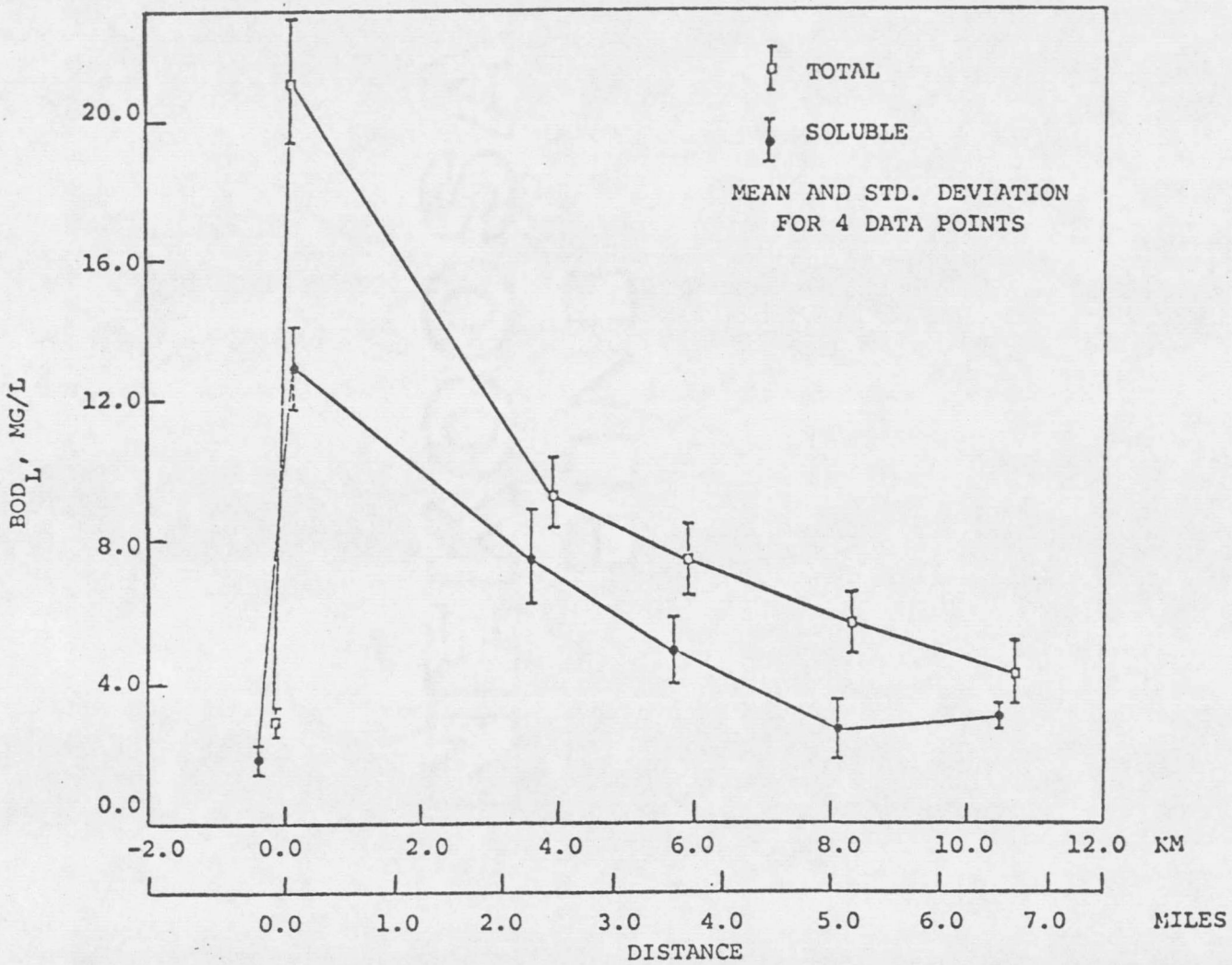


Fig. 35. Distribution of Soluble and Total BOD_L Under Steady State Flow Conditions, 1980.

BOD_L returns to the background concentration within 6 to 7 miles. The soluble BOD_L is nearly 60% of the total at EG 2. The proportion of soluble to total BOD_L subsequently increases due to the reduction in particulates by Site 3. Except for a small drop in DO below the outfall, Figs. 36 and 37 indicate high levels of DO ranging from 9 to 12 mg/l at the different sites during the sampling period. pH variation in the study reach showed a drop in pH at Site 2 possibly caused by the sewage effluent as evident from Figs. 36 and 37. The increased pH levels further downstream could be a result of photosynthetic activity. Generally, an increasing trend can be seen for the solids as shown in Figs. 38 and 39. While the increase at Site 2, which is more for VSS than SS could be attributed to the sewage effluent discharge, downstream increases in SS and VSS can be significantly due to the scouring activity at the stream bed considering a near constant stream discharge and high velocity of flow in the study reach. Nitrogen data collected during 1980 as shown in Table 18 for comparison with the preliminary study results indicate an increase of NO_3-N and a decay of NH_3-N below the sewage outfall. This is in general agreement with the preliminary study results where possible reasons for these changes have already been discussed.

Water quality parameter measurements thus far described were made during specific times of a day on various occasions. An attempt was made to study the changes during a day of some selected parameters. The choice of parameters and the frequency of measurements were based on the relevance of such parameters to the objectives of the study, cost and the ease with which samples can be taken and analyzed. Diurnal variations shown in Fig. 40 for SOC indicate that Site 2 variations follow a pattern similar to the sewage effluent discharge. Significant changes in sewage outfall discharge and SOC at Site 2 seem

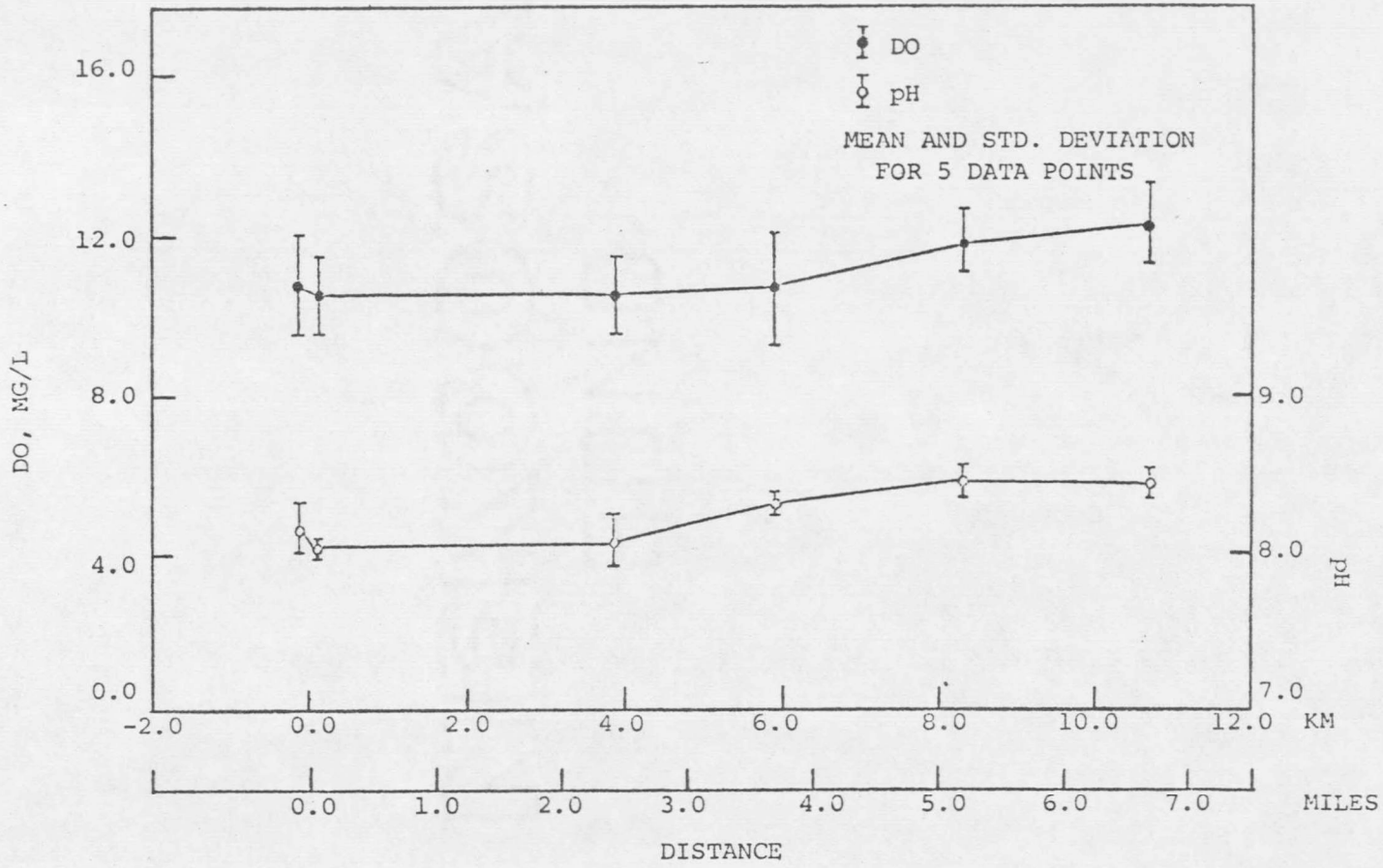


Fig. 36. Plots of Steady State DO and pH at the Chosen Sites in the River, 1980.

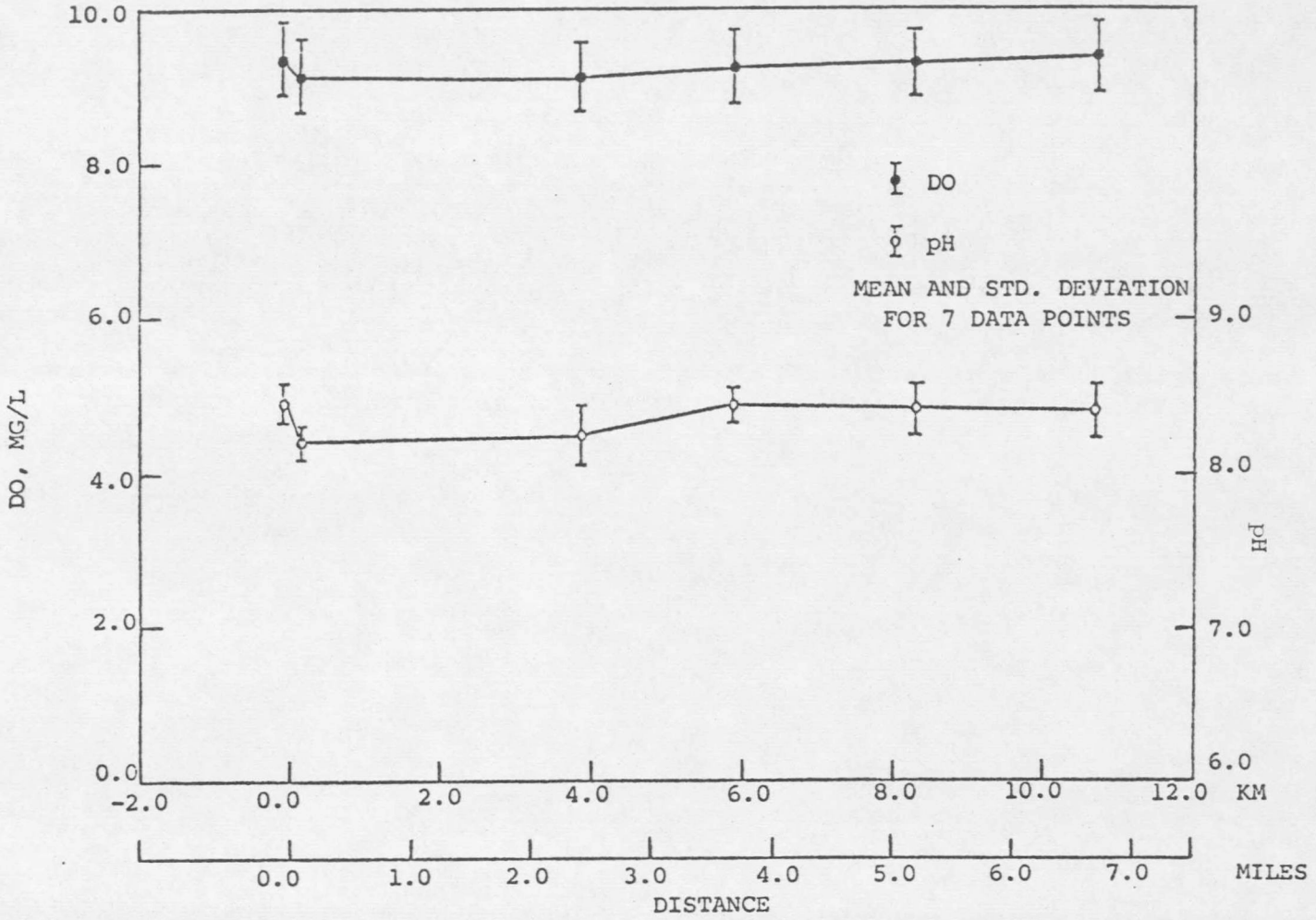


Fig. 37. Plots of Steady State DO and pH at the Chosen Sites in the River, 1981.

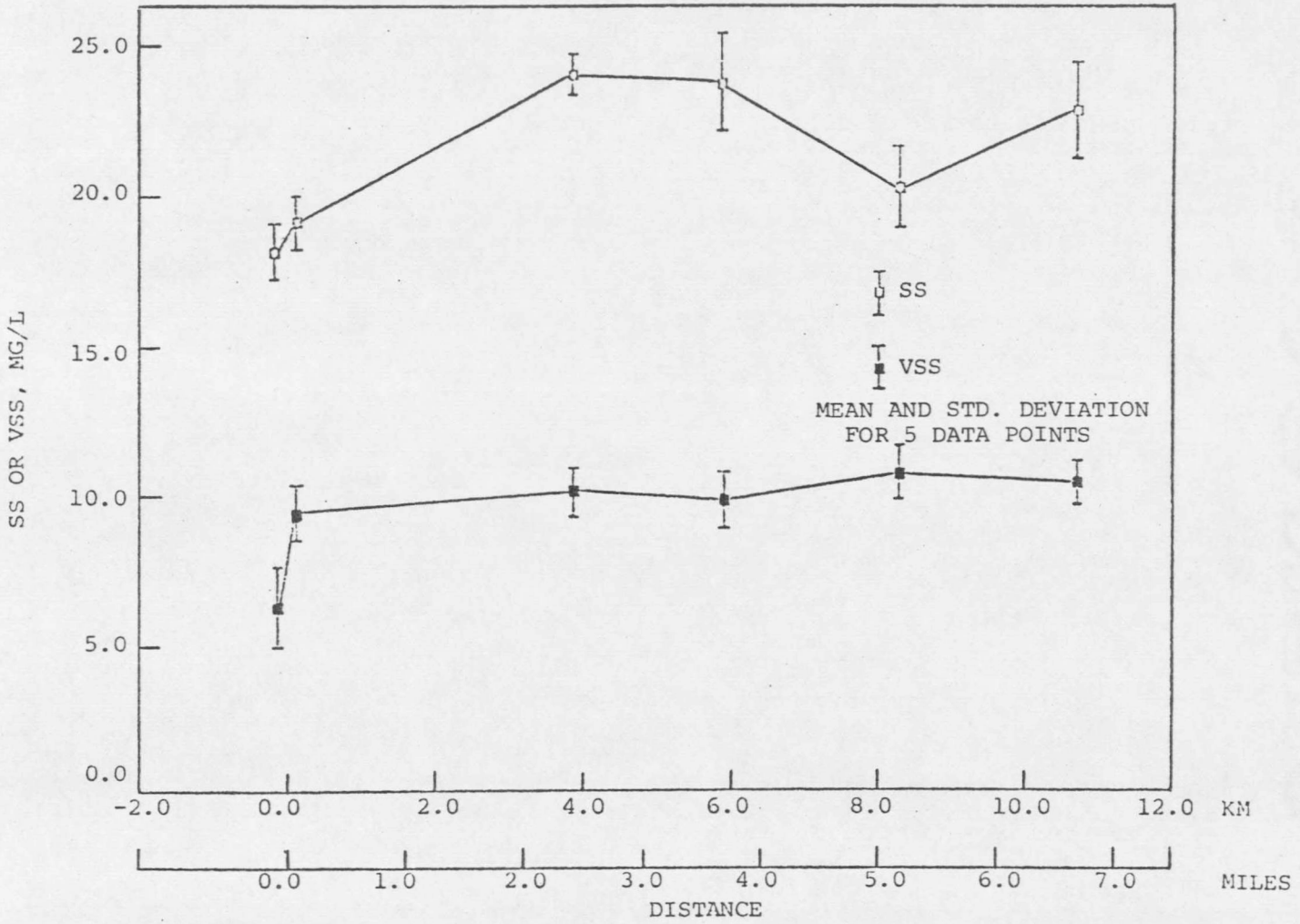


Fig. 38. Distribution of Solids Under Steady State Flow Conditions, 1980.

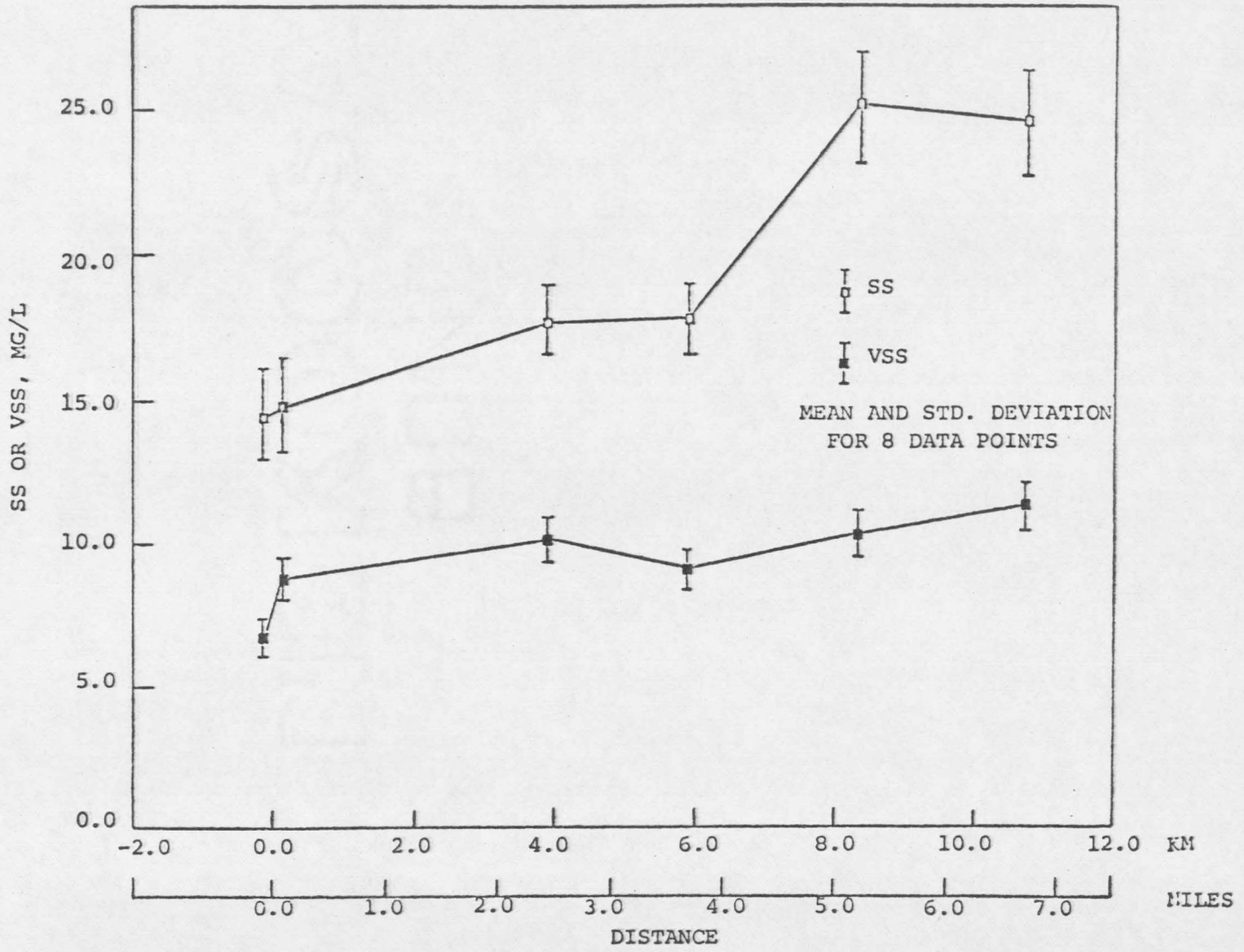


Fig. 39. Distribution of Solids Under Steady State Flow Conditions, 1981.

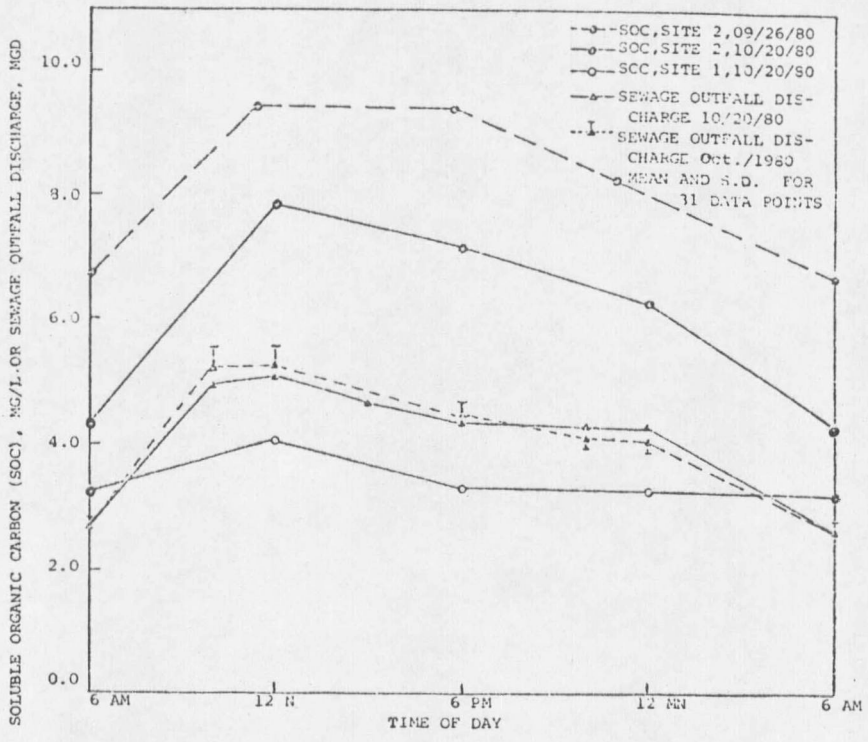


Fig. 40. Diurnal Variation of Organic Carbon and Sewage Outfall Discharge at Selected Sites, 1980.

Table 18. Mean Values for Nitrogen During Steady State Flow Period, 1980.

Site	NO_3-N mg/ℓ	NH_3-N mg/ℓ
1	0.54	0.40
2	0.57	1.64
3	0.63	1.14
4	0.71	1.06
5	0.76	0.47
6	0.84	0.45

NOTE: Mean values given in this table were calculated using 3 measurements taken during August.

to occur after midnight. SOC measurements at Site 1 above the outfall however, do not show major changes during the same period. Diurnal variations of SOC will be discussed further in relation to unsteady state modeling under the discussion. Since more information on diurnal variation of sewage outfall discharge can be obtained from the records of the Bozeman Wastewater Treatment Plant at no cost, an effort was made to study the variations further in detail over the whole month of October. This is also shown in Fig. 40. The objective of this additional analysis to check whether the pattern shown for a particular day reflected the variation over a longer period during the steady state flow period studied. The pattern shown in Fig. 40 for sewage effluent discharge and further examination of the flow charts for the steady state flow period indicated very similar pattern. This shows a good similarity between the diurnal variations obtained over selected days in

this study and diurnal variations of sewage outfall discharge over longer periods during the study period. The diurnal variations of DO shown in Fig. 41 seem to indicate at least 8 mg/l in the nights with a range of 8 to 12 mg/l over a day. Supersaturated levels of DO and higher pH shown in Fig. 41 during the afternoon may be due to the photosynthetic activity of the microbial community in the river.

Biofilm data. Electron microscopy: Several magnifications of the electron micrographs obtained at Sites 1, 2 and 3 are shown in Figs. 42 to 45. The higher magnification at Site 1 as shown in Fig. 42(b) indicates striations of the cells characteristic of diatoms.

The low magnification picture at Site 2 shows the attached algal community as evident from Fig. 44 while the higher magnifications show the attachment of bacterial rods and the general structure of the biofilm as seen in Fig. 43. The critical point drying procedure does not permit the polysaccharide matrix surrounding the microbes to be seen. This material might have shrunk in the course of the drying process and may appear as fibers in the micrographs shown. Electron micrographs at Site 3 support the interesting phenomenon that algal surfaces provide suitable surfaces for subsequent bacterial colonization. Fig. 45(a) shows the attachment of bacterial rods to a diatom which when magnified appear as given in Fig. 45(b). These electron micrographs are useful in understanding the general structure of the biofilms and the attachment of the organisms.

Biofilm characteristics: The variation of biomass measured as ash free weight (AFW) with time given in Figs. 46 and 47 for 1980 and 1981 clearly demonstrate the different phases of a growth curve. The accumulation of biomass is slow up to about 20 days after which it drastically increases possibly corresponding to a logarithmic growth. This is also

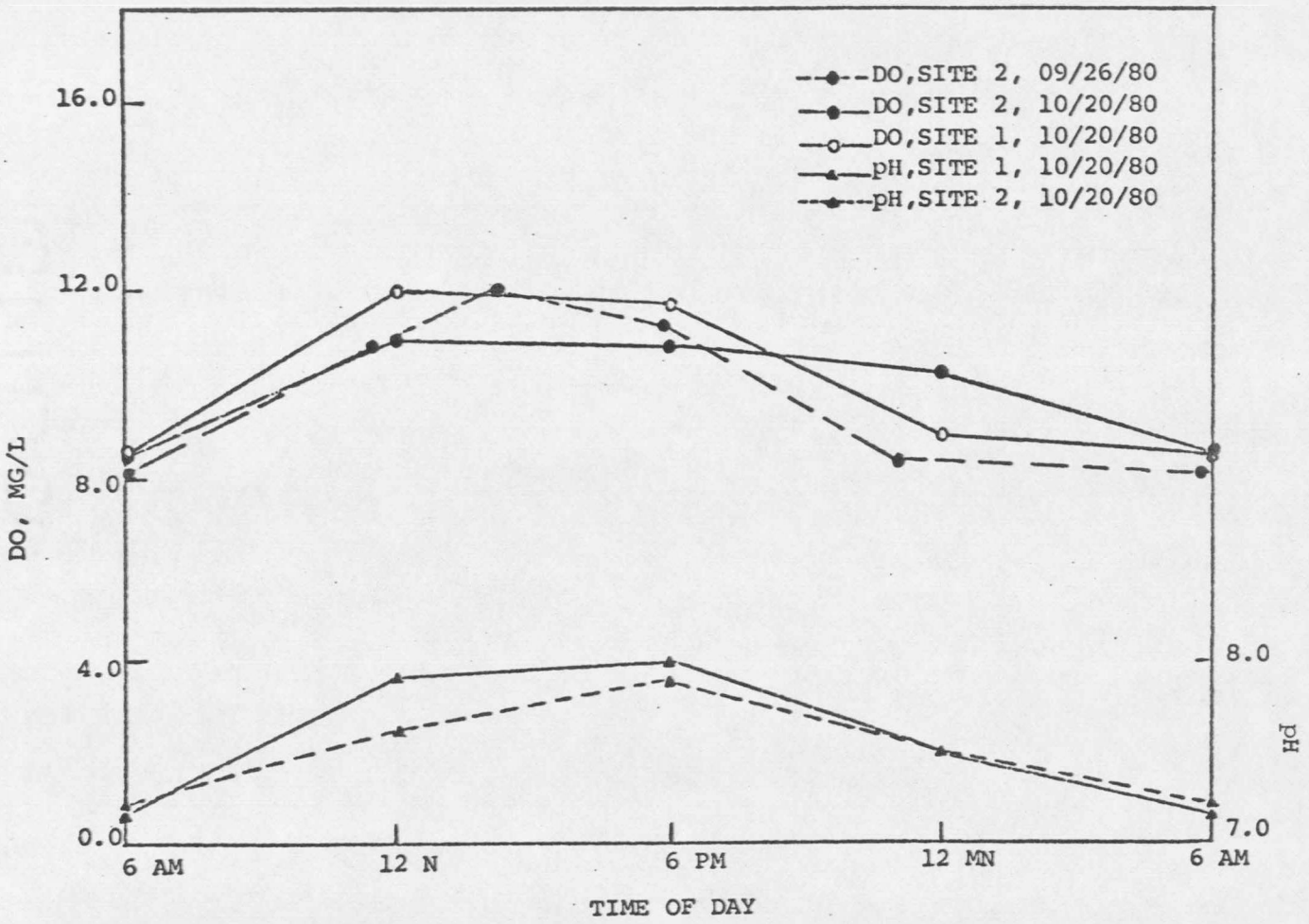
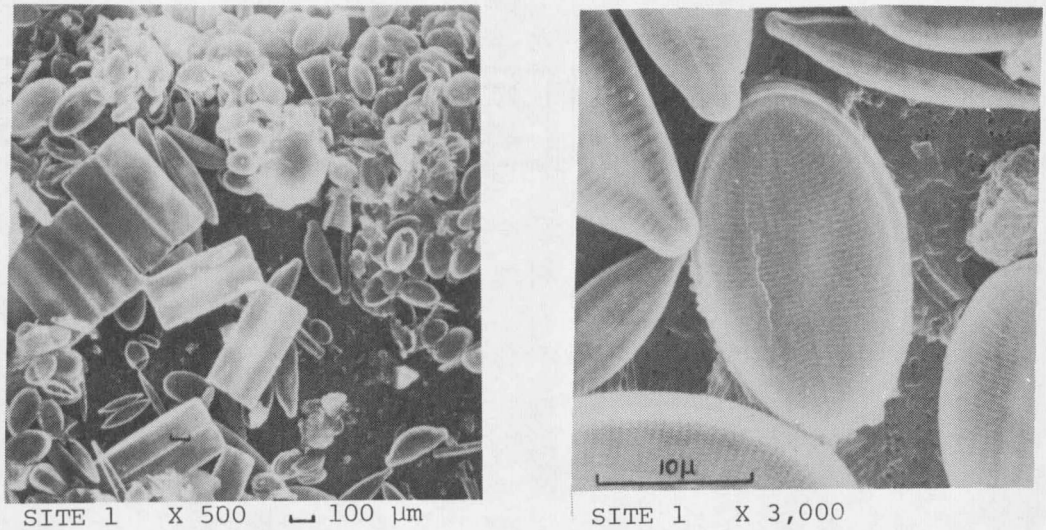
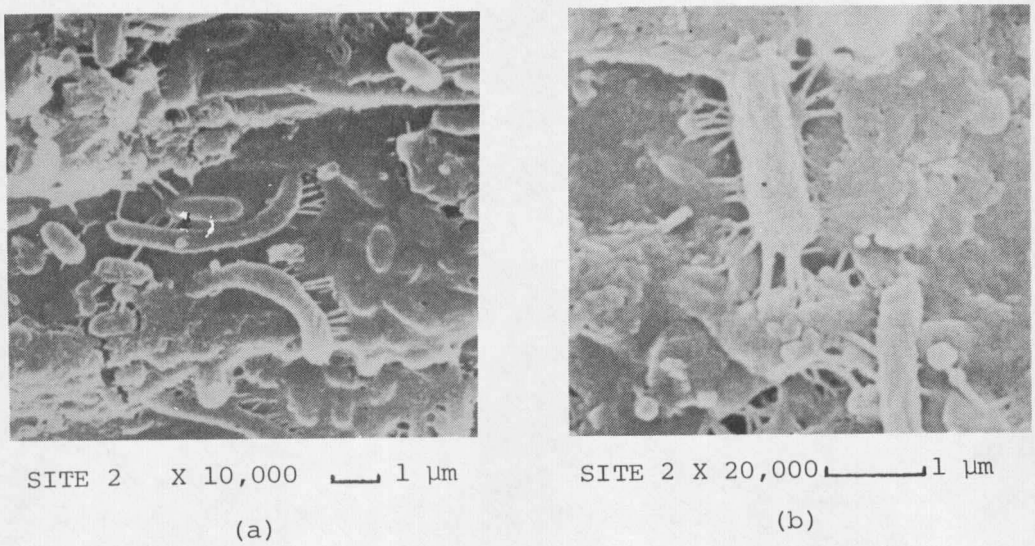


Fig. 41. Diurnal Variation of DO and pH at Selected Sites, 1980.



(a) (b)

Fig. 42. (a) Algal Community at Site 1; (b) Magnified View of Diatoms.



(a) (b)

Fig. 43. (a) Attachment of Bacterial Rods at Site 2; (b) Magnified View of the Rods.

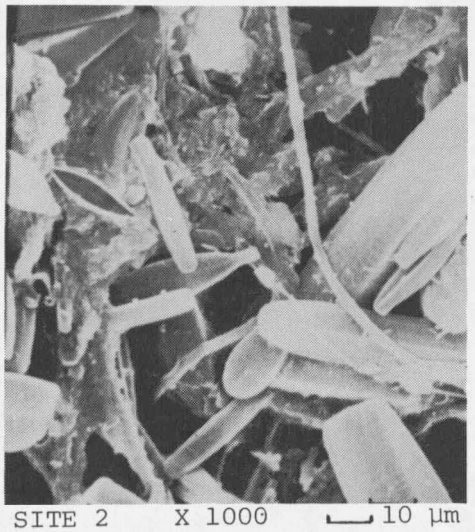
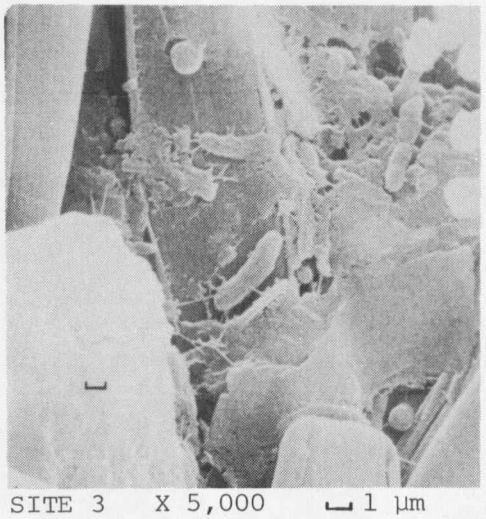
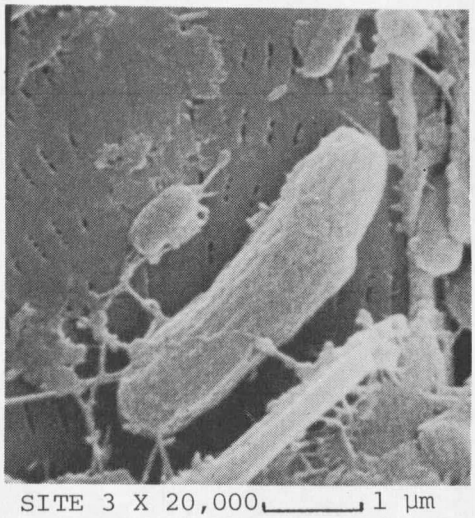


Fig. 44. Algal Community at Site 2.



(a)



(b)

Fig. 45. (a) Attachment of Bacterial Rods to Diatoms at Site 3. (b) Magnified View of an Attached Rod to a Diatom.

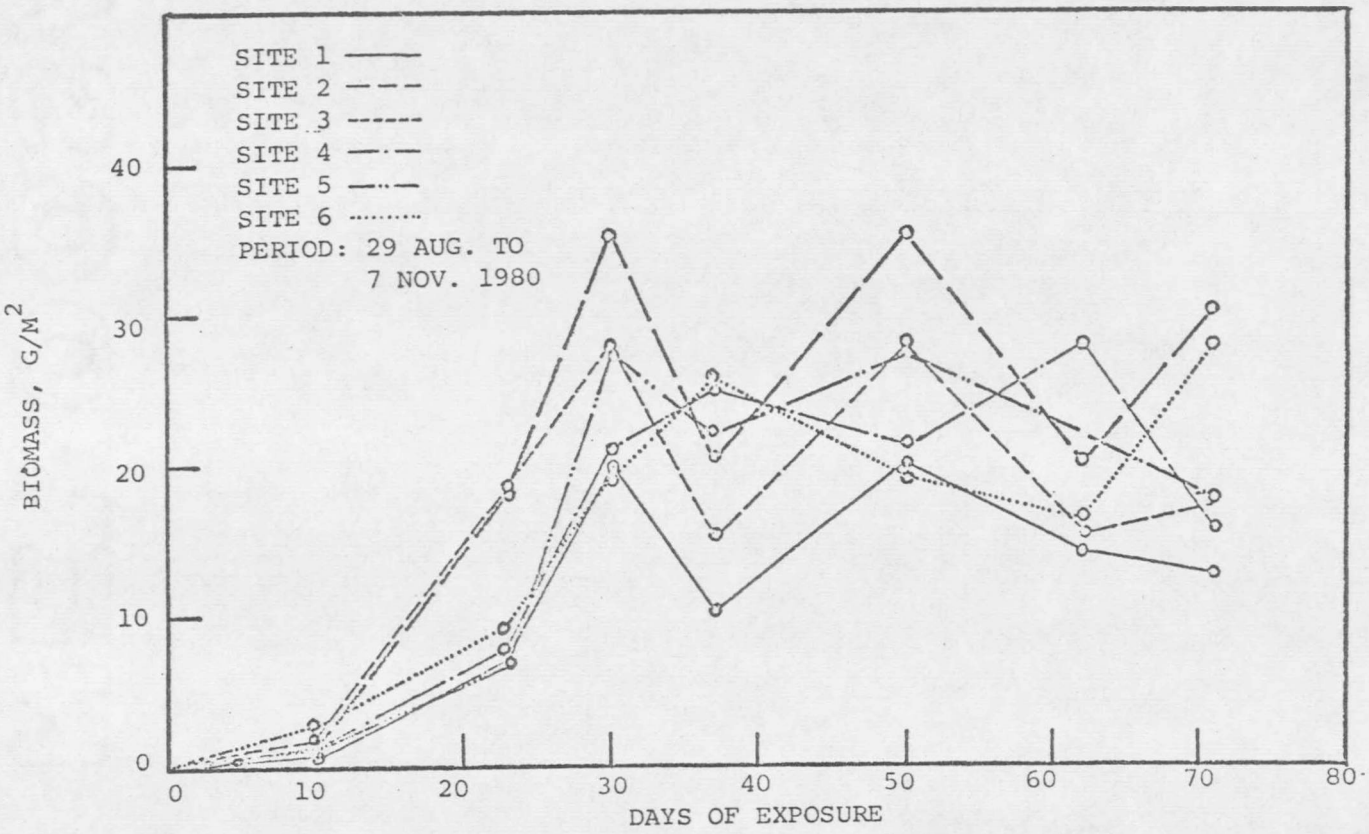


Fig. 46. Variation of Biomass at the Selected Sites, 1980.

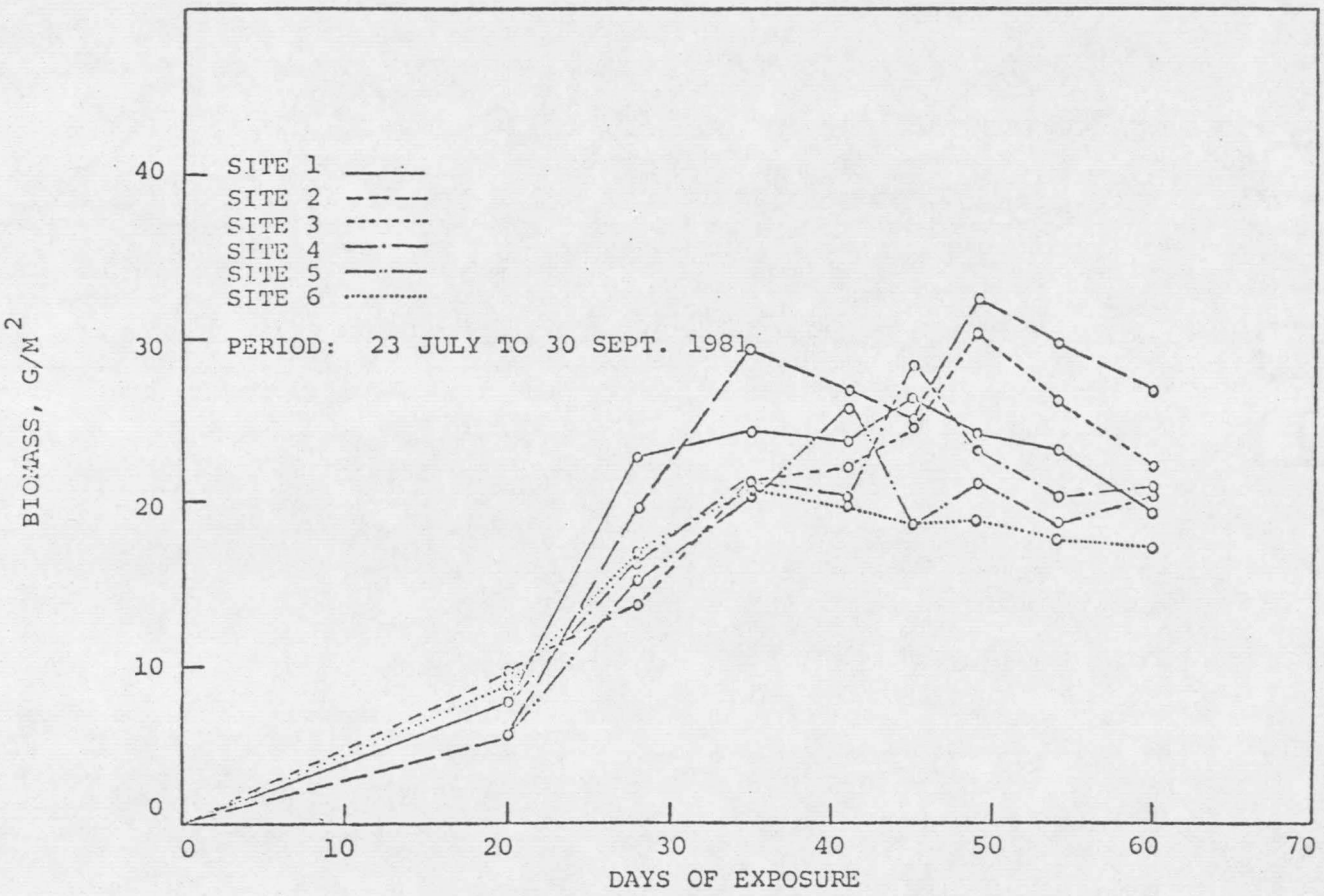


Fig. 47. Variation of Biomass at the Selected Sites, 1981.

evident from Tables 19 and 20 given subsequently for biomass productivity. The plateau phase of the curves shown in Figs. 46 and 47 exhibit an oscillatory behavior possibly caused by scour and growth. The mean film thickness curves in Figs. 48 and 49 show lower amplitude for the oscillations but all four curves shown in Figs. 46 to 49 establish the beginning of a plateau phase after 30 to 35 days of exposure. Fig. 48 also shows the similarity of the plateau phase of films grown on plexiglass plates closer to Site 2 and the glass slide growths at Site 2. Initially, however, the thickness development had been slower on the plexiglass plate. It must be pointed out that for purposes of thickness measurements, glass slides were glued to the plexiglass plate so that they could be removed easily and viewed under the microscope.

Biomass and thickness measurements during the plateau phase can be used to determine the steady state values corresponding to steady state conditions as given in Tables 19 and 20. A period of 30 to 62 days was used for this purpose to be consistent in determining the steady state values for 1980 and 1981. These measurements were based on two different sets of slides placed at different times during the steady state flow period. Vandalism affected biofilm studies at Sites 2 and 5 for the first set and only Site 1 during the second set during 1980. The bricks with slides therefore had to be moved for the first set in 1980 and hydraulic conditions were not monitored to assure similarity at all sites. The first set therefore is not included in the analysis for 1980. For the second set during 1980, vandalism affected Site 1 results and may be the possible cause for a lower equilibrium biomass and biodensity compared to those of 1981 as shown in Tables 19 and 20. Vandals broke many glass slides or turned the bricks upside down. Vandalism, however, did not affect the biofilm studies of 1981. Data on biomass productivity are also given in Tables

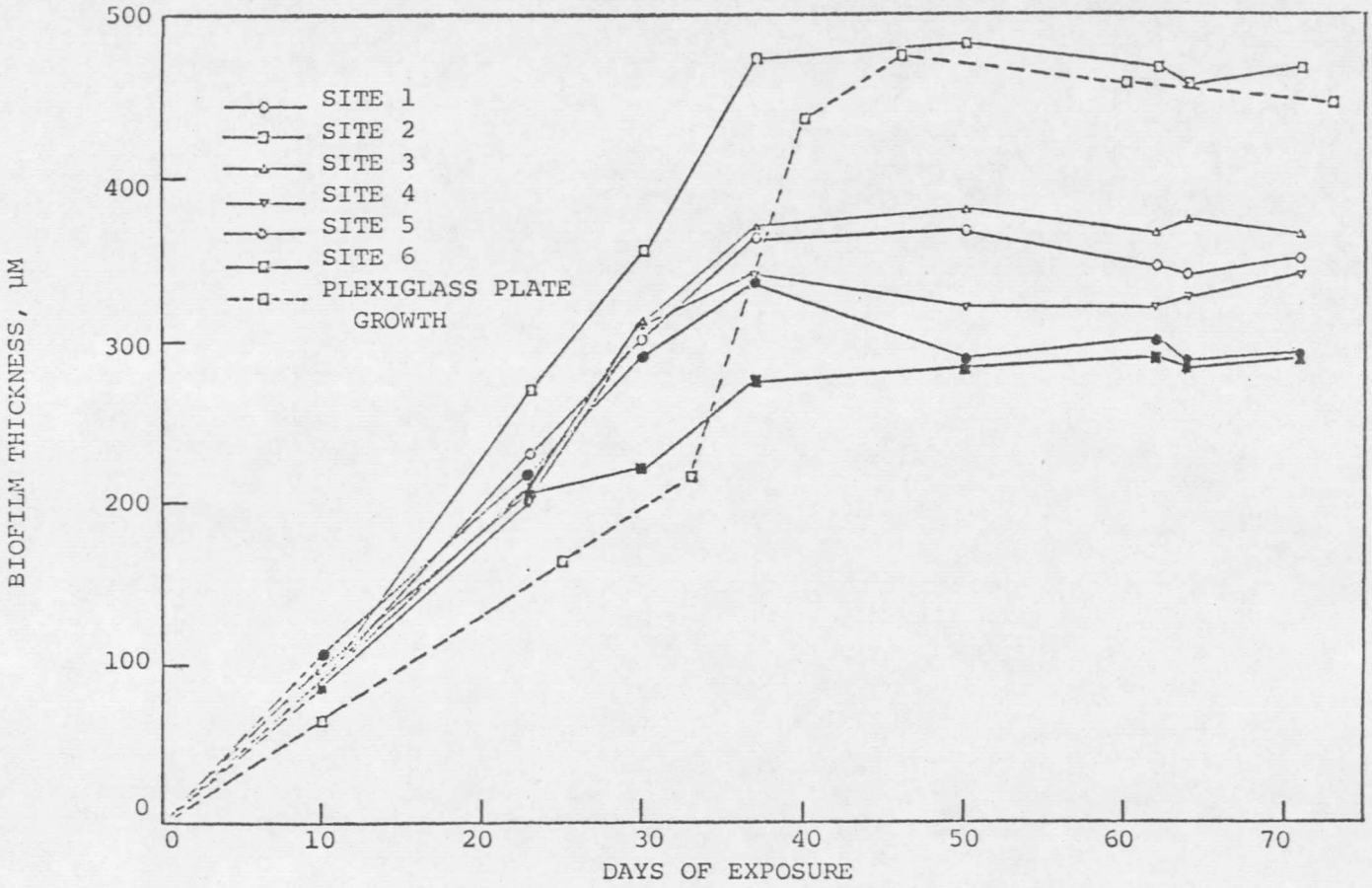


Fig. 48. Plot of Mean Biofilm Thickness at Various Sites vs. Time, 1980.

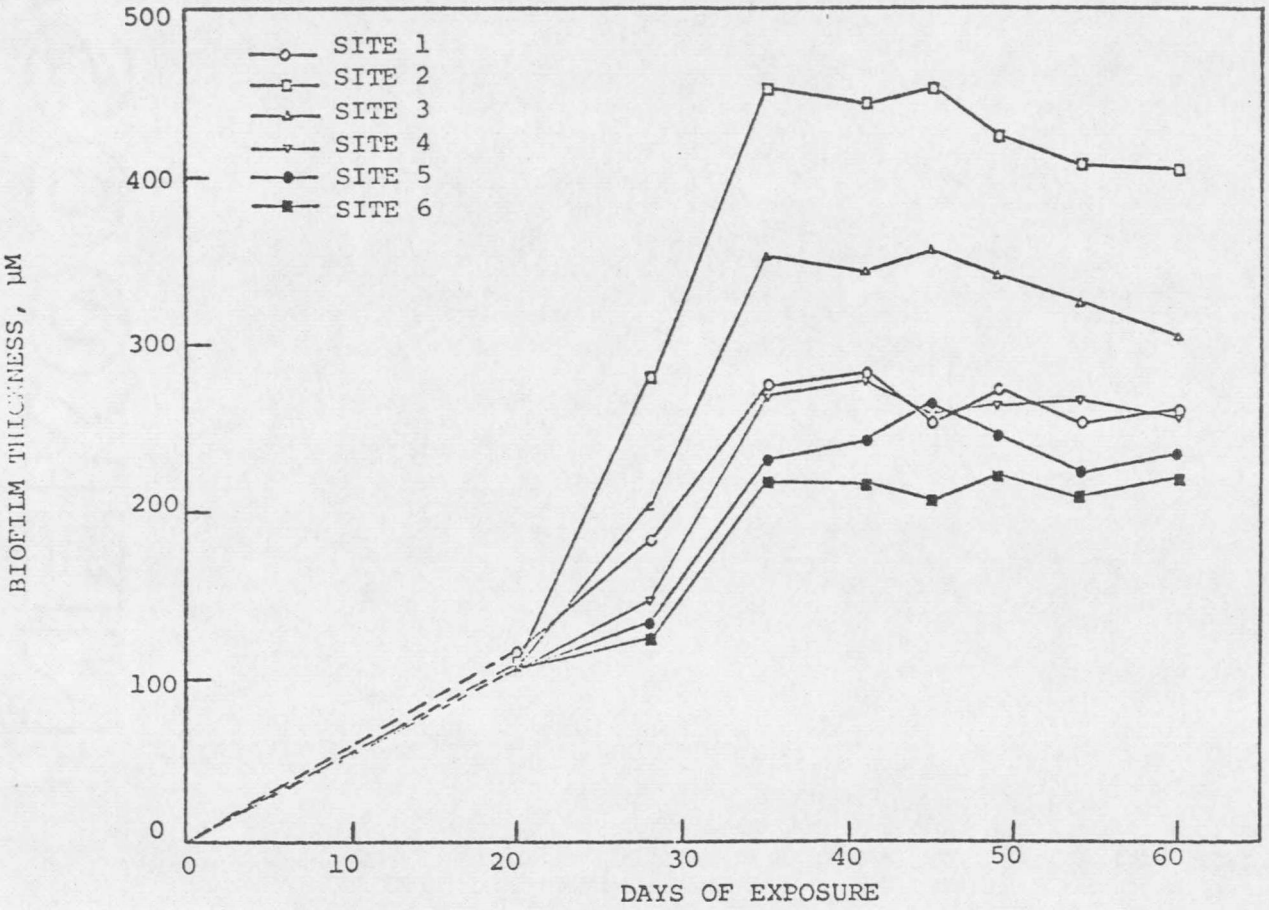


Fig. 49. Plot of Mean Biofilm Thickness at Various Sites vs. Time, 1981.

Table 19. Steady State Biofilm Thickness and Biomass, Biomass Productivity and Biodensity at Various Sites, 1980.

Site	Rate of Biomass Production g/m^2-day 0-10 days	10-30 days	†Steady State Values Biomass g/m^2	S.D. g/m^2	Thickness μm	S.D. μm	Biodensity kg/m^3
1	0.06	0.98	16.21	4.92	344	31	47.12
2	0.10	1.74	28.02	8.81	444	59	63.11
3	0.18	1.32	21.75	7.55	357	31	60.92
4	0.14	1.01	24.03	3.12	323	13	74.30
5	0.11	1.35	25.00	3.38	304	24	82.23
6	0.28	0.84	20.18	3.77	267	32	75.58

†Steady State values are determined by averaging the film thickness and biomass measurements made during the period of 30 to 62 days of exposure.

Table 20. Steady State Biofilm Thickness and Biomass, Biomass Productivity and Biodensity at Various Sites, 1981.

Site	Rate of Biomass Production g/m^2-day		†Steady State Values				Biodensity
	0 - 20 days	20 - 35 days	Biomass g/m^2	S.D. g/m^2	Thickness µm	S.D. µm	Kg/m^3
1	0.38	1.12	23.57	2.19	266	13	88.6
2	0.28	1.57	28.29	2.66	432	21	65.5
3	0.47	0.77	24.42	3.40	342	12	71.4
4	0.38	0.89	22.67	2.90	265	10	85.5
5	0.28	0.97	20.81	2.64	239	13	87.0
6	0.45	0.77	18.80	1.30	215	6	87.4

†Steady State values are determined by averaging the film thickness and biomass measurements made during the period of 35 to 60 days of exposure.

19 and 20 along with the biodensities determined using equilibrium biomass and thicknesses. The increase in biodensities can be seen with increasing distance from the sewage outfall. The mean and standard error for the biodensities in the study reach required for subsequent modeling efforts are tabulated for the years 1980 and 1981 in Table 21.

Autotrophic Index (AI): Measurements of Chlorophyll a (Chl. a) and ashfree weight (AFW) were used in determining the Autotrophic Index (AI) values calculated according to Weber (136) and are given in Tables 22 and 23 for slide and cobble growths.

$$\text{Autotrophic Index (AI)} = \frac{\text{Biomass as AFW}}{\text{Chl. a}} \qquad (42)$$

Figures 50 and 51 clearly show highly heterotrophic films below the sewage outfall for both the slide and cobble growths. AI values for the biofilms appear to be at almost the same level at Sites 1, 4, 5, and 6.

An effort was made to compare the biomass accumulation and compositional variation for cobbles and glass slides at Site 2 during 1980. A clean cobble was monitored for this purpose after placing it on the river bed close to the bricks with slides. The plots shown in Fig. 52 show films becoming highly heterotrophic during the plateau phase of their growth even though they appear to be more autotrophic at the beginning. This may suggest more algal cells colonizing and providing a favorable surface thus promoting substantial heterotrophic accumulation. More data will be ncessary to substantiate this observation.

ATP measurments: During 1981, ATP measurements were made as they provide estimates of biomass without a bias from inorganic sediments or organic detritus. With the available reagents for ATP, 3 sets of measurements on cobble and glass slide growths were

Table 21. Mean Biodensity Below Sewage Outfall.

Year	†Mean Biodensity Kg/m^3	S.E. Kg/m^3
1980	71.23	4.01
1981	79.36	4.56

† Mean biodensity for the study reach below the sewage outfall is determined by averaging biodensities between Sites 2 and 6.

Table 22. Determination of Autotrophic Index (AI) for Cobble Growths, 1980.

Site	Chl.a (mg/m^2)		Biomass $(g)m^2$		AI		Mean AI
	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2	
1	297	450	38.65	48.86	130	109	120
2	306	419	92.48	99.36	302	238	270
3	293	394	95.75	86.74	327	220	274
4	319	455	38.18	49.29	120	108	114
5	402	293	46.63	46.77	116	159	138
6	138	356	19.83	44.55	144	125	135

NOTE: Samples for Set 1 were collected on August 5, 1980 and Set 2 on August 18, 1980.

Table 23. Determination of Autotrophic Index (AI) for Slide Growths, 1980 and 1981.

Site	Days of Exposure		Set		Chl.a (mg/m^2)		Biomass (g/m^2)		AI		Mean AI	
	1980	1981	1980	1981	1980	1981	1980	1981	1980	1981	1980	1981
1	50	60	1	1	116.4	118	14.44	19.47	124	166		
	71	55	2	2	125.0	204	12.73	24.13	102	119		
	-	60	-	2	-	182	-	19.55	-	107	113	130
2	50	60	1	1	93.6	116	32.61	29.50	348	255		
	71	55	2	2	96.4	108	30.49	32.20	316	298		
	-	60	-	2	-	99	-	24.14	-	243	332	265
3	50	60	1	1	48.5	114	13.78	22.97	284	203		
	71	55	2	2	73.7	140	17.47	28.88	237	207		
	-	60	-	2	-	110	-	21.29	-	194	261	201
4	50	60	1	1	81.4	137	11.25	18.70	188	138		
	71	55	2	2	120.0	211	15.98	23.17	133	110		
	-	60	-	2	-	142	-	22.88	-	161	135	136
5	50	60	1	1	186.0	254	18.60	17.62	100	69		
	71	55	2	2	193.0	340	17.14	19.43	89	57		
	-	60	-	2	-	317	-	23.62	-	74	94	66
6	50	60	1	1	234.0	199	12.91	18.50	55	93		
	71	55	2	2	240.0	265	28.43	18.67	118	70		
	-	60	-	2	-	228	-	15.51	-	68	86	77

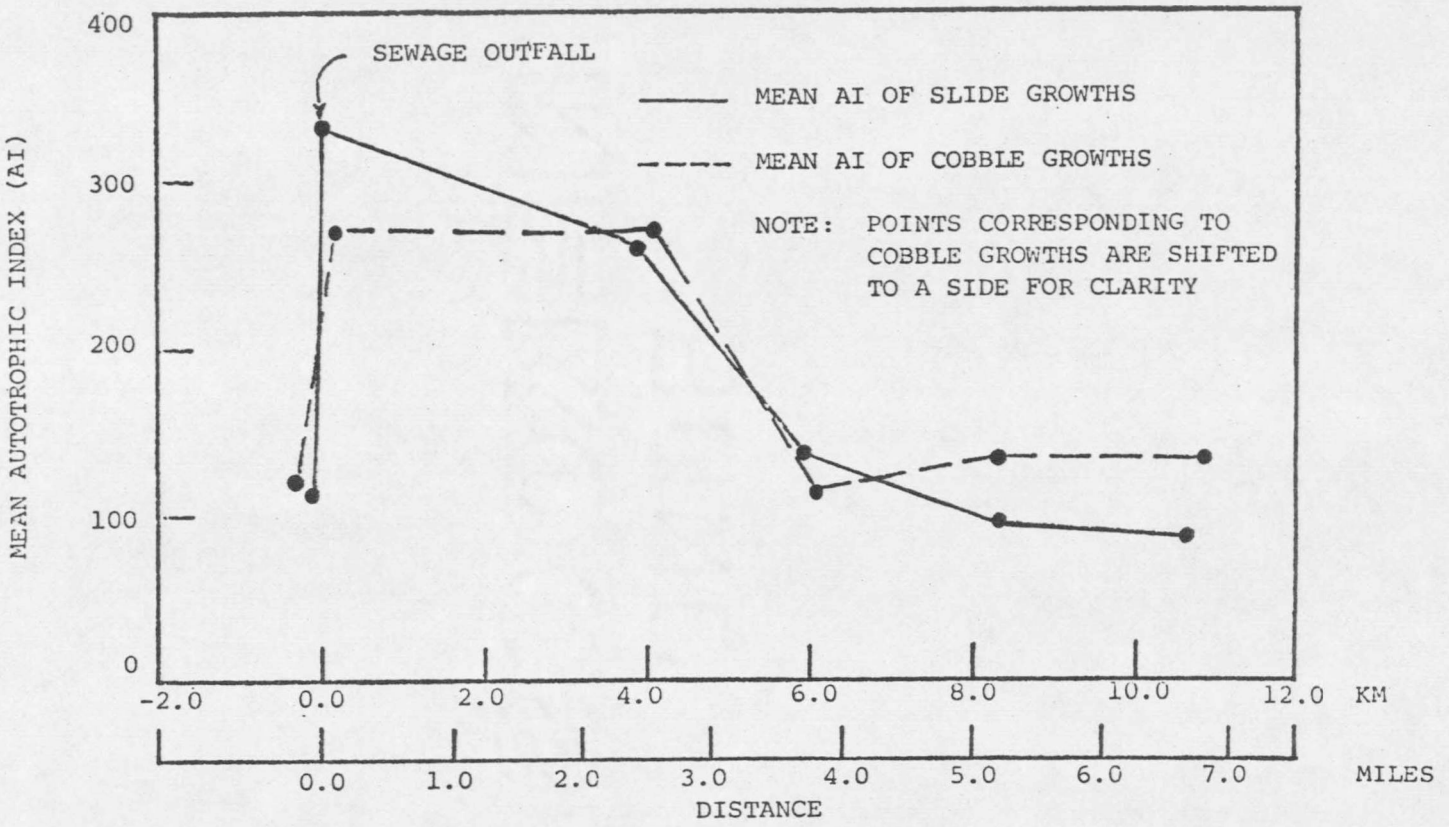


Fig. 50. Compositional Variation of Biomass Below the Sewage Outfall, 1980.

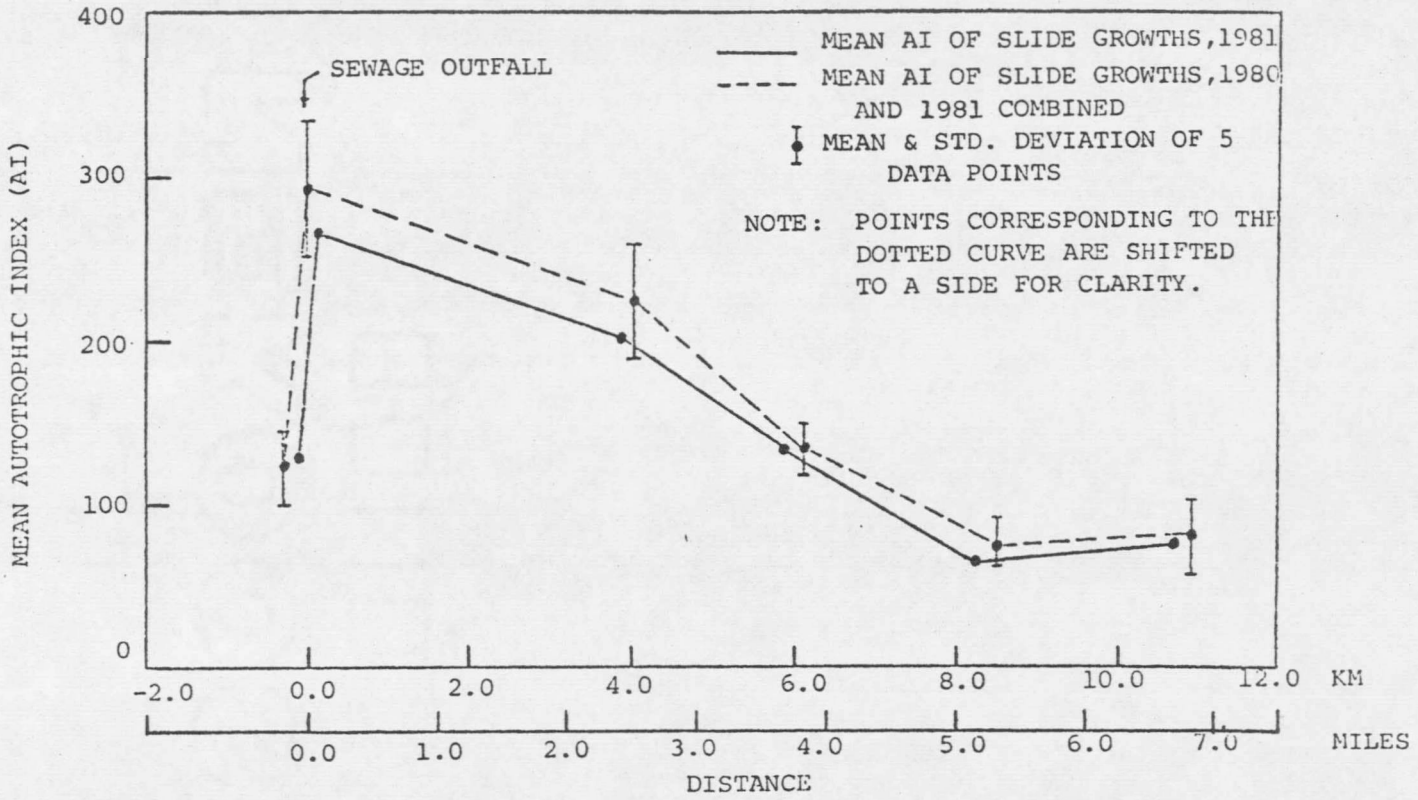


Fig. 51. Compositional Variation of Biomass Below the Sewage Outfall, 1981.

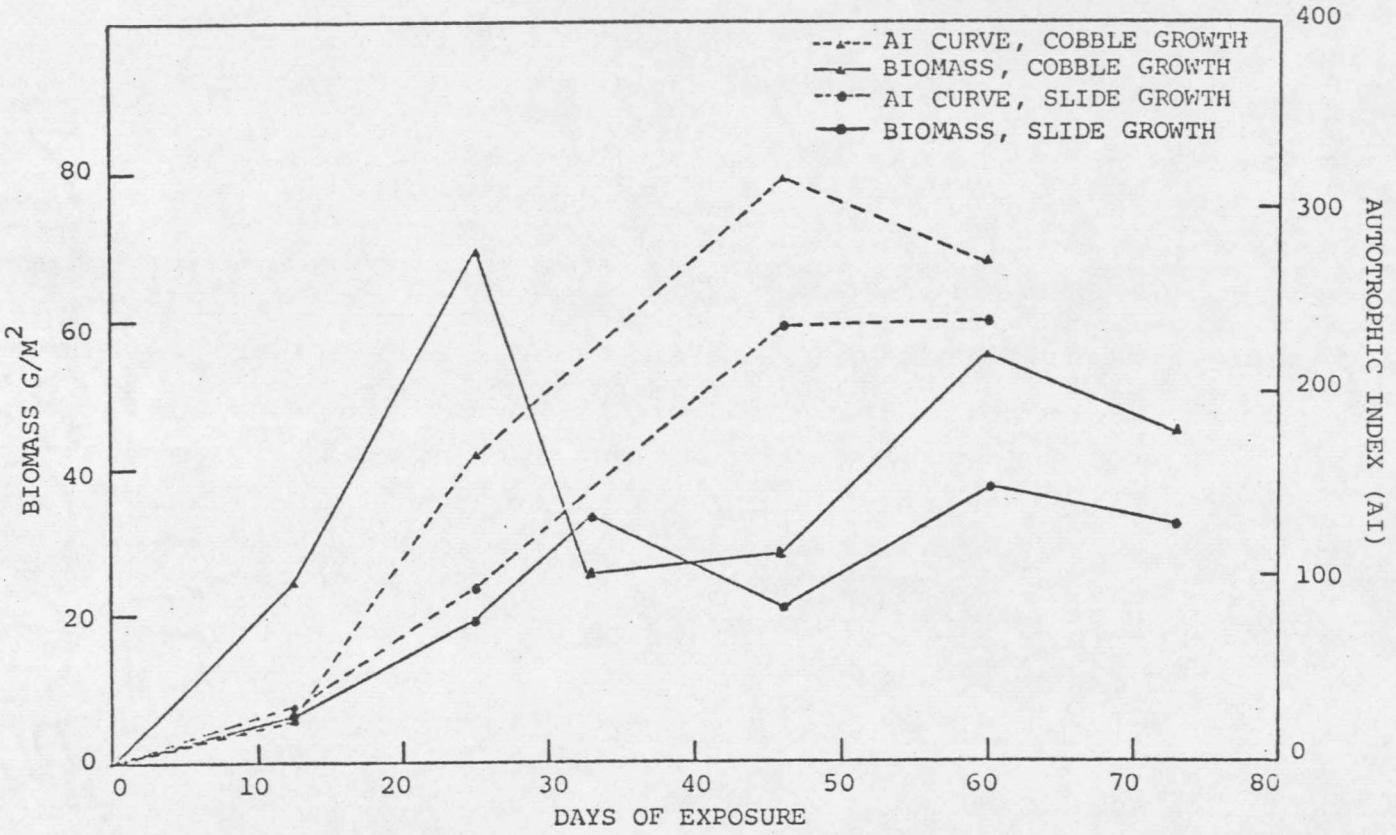


Fig. 52. Variation and Comparison of AI and Biomass for Cobble and Slide Growths for Site 2, 1980.

made. Table 24 gives a comparison of ATP to AFW on the same samples after converting both to an areal unit. ATP biomass estimates appear to be much smaller compared to the AFW estimates. Using appropriate conversion factors, both ATP and AFW can be converted to a common unit such as organic carbon in establishing a viability index as shown under the discussion.

Table 24. Comparison of ATP and Ash-free Weight (AFW) for Cobble and Slide Growths, 1981.

Type of Substrate	Site	ATP mg/m^2	AFW g/m^2	ATP/AFW $\times 10^{-3}$
1. Cobbles	1	15.2	51.5	0.30
	2	27.5	74.5	0.37
	3	20.5	60.0	0.34
	4	18.2	60.8	0.30
	5	16.2	48.2	0.34
	6	13.9	44.6	0.31
2. Cobbles	1	14.3	61.0	0.23
	2	16.6	63.2	0.26
	3	12.7	49.2	0.26
	4	16.0	52.9	0.30
	5	15.4	56.4	0.27
	6	15.0	51.6	0.29
3. Glass Slides	1	2.4	26.6	0.09
	2	9.6	32.8	0.29
	3	5.4	30.0	0.18
	4	8.2	28.5	0.28
	5	4.4	28.6	0.15
	6	6.6	24.2	0.27

Model Verification

The models proposed earlier will be verified in this section. The simultaneous solutions of Eqs. 19, 20 and 21 given under theoretical considerations were solved assuming

steady state conditions and programmed using numerical methods. The computer program is annexed in the Appendix. Program 'DECAY 2' describes steady state variations below the sewage outfall for the substrate measured as SOC, suspended biomass as VSS and Biofilm thickness. For the substrate program, 'DECAY 4' was written to provide diurnal variations corresponding to the changes in the point source of pollution contributed by the sewage outfall and is also annexed in the Appendix. However, only the steady state models will be presented here and steady vs unsteady state models will be discussed subsequently under the Discussion.

The extensive data collection done over 1980 and 1981 and presented in the results section are used in validating the steady state models. The predicted and the measured values for the three model parameters S, X and Th are shown in Figs. 53 and 54. The parameters given in Table 25 were required as input to the mathematical models for solution of Eqs. 5, 6 and 7.

Methods to estimate the longitudinal dispersion coefficient D_L have been given under the literature review. Some of the methods used for estimating D_L values and their results are given in Table 26.

In the formulas given in Table 26, f = an empirical coefficient varying from 50 in the streams with a uniform velocity distribution to 700 in wide estuaries with very large lateral variations in velocity, assumed 100 in this study; R_H = hydraulic radius, U_* = shear velocity, n = Mannings Coefficient = 0.04 from Chow (20) for a mountain stream with gravel, cobble and few boulders; S_e = slope of energy gradient; W = channel width, Q = stream flow, H = mean flow depth, β_D = dimensionless dispersion coefficient and U = mean flow velocity. Elder (35) gives the lowest estimates for D_L and the largest values were obtained

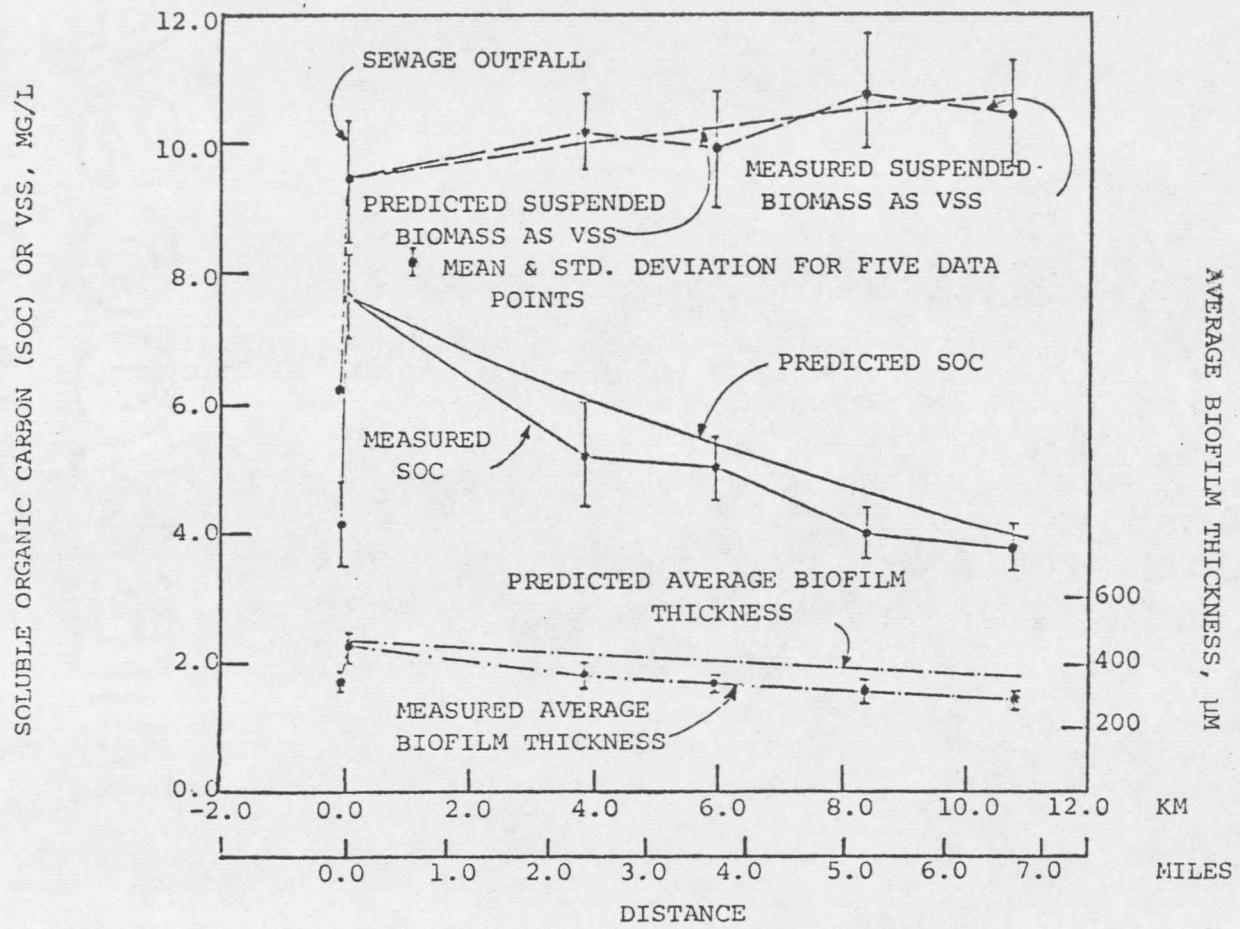


Fig. 53. Measured and Predicted Distributions for Organic Carbon, Suspended Biomass and Biofilm Thickness Below Sewage Outfall, 1980.

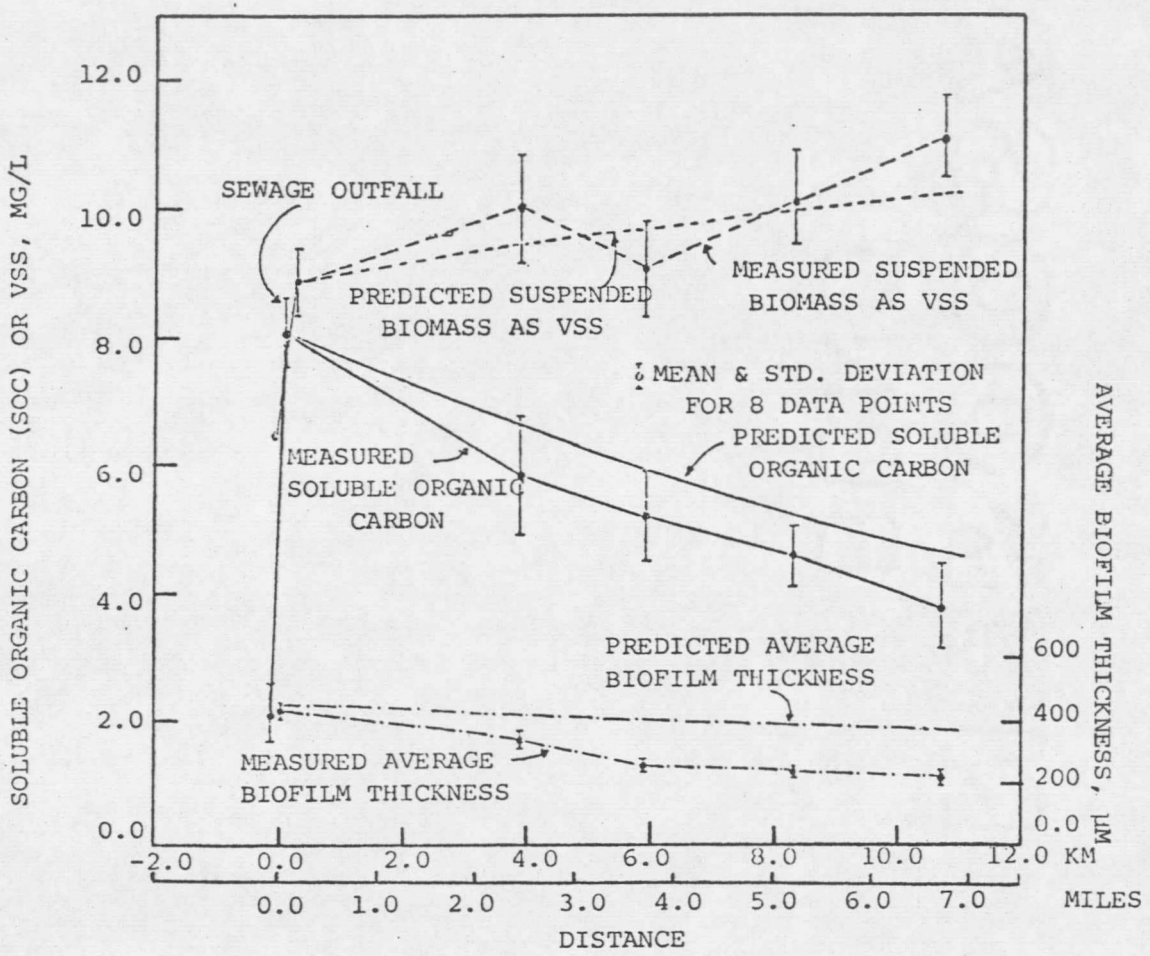


Fig. 54. Measured and Predicted Distributions for Organic Carbon, Suspended Biomass and Biofilm Thickness Below Sewage Outfall, 1981.

Table 25. Parameters Required as Input to the Steady State Models.

	Hydraulic and Biofilm Parameters				
Year	D_L m^2/h	U m/h	H m	ρ Kg/m^3	T °C
1980	7923	2195	0.22	71.23	18.8
1981	9502	2415	0.25	79.36	19.9

$R_{B20} = 0.0126$ S g/m^2-h ; $\theta = 1.098$

$R_D = 1.47 \times 10^{-5} M^{2.46}$; $\alpha_D = 1.47 \times 10^{-5}$, $\beta = 2.46$

$Y_A = 0.42$

Table 26. Methods for Estimating Longitudinal Dispersion Coefficient.

Method	Formula	D_L (m^2/h)	
		1980	1981
Fischer (37)	fRU_*	7923	9502
Elder (35)	$22.6n\ UH^{0.833}$	471	570
McQuivey & Keefer (82)	$\frac{0.058\ Q}{S_e\ W}$	6740	7629
Liu (69)	$\beta_D \frac{Q^2}{U_* R_H^3}$	17,621	12,209
	$\beta_D = 0.018 \left(\frac{U_*}{U}\right)^{1.5}$		

by using Liu (69). The value of β_D defined by Liu (69) does not depend on the size of stream while the other simpler methods by Fischer (37) and Elder (35) use coefficients depending on stream sizes. Liu's (69) method may thus be considered more refined than the other simpler methods. In this study however, intermediate values given by Fischer (37) were used for modeling and the impact of using a range of D_L values on the substrate decay were tested subsequently by a sensitivity analysis. Variations of several other parameters in affecting substrate decay were also studied under sensitivity analysis.

The plots on Figs. 53 and 54 show good agreement of the predicted and measured values. The higher film thicknesses predicted below the sewage outfall may be due to the usage of a smaller constant biodensity even though increased biodensities are seen with distance from the outfall. Besides there may be greater scour in the river causing smaller measured film thicknesses. The model predictions for the SOC using mean hydraulic and biofilm parameters are generally higher than the measured values in the river. Suspended biomass predictions are closer to the measured values. It is easy to recognize that, in terms of measurements the river biofilm thickness is the most difficult parameter in the validation of the models because of the necessity to use artificial substrates such as glass slides to monitor the growth.

Sensitivity analysis. The objective of this section is to analyze which parameter or parameters are very sensitive in the substrate decay models. The variation and sensitivity of hydraulic and biofilm parameters are shown in Figs. 55 to 58 for 1980 and 1981.

Hydraulic parameters: A large range of variation to accommodate all the different methods given earlier was used for the longitudinal dispersion coefficient D_L and was

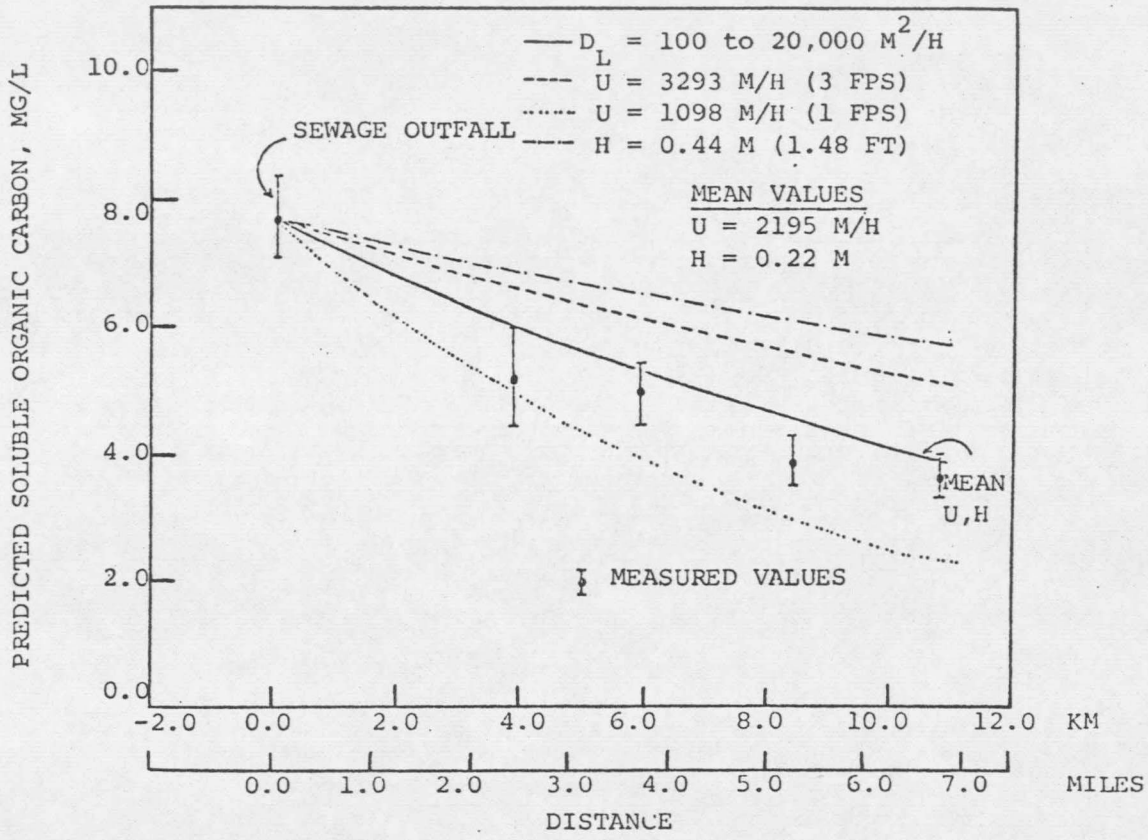


Fig. 55. Sensitivity of Hydraulic Parameters in the Model for Substrate Decay, 1980.

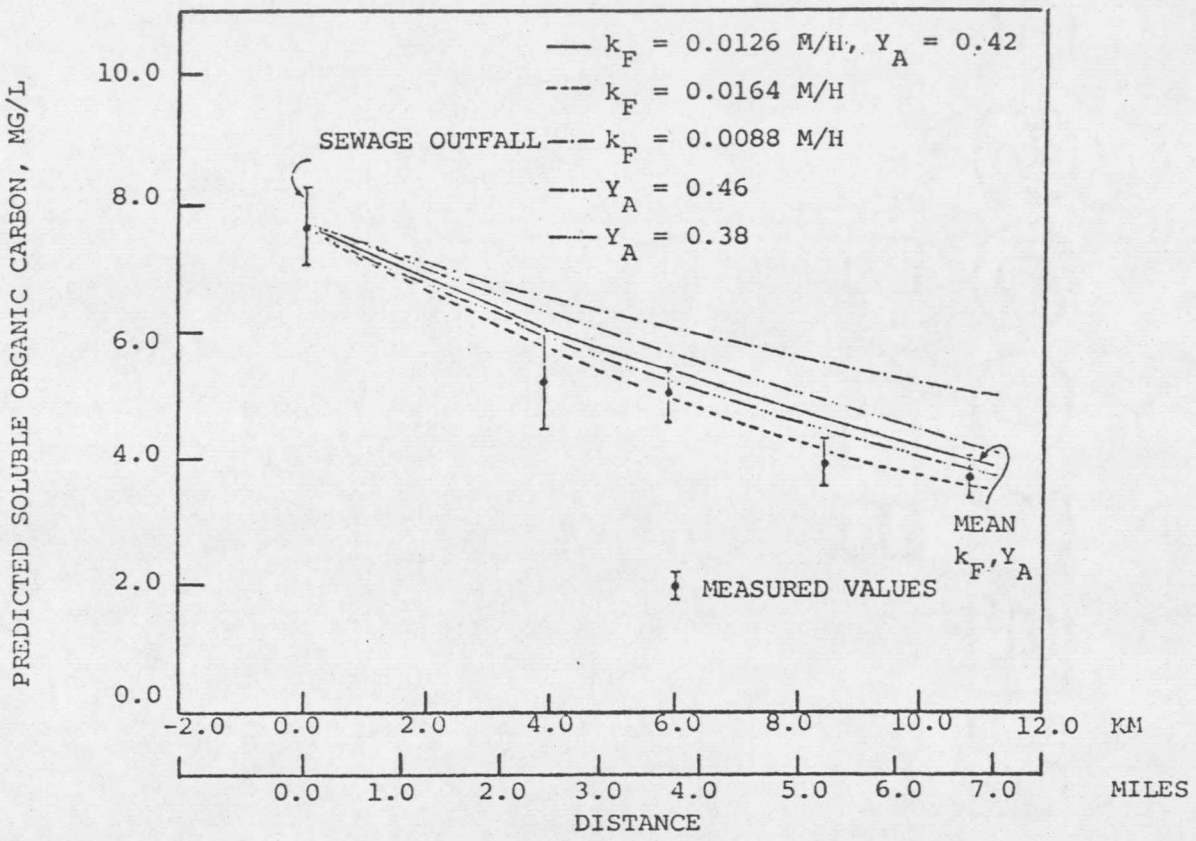


Fig. 56. Sensitivity of Biofilm Parameters in the Model for Substrate Decay, 1980.

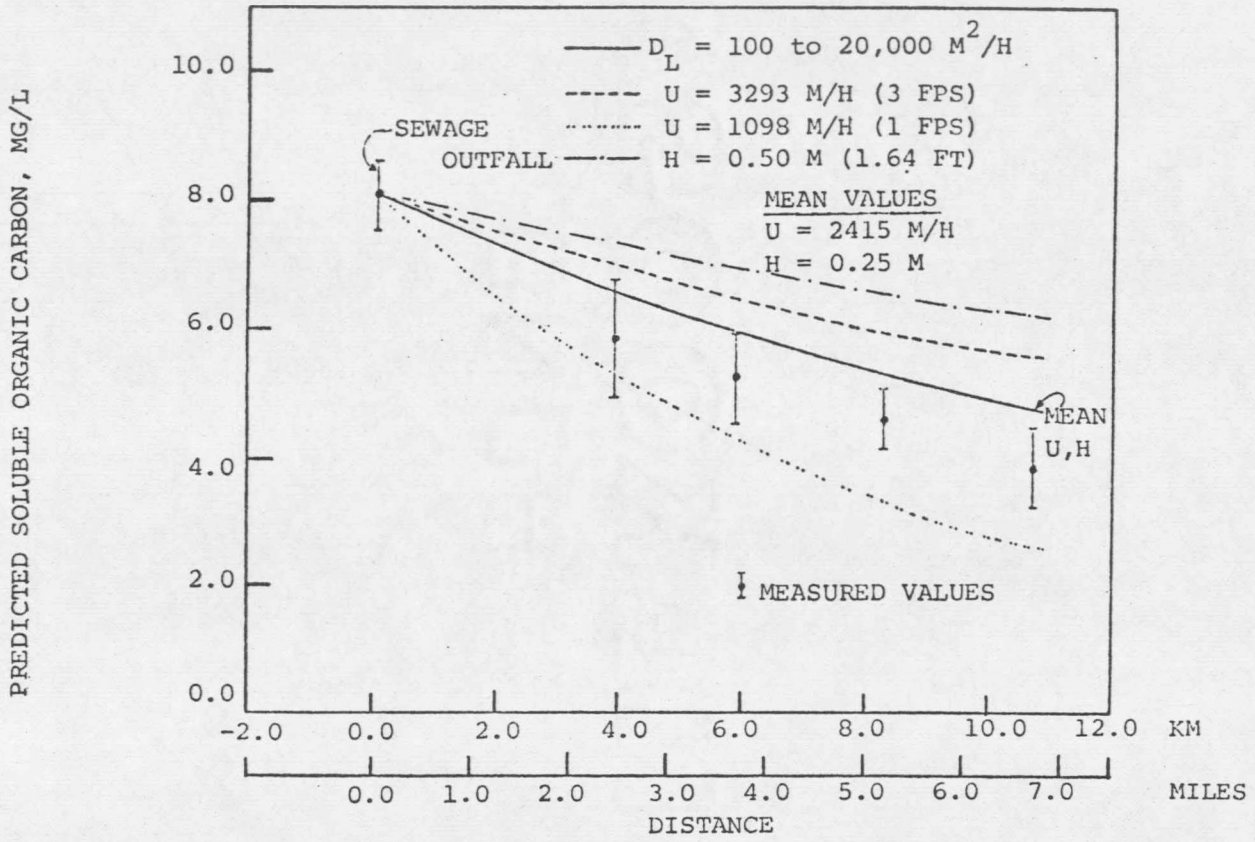


Fig. 57. Sensitivity of Hydraulic Parameters in the Model for Substrate Decay, 1981.

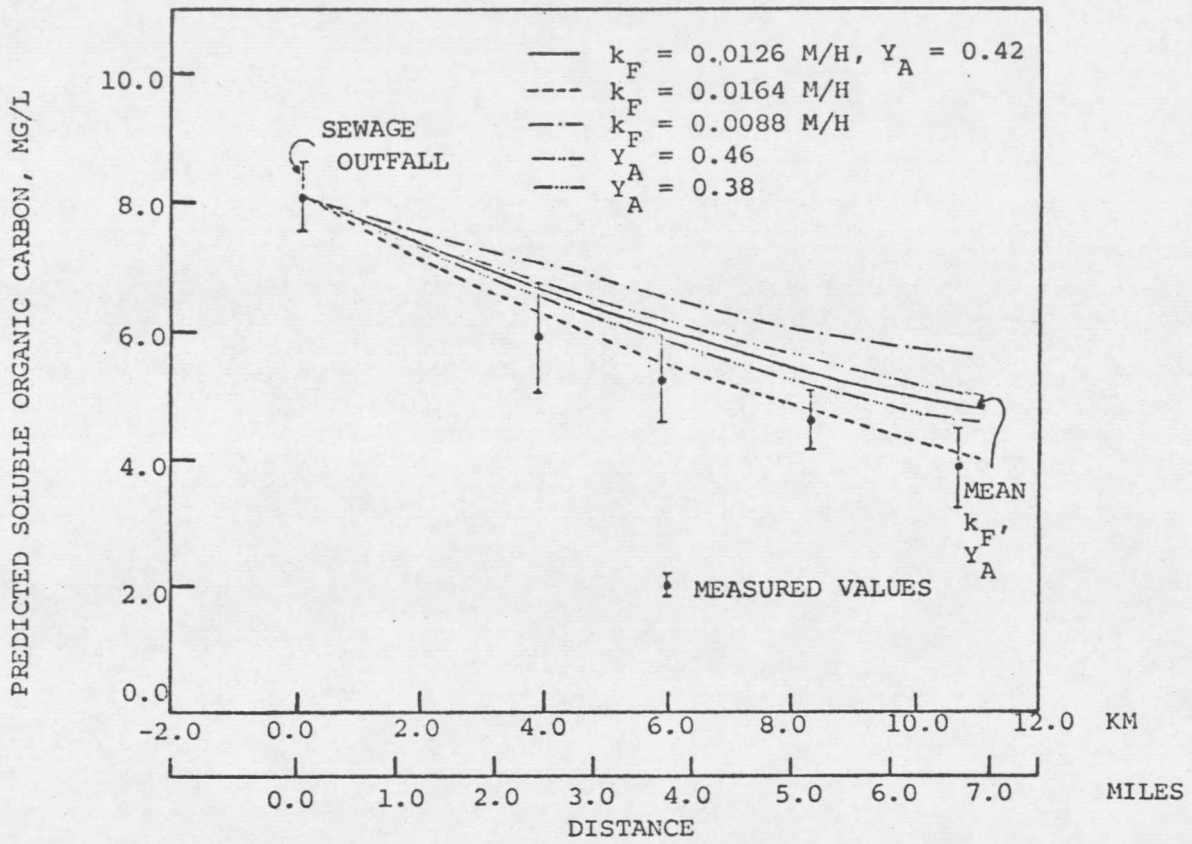


Fig. 58. Sensitivity of Biofilm Parameters in the Model for Substrate Decay, 1981.

found to have a negligible variation as shown in Figs. 55 and 57 for 1980 and 1981. This is not surprising since a small river has very little longitudinal dispersion caused by the smaller lateral velocity variations as compared to an estuary or a very large river. Larger flow velocities and depths decreased the substrate decay. These larger variations were imposed on the substrate model to study their impact on the soluble organic carbon in the river. Larger flow velocities, by reducing the flow time in the study reach would decrease the substrate decay while biofilms have a larger effect on smaller flow depths in the reduction of SOC. However, using the mean values for the hydraulic parameters, good agreement can generally be found.

Biofilm parameters: In this section, mean values of hydraulic parameters are used and the biofilm parameters varied. The plots on Figs. 56 and 58 show the sensitivity of the parameters. The value of k_F in $R_{B20} = k_F S$ was varied within the 95% confidence intervals obtained. Values between the upper limit of k_F = 0.0164 m/h and the mean k_F = 0.0126 m/h appear to best fit the measured values for both 1980 and 1981. The variations in Y_A in the established range of 0.38 to 0.46 has very little impact on the predicted substrate decay.

DISCUSSION

In the previous chapter, the results obtained over the three summers of 1979, 1980 and 1981 have been analyzed and their significance discussed. This chapter will deal with a discussion of topics that are related to this study and were not covered in the previous chapter.

Diffusional Limitations

The importance of diffusional limitations of the electron donor and acceptor cannot be overemphasized. The general substrate diffusional limitations can cause endogenous decay of the biomas and that of oxygen would result in product formation. Substrate and oxygen diffuse into the biofilm until the thickness becomes large enough to impede their passage to the bottom layer. The film becomes stratified when there is enough oxygen in one layer causing an aerobic portion while the oxygen deficient layer becomes anaerobic. Substrate or the food may still diffuse to the basal layer even when there is restriction of oxygen. Even if the substrate diffusion is limited, there may be enough nutrients that were adsorbed and not utilized before the formation of limiting thicknesses (81,108). The methods of determining oxygen limitations and their consequences on the system studied hare already been dealt with under the review of literature. Unless oxygen profiles using a micro probe are studied in a slime layer, it is difficult, if not impossible to make any conclusive statement about oxygen limitations. However, an effort will be made to check the limitations using some theoretical developments and available parameters in the literature. Williamson and McCarty (146) used the following equation,

$$S_{oa} < \frac{D_{cd}\,\nu_a\,MW_a}{D_{ca}\,\nu_d\,MW_d} S_{od} \qquad (42)$$

to predict whether DO is flux limiting in heterotrophic biofilms. The symbols in this equation have already been defined under the review of literature. Since the exact stoichiometry in a mixed river biofilm is not known, a simple reaction may be assumed as follows:

$$C + O_2 \rightarrow CO_2 \qquad (43)$$

This would yield $\nu_d = \nu_a = 1$, $MW_d = 12$, $MW_a = 32$ where the electron donor is carbon and the electron acceptor is oxygen. Assumptions on the values for D_{cd} and D_{ca} have to be made and can be based on available values in the literature. Bungay (17) determined $D_{O_2} = 0.035$ cm^2/day for heterotrophic biofilms. Williamson and McCarty (146,147) reported D_{O_2} values in the range of 1.9 to 2.6 cm^2/day. The value of D_{O_2} suggested by Bungay (17) appears to be very small compared to other reported values. Williamson and McCarty (146,147) also assumed $D_{cd} = 0.5$ cm^2/day for carbonaceous waste diffusion coefficient which is very close to the value suggested for glucose ($D_{cd} = 0.6$ cm^2/day) using $D_{O_2} = 0.035$ to 2 cm^2/day, $D_{cd} = 0.5$ cm^2/day, $\nu_d = \nu_a = 1$, $MW_d = 12$, $MW_a = 32$ and Eq. (2),

If $D_{O_2} = 0.035$, $S_{oa}/S_{od} < 38$,

$D_{O_2} = 2.0$, $S_{oa}/S_{od} < 0.67$ for oxygen limitations.

This means the bulk liquid concentration ratio of oxygen:carbonaceous waste must be less than or equal to 38 or 0.67 depending on whether $D_{O_2} = 0.035$ cm^2/d or 2.0 cm^2/d.

Based on the bulk liquid DO values obtained in the river and assuming S_{od} = 10 mg/l, maximum allowable carbonaceous waste concentration without inducing oxygen flux limitations would be 0.26 mg/l or 14.93 mg/l depending on the value of D_{O_2}. The use of a much smaller value reported by Bungay (17) for D_{O_2} would produce oxygen limitations at very small carbonaceous waste cooncentrations whereas use of higher values for D_{O_2} would accommodate higher waste concentrations without introducing oxygen limitations. It may be noted that the SOC in the river never exceeded 12 mg/l. It should also be recognized that the above analysis was simplified to study a complex heterotrophic microbial film and a mixed undefined substrate and therefore the analysis is not in the least conclusive. The analysis however, does show the problems in such analyzes because of the unknown stoichiometry of the reaction and the lack of reliable diffusional coefficients for the particular system studied.

It is difficult if not impossible under these circumstances to confirm the non-existence of oxygen limitations in the film. However, in view of the supersaturated levels of DO above and below the outfall most of the day, an almost constant yield coefficient for biomass at steady state and the existence of green pigments observed in the basal layers, it is reasonable to assume that major oxygen limitations were absent in the films studied. The results were analyzed on the basis of no oxygen limitations.

Steady and Unsteady State Models

Steady state conditions were assumed for the proposed models in predicting the three main parameters S, X and Th for purposes of validation of models. Unsteady state models for purposes of verification would require extensive data collection at all the sites with

data for diurnal variations on several days. Besides, steady state assumptions reduce complexity of the mathematical treatment. An unsteady state modeling exercise for the substrate will be described in this section. Eq. (19) given under the theoretical considerations was programmed and solved using numerical methods. The computer program 'DECAY 4' is annexed in the Appendix.

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

The diurnal variation curve at Site 2 shown in Fig. 40 for Sept. 26, 1980 was used in order to formulate an input function. Using the subroutine available in the TI programmable 58/59 calculator, a Fourier function was developed as follows:

$$S = 8.26 - 1.48 \cos\left(2 \frac{\pi \cdot T}{9}\right) \qquad (44)$$

This was used as the input function varying diurnally at Site 2. The results of this exercise are plotted as shown in Fig. 59. The plots in Fig. 59 show diurnal variations at two sites further downstream of Site 2 given the variation below the outfall. The further the site from the outfall, the variations over a day become minimal. The maximum variations in SOC corresponding to the diurnal variation in the sewage outfall discharge occur at Site 2. In the unsteady state model, the diurnal variation of SOC at Site 2 is taken into account assuming constant hydraulic conditions. It can also be seen from this exercise that extensive data collection is needed to verify the unsteady state model. This is beyond the available resources for this study. Sampling for organic carbon and solids were done during steady state flow conditions. Equilibrium film thicknesses and biomass were obtained during the plateau phase of the growth. As have been shown, the diurnal variations in

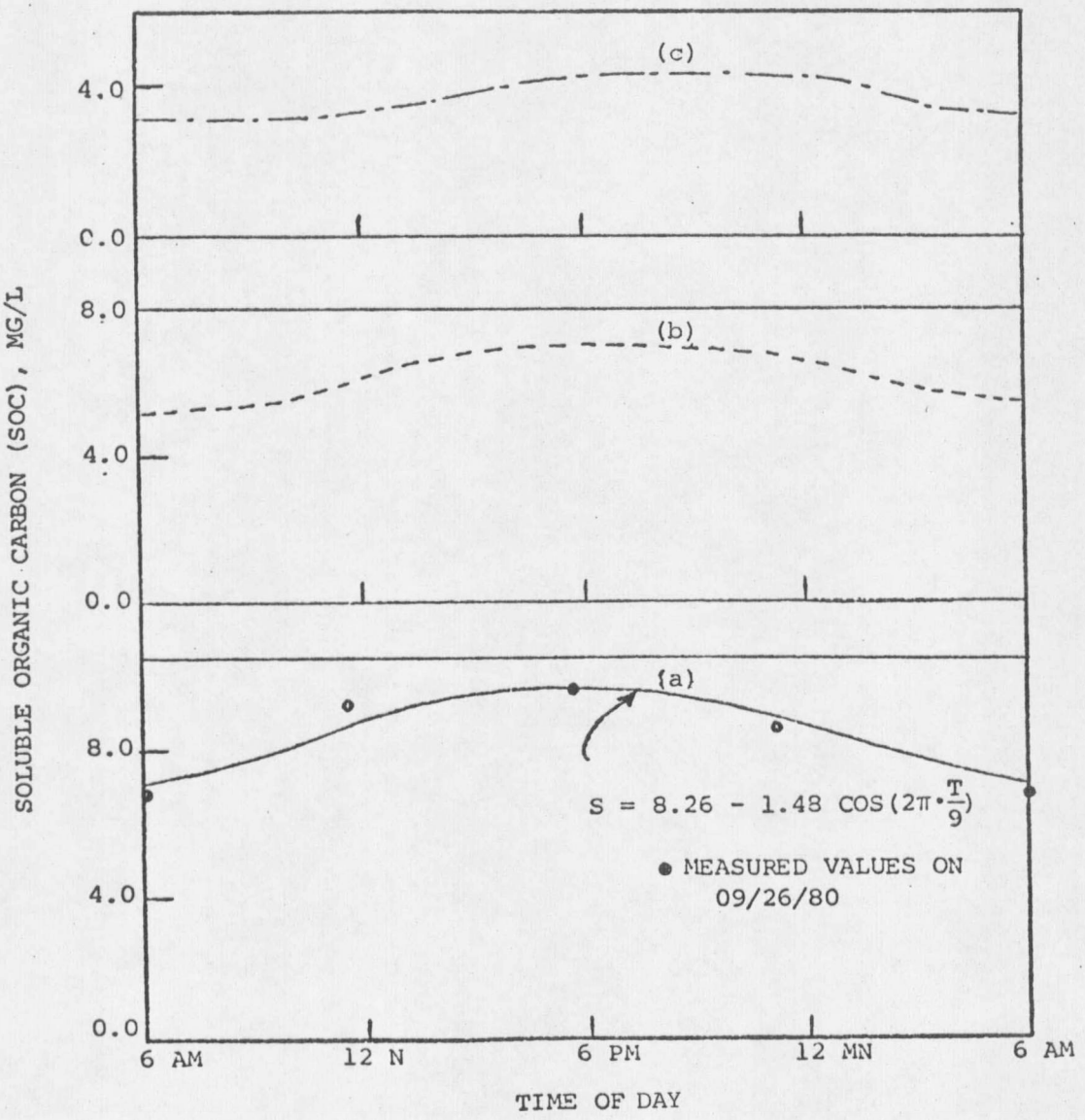


Fig. 59. Unsteady State Model Results Using Program 'DECAY 4'. (a) Diurnal Variation of SOC at Site 2, (b) Diurnal Variation of SOC at 4 Km Below Site 2, (c) Diurnal Variation of SOC at 10 Km Below Site 2.

sewage outfall discharge appear to have caused major variations in SOC at Site 2 with only smaller variations further downstream from the outfall. The largest variation in SOC appears to occur at Site 2 during a day between 4 A.M. and 10 A.M. Samples were collected during the day time generally between 10 A.M. and 4 P.M. The flow time between Sites 2 to 6 is about 5 hours during which time the variation at Site 2 for SOC is smaller compared to the total removal in the same stretch during the sampling period on a day. Considering all these factors, it is justifiable to use steady state assumptions in the proposed models to study and validate the models for the critical period of a day when the organic carbon concentrations are the highest.

Kinetic Expressions

In this study, the kinetics of substrate uptake was determined to be a first order reaction rate. However, the use of saturation kinetic expressions in describing such a reaction rate is possible with appropriate values for the coefficients since under certain conditions saturation kinetic formulations can be shown to degenerate to first and zeroth order. This depends on the ratio of the concentration of the water quality parameter studied to the corresponding half-saturation constant. An attempt was made to determine what range of values would be obtained for the parameters by solving Atkinson's (4) biofilm model described under the review of the literature. This model uses an effectiveness factor λ and accounts for solid phase diffusional limitations. The problem of using this model lies mainly in the number of parameters required, which are mostly unknown. The Eqs. (6) to (11) given earlier are used for this discussion. Even assumptions of values for effective diffusion coefficient within the microbial mass, D_e or half-saturation constant, K_s

are difficult for a natural system for an undefined substrate. The range of K_s values for example can be 5 to 500 mg/l. In order to see what range of values are possible for the half saturation constant K_s and the maximum substrate consumption rate per unit volume of film, 'αa', a curve fitting procedure was used to determine values of the rate of substrate consumption per unit interfacial area (N) using the assumed range of values for D_e and K_s and compared to the values of N obtained in this study. The following values were assumed for this exercise:

αa = 800 to 4000 g/m^3-h; K_s = 5 to 500 mg/l; $D_e = 2.5 \times 10^{-6}$ m^2/h (glucose); Th = 444 μm, S = 5 to 15 mg/l.

The Eqs. 6 to 11 were programmed on a TI programmable 59 calculator and the K_s values were varied for a particular value of 'αa' and the values of N were calculated. From this exercise, a range of 5 to 10 mg/l for K_s and 'αa' = 1500 to 2000 g·m^3-h were obtained that would give values of N close to the values obtained in this study. This is however, merely an exercise in determining possible values for K_s. The value of the half-saturation constant K_s is a result of two principal activities, one being the transfer of substrate from the bulk solution to the biomass and the other being the substrate utilization by biochemical reactions of the biomass. Very high values for K_s would suggest substrate diffusional limitations. This model has its limitations in the application to biofilms containing filamentous organisms because of its assumption that discrete viable organisms are dispersed in the slime. However, this model was chosen for this exercise because it incorporates substrate diffusional resistance. In the context of this discussion, the specific biomass production rate curve given in Fig. 31 can be compared with a simple monod function given as

$$r = \frac{\mu_m S}{K_s + S} \tag{45}$$

when

$$S \ll K_s, \mu = (\frac{\mu_m}{K_s})S$$

or

$$\mu = K.S \text{ where } K = \mu_m/K_s = \text{constant} \tag{46}$$

In the range of concentrations of substrate very much less than the values of the half-saturation constant, the reaction rate will be first order according to Eq. (46). This, however, may imply higher values for the half-saturation constant and therefore possible substrate diffusional limitations.

Characterization of Mixed River Biofilms

River biofilms are a complex community of algae, bacteria, protozoa and other micro and macro organisms. They are involved in several functional processes including nutrient cycling and primary productivity. Investigators generally consider or choose to analyze one group of organisms within a community because it is impossible to characterize all the species. However, it is their complexity in composition that helps in survival and persistence under different stressed situations in aquatic systems. In this study the emphasis was on the overall soluble organic carbon removal by the biofilms and their productivity. Since river water has several organic compounds and cannot be defined easily, SOC was used as the substrate for the biofilms with the availability of other nutrients. Non-taxonomic structural analyses such as gravimetric and pigment analyses were used in estimating

biomass production and partitioning of the major groups of organisms. Conventionally biomass values have been expressed as organic matter, organic carbon or caloric values. In this study ashfree weight (AFW) giving the organic matter was used in estimating biomass because it has been used as a standard technique, requires minimal equipment and costs are low. AFW estimates organic content of all biological and other organic components of the biofilm without distinguishing between viable and non-viable components of the samples. Acetone extracts of biofilm samples have been conventionally used in calculating Chlorophyll values which can be converted to estimate algal biomass. It must be recognized however, that Chlorophyll is a very dynamic cellular constituent and affected by factors such as algal cell's age, physiological conditions and nutrient deficiencies. Rapid ATP degradation after cellular death makes ATP specific for living organisms. Besides ATP provides estimates of viable cell mass. Organic carbon biomass estimates can also be obtained from ATP measurements using appropriate conversion factors even though ATP-to-cell-carbon ratios may vary with nutrient conditions and among the different taxonomic divisions. Using all these measurements and recognizing the biomass associated with each method, biofilm samples could be partitioned into autotrophic-heterotrophic and living-nonliving organic matter. This will be useful in the general characterization of river biofilms.

Ash-free dry weight, Chlorophyll a and ATP values can be related to the organic carbon content thus providing a basis for comparison of biomass. A study was carried out in 1981 measuring ATP and AFW on the same biofilm samples grown on cobbles and glass slides. These measurements converted to an areal basis are given in Table 27. Clark (21) defined Viability Index as follows:

Table 27. Organic Carbon Estimates and Viability Index.

Type of Substrate	Site	ATP-derived Organic Carbon g/m^2	AFW-derived Organic Carbon g/m^2	Viability Index (VI)
1. Cobbles	1	3.8	25.8	0.15
	2	6.9	37.3	0.18
	3	5.1	30.0	0.17
	4	4.6	30.4	0.15
	5	4.1	24.1	0.17
	6	3.5	22.3	0.16
2. Cobbles	1	3.6	30.5	0.12
	2	4.2	31.6	0.13
	3	3.2	24.6	0.13
	4	4.0	26.5	0.15
	5	3.9	28.2	0.14
	6	3.8	25.8	0.15
3. Glass Slides	1	0.6	13.3	0.05
	2	2.4	16.4	0.15
	3	1.4	15.0	0.09
	4	2.1	14.3	0.15
	5	1.1	14.3	0.08
	6	1.7	12.1	0.14

$$\text{Viability Index (VI)} = \frac{\text{ATP - derived organic carbon}}{\text{AFW - derived organic carbon}} \qquad (47)$$

It must be noted that organic carbon content of biofilm samples can be measured by direct chemical analysis. Conversion factors for AFW and Chlorophyll a to organic carbon have been described under the results. ATP has been found to maintain a relatively constant ratio with cellular carbon (56). The ratio of μg cellular organic carbon/μg ATP has been reported to vary from 250 to 276 (21,56). Since the ratio of 250 is commonly used, this will be used in this analysis. ATP-derived organic carbon appears to be much smaller than AFW-derived values and consequently smaller viability Index values are given in Table 27

at the different sites. However, this is based on the conversion factors available in the literature and three sets of measurements. Besides, if ATP extraction is not complete, it may provide lower biomass estimates as organic carbon. It is obvious that AFW-derived organic carbon would yield higher estimates because it includes all components of organic matter. Viability Index values can be used as a measure of the viability of biomass. However, if direct measurements of organic carbon are coupled with the measurements described so far, a reliable procedure for partitioning a river biofilm on the basis of their trophic levels and viability can be done. This would be very helpful in a better characterization of a mixed community river biofilm.

Limitations of the Proposed Models

Models have their own limitations. The limitations have to be recognized before they are applied to different situations.

(1) The proposed models for predicting soluble organic carbon and biomass variations are mathematical models incorporating parameters and kinetic expressions developed empirically from pilot plant studies. It is therefore important to recognize the valid ranges of the parameters. Expressions for R_B vs. S, R_D vs. M and coefficients of yield were determined under constant hydraulic conditions for a swift moving, shallow stream with the variation of soluble organic carbon in the range of 5 to 17 mg/l and a detention time of one hour. The effects of variable hydraulic conditions on R_B or R_D are not incorporated in the models. Caution must be exercised when using the models outside the specified ranges.

(2) Naturally grown river biofilms were used in the pilot plant study because it was a

cost-effective method of developing such a film in an outdoor channel. Besides it was developed in the system that would be subsequently modeled. However, the limitation of this procedure lies in the fact that the exact composition of such a film is difficult, if not impossible to define. But it can also be seen that parameters developed using a well defined laboratory film cannot be used to closely simulate a natural system either. Studies done with well defined films have been discussed under the literature review.

(3) Models were formulated for a specific shallow river system and tested under steady state conditions. The performance of the models using unsteady state conditions or in an alternate but similar system is not known.

(4) When there are several point sources of pollution in a study reach, only one point source at a time can be considered in the model. However, the model can be extended in a piece-wise fashion.

(5) The prediction of biofilm depth is a difficult parameter to verify in a river system, because the measured film depths are based generally on biofilm samples grown on artificial substrates such as glass slides. Biomass estimates obtained on samples from artificial and natural substrates in this study indicate higher accumulations on natural substrates. The limitations of the model in predicting biofilm depth is its requirement of an estimate for biodensity which has to be available.

Any model simulating a natural system can be expected to have several limitations in its application to other systems. The objective of this investigation was not to develop a universally applicable model, but to lay down the methodology for realistically modeling a specified river system by generating kinetic data in a pilot plant study and incorporating

this data into the substrate-biomass models formulated based on firm theoretical considerations.

Original Contribution of This Research

The importance of sessile microbial communities in river systems has been established by several investigators as reviewed previously. However, the explicit use of biofilms in modeling river systems where they dominate organic degradation has not been attempted. This study demonstrates the importance of biofilms in determining the kinetics of soluble organic carbon uptake in the East Gallatin River. The study also shows that when organic pollutional loads are discharged into a river, the variations of organic carbon concentration and the biomass can be realistically modeled using the proposed three parameter substrate-biomass formulation. The model is based on theoretical considerations with supporting kinetic data for the biofilms generated from pilot scale studies. In the opinion of the author, a study of this nature has not been completed in the past.

Significance of Some Results in This Study to the Bozeman Sewage Treatment Plant Expansion

In the current expansion of the Bozeman Sewage Treatment Plant, additional secondary treatment facilities are being built and tertiary treatment facilities to remove ammonia have also been proposed for the near future. In this discussion, tertiary treatment to remove ammonia will be considered. Very low ammonia concentrations in the river are expected below the sewage outfall after tertiary treatment. It is important to recognize the significance of the background concentrations of ammonia which will not be reduced by

introducing tertiary treatment. Results of this study indicate that the low concentrations of ammonia which will not be reduced by introducing tertiary treatment. Results of this study indicate that the low concentrations of ammonia below 0.1 mg/l anticipated after teriary treatment are difficult, if not impossible to be achieved considering the background ammonia levels in the range of 0.1 to 0.4 mg/l. If the East Gallatin River is receiving enrichment above the outfall and has higher background ammonia levels, providing an expensive tertiary treatment facility will not permit the achieving of the low level concentrations anticipated below the outfall. Besides, the high ammonia concentrations in the river presently decays rapidly in a short stretch of the river and no fish kills have been observed by the Department of Fish and Wildlife below the outfall in the past few years. In fact, personal communication with a department official indicates an increase in the fish population with a reduction in fish length. It is therefore questionable whether expensive tertiary treatment would result in the low ammonia concentrations anticipated after completion of the facility.

SUMMARY AND CONCLUSIONS

Summary

The study was directed towards the goal of establishing the growth and substrate utilization kinetics of heterogenous natural biofilms in multi-substrate environments. The objectives of this particular research were designed to determine the substrate (Soluble Organic Carbon) uptake by river biofilms with pilot channel studies and incorporate the kinetic expressions developed into the proposed models for describing organic carbon degradation in streams. The entire experimental investigation including a pilot plant scale experimental study and river data collection was tailored to achieve these objectives. In the following section the results of this study conducted over the summers of 1979, 1980 and 1981 will be summarized.

The preliminary study results obtained during 1979 laid down the foundation for the detailed investigations carried out subsequently. The measurements of several water quality and biofilm parameters taken above and downstream of the sewage outfall established that the river biofilms are the major component of the assimilative capacity of the shallow river system and were responsible for the rapid degradation of organics within 7 miles below the sewage outfall. In the stretch of 7 miles below the sewage outfall the results exhibited constant stream flow conditions while major variations in substrate concentration and biofilm growth occurred.

Based on the conclusions of the preliminary study, the major hypothesis to be tested was whether in determining the kinetics of organic carbon uptake, river biofilms are the most significant microbial community in comparison to the suspended biomass. An exper-

imental pilot plant scale channel study was completed to determine kinetic expressions for the biofilms. Twenty experimental runs including 3 control runs and a run with tap water were made. The variations of SOC, VSS, DO, pH, temperature were studied for each run. Negligible variations in SOC, VSS and DO were shown for the control runs without the attached biomass in comparison to the regular runs. The range of influent SOC concentrations were varied from 5 to 20 mg/l. The attached biomass production rate R_B, the detachment rate R_D and the biofilm yield coefficient Y_A were determined for the runs under steady state conditions. A mean biofilm yield coefficient Y_A was determined in the range of SOC concentrations studied. Functional relationships of Y_A vs. S or $(S_i - S)$ showed no significance. A comparison of measured Y_A values and calculated Y_A values under steady state conditions showed a fairly good agreement even though the measured Y_A values were generally larger. For this comparison, the measured Y_A values were corrected for autotrophic contribution to organic accrual using two different methods.

Temperature corrections for R_B and R_D were determined using a coefficient θ in $R_B = R_{B20}\,\theta^{T-20}$. The relationship between R_B and S (measured as SOC) established first order kinetics yielding $R_{B20} = k_F \cdot S$ in which $k_F = 0.0126$ m/h. Biofilm detachment rate was related to the biomass (measured as ash-free dry weight) providing values for detachment rate coefficients α_D and β in the expression $R_D = \alpha_D M^{\beta}$. The regression statistics for the relevant parameters was also included in the results. Converting R_B into a specific biomass production rate, $r_s (r_s = R_B/M_A)$ the variation of r_s with S appeared to be linear. This agrees with the fact that saturation kinetic expressions reduce to linear formulations under certain conditions as explained under discussion. The plot giving the SOC removal

with the SOC loading rates, R_L, showed a large increase in SOC removal in the range of R_L from 0.7 to 1.0 g/m^2-h and stabilization subsequently.

The river data collection during 1980 and 1981 was done in order to test the usefulness of the kinetic expressions developed in the proposed models under steady state conditions. Based on the preliminary study, the study reach was reduced to 7 miles below the sewage outfall. Hydraulic, water quality and biofilm parameters were measured at 6 sites, one above and five below the sewage outfall within the study reach. Mean values of hydraulic parameters namely flow velocity and depth based on measurements at the different sites were compared with the estimated average flow velocity and depth obtained for the whole study reach as stream flow was almost a constant. The water quality parameter measurements included TOC, SOC, BOD_L, DO, pH, NH_3-N, NO_3-N and water temperature. Organic carbon measurements showed that during the low flow period, the major proportion of the organic carbon in the river is soluble. The highest particulate organic carbon concentration existed at Site 2 below the outfall, of which a significant reduction occurs by Site 3 or 4. The BOD_L changes obtained during 1980 followed a decay pattern similar to that obtained during the preliminary study. The SOC decay patterns in 1980 and 1981 were similar to the BOD_L data of the years 1979 and 1980. Except for a small drop in DO below the outfall, high levels of DO in the range of 9 to 12 mg/l were obtained during the sampling periods. Increased solid concentrations measured as SS and VSS were observed below the outfall due to increased scouring activity. Ammonia decayed to the background levels within the study reach while nitrate values increased in SOC during a day appeared to occur after midnight. Incorporating such variations into the organic carbon model were illustrated.

Electron Micrographs done on biofilms above and below the outfall showed its general structure. The biofilm characteristics were studied by measuring film thickness, ash free weight, chlorophyll and ATP. Ash free weight was used as a measure of biomass. The variation of biomass and thickness showed that the plateau of growth was reached within 30 to 35 days of exposure. Steady state equilibrium film thicknesses and biomass were determined and used in the determination of biodensities. Biomass productivity appeared to correspond to the organic loadings in the river. Autotrophic Index measurements in the study reach showed highly heterotrophic biofilms below the sewage outfall. ATP measurements done on biofilms in 1981 provided lower biomass and organic carbon estimates yielding low Viability Indices. The equations given below as a proposed model were solved under steady state conditions and the computer program 'DECAY 2' used to predict variations is annexed in the Appendix. Plots showed good agreement of the predicted and measured values.

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

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

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

A sensitivity analysis of the hydraulic parameters in the decay of organic carbon indicated negligible variations for an estimated D_L range of 100 to 20,000 m^2/h. Increased average velocities and flow depths decreased the substrate decay below the outfall. The biofilm parameters were varied within the confidence intervals for the parameters. Values of k_F

in the range of 0.0126 to 0.0164 appear to best fit the measured decay profiles while variation in Y_A in the range of 0.38 to 0.46 did not have a significant impact on the predicted SOC values.

Conclusions

The following conclusions were drawn based on the field and pilot plant scale experimental studies.

1. Substrate (Soluble Organic Carbon) uptake by the heterotrophic river biofilms followed first order kinetics in the specified range of substrate concentrations.
   River biofilms under polluted conditions, in comparison to suspended biomass, were the most significant microbial community and dominated the degradation of organic carbon.
2. Good agreement was found between the predictions of the proposed model and the measured organic carbon decay and biomass variations below the sewage outfall.

Future Studies—The following are possible studies recommended for the future.

1. Artificial stream channel studies to determine the effects of hydraulic parameters such as flow velocity, shear velocity, depth and temperature on substrate removal and biofilm characteristics. This would permit a comparison of the substrate removal capabilities of biofilms between swift shallow streams and sluggish deep rivers.
2. Controlled studies of mixed substrate utilization in heterogeneous microbial communities. Studies on the pure and mixed substrate utilization by known propor-

tions of algal bacterial mixes will be helpful in understanding the complex microbial communities in the natural environments.

APPENDICES

APPENDIX A

Experimental Channel Runs Not Included in the Results

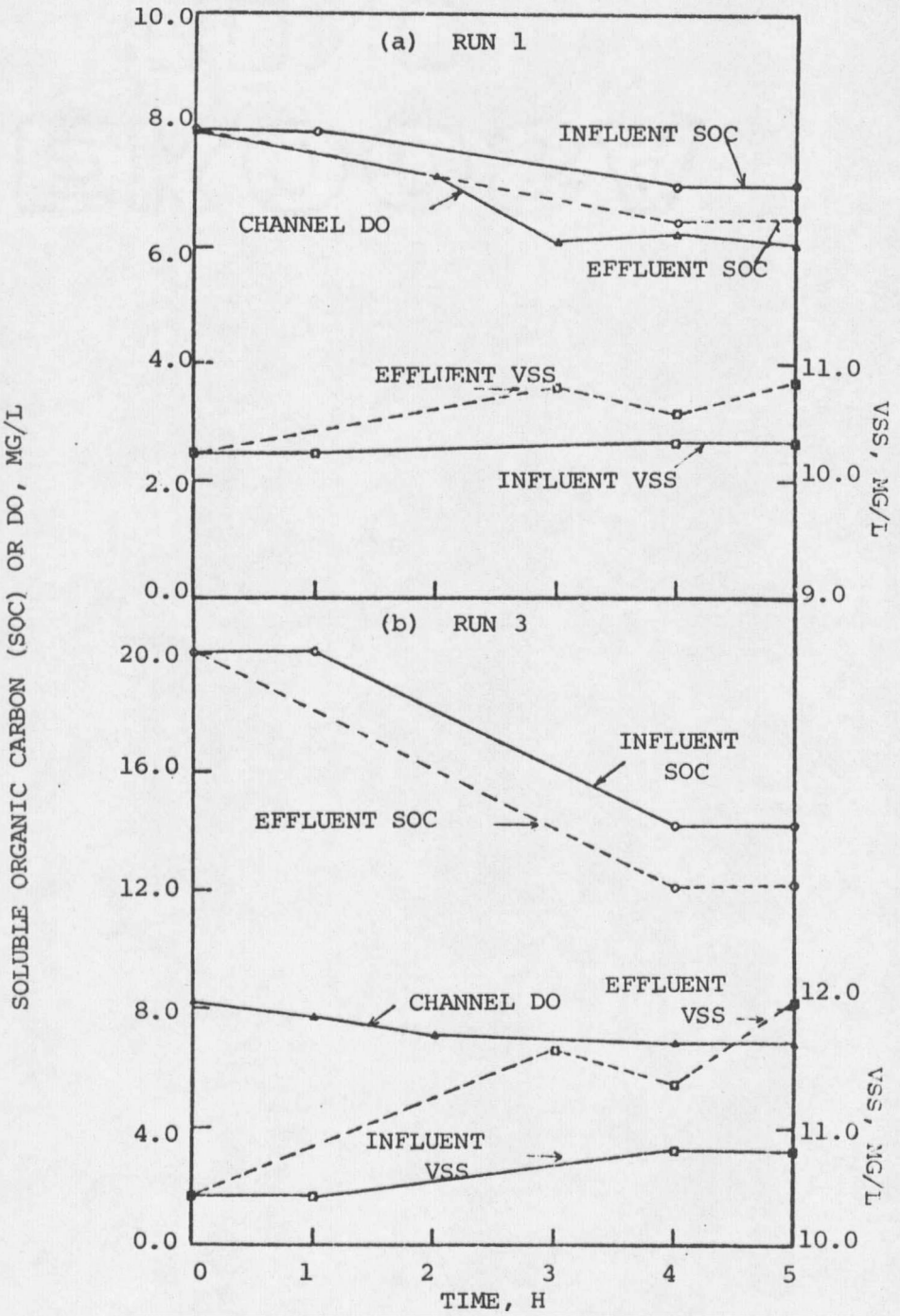


Fig. A1. Organic Carbon, Channel DO and VSS Variation with Time for (a) Run 1 and (b) Run 3.

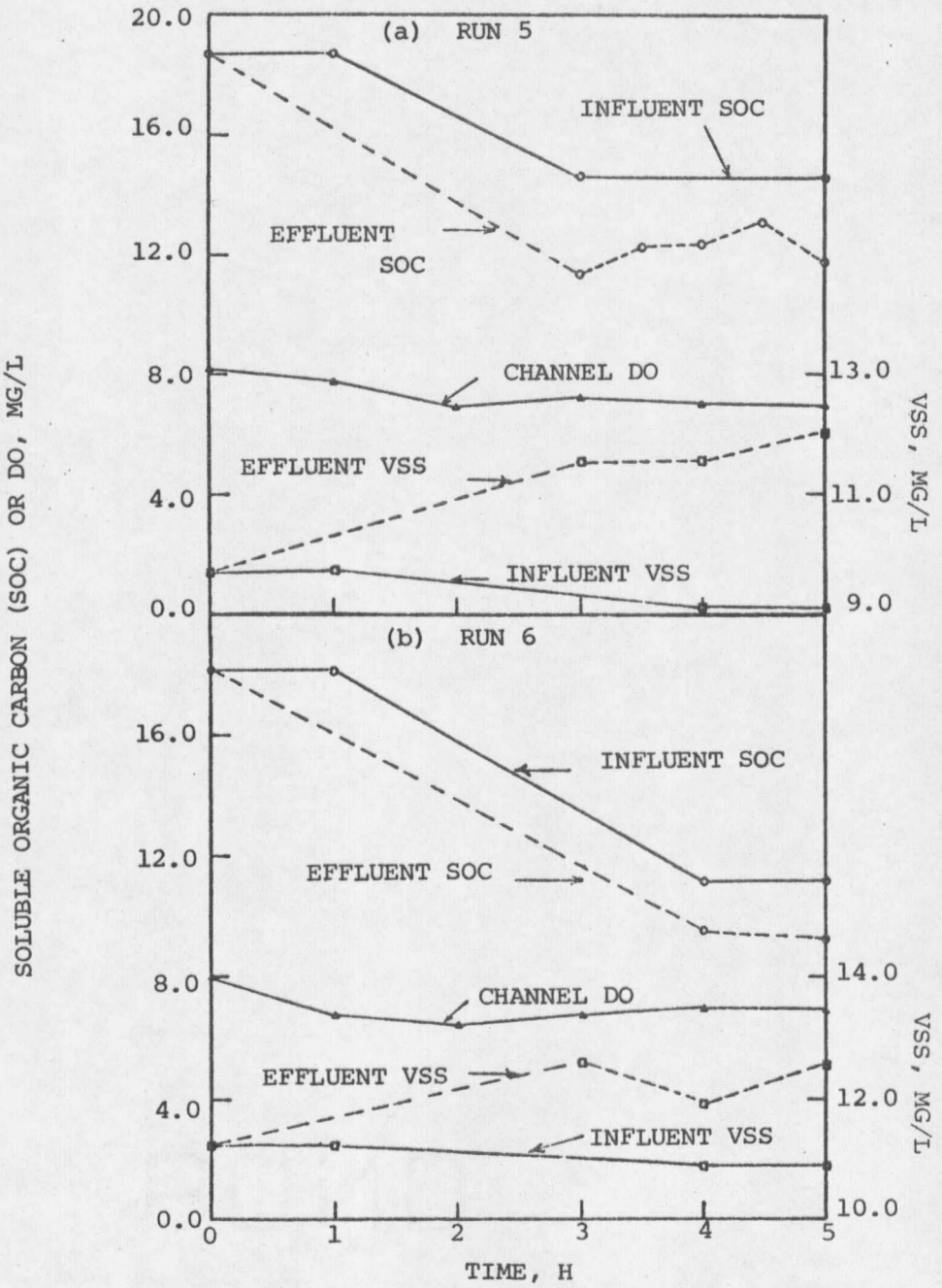


Fig. A2. Organic Carbon, Channel DO and VSS Variation with Time for (a) Run 5 and (b) Run 6.

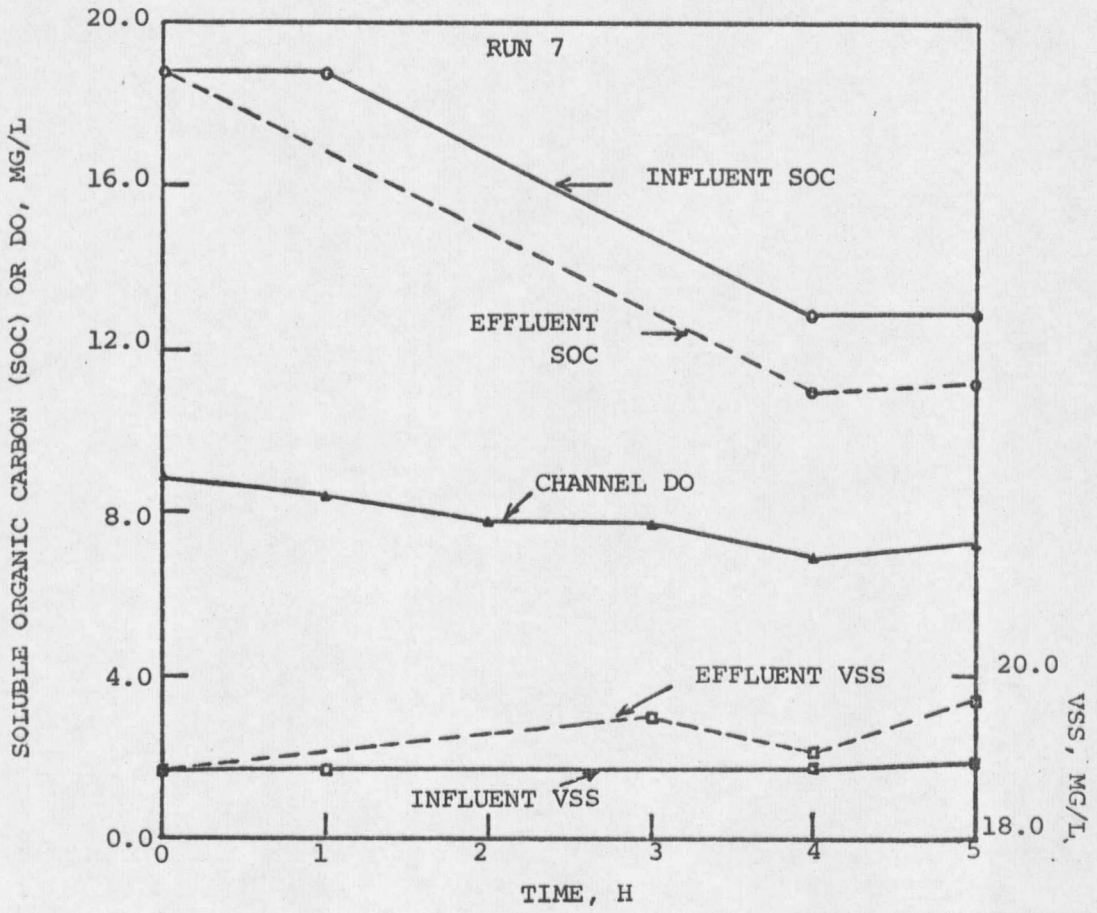


Fig. A3. Organic Carbon, Channel DO and VSS Variation with Time for Run 7.

APPENDIX B

River Bed Elevations

The following bed elevations were established by a survey during the preliminary study.

Site	Elevation(ft)	Description of Site
EG 1	4610.02	Bridge on Spring Hill Road
EG 2	4609.70	Small bridge on country club golf course
EG 3	4452.64	Bridge on Airport Road
EG 4	4341.02	Steel bridge on Hamilton Road
EG 5	4326.75	Concrete bridge on Broadway about 4 miles from Belgrade
EG 6	4246.65	Steel bridge on Drycreek School Road

APPENDIX C

Computer Programs

DECAY 2–Steady State Dispersion Model

DECAY 4–Unsteady State Model

```
15:09 MAR 03 '82 DECAY2.RCMAA

C       INITIATION***DECAY2-STEADYSTATE DISPERSION MODEL
        PROGRAM LIST
        INPUT DZ,PI,PF,IO
5       INPUT SO,XO,U,Y,H,T,DE,DL,A,B
        BE=2.46;ALD=.0000147
        IF (SO.EQ.0.) STOP
        Z=0.
        S=SO
        X=XO
4       RB=(A*S)+B
        RBT=RB*1.098**(T-20.)
        TH=(RBT/(ALD*(DE**BE)))**(1./BE)
        V1=U/DL
        A1=(A*1.098**(T-20.))/(Y*H*DL)
        B1=(B*1.098**(T-20.))/(Y*H*DL)
        AM1=(V1+(V1**2+(4.*A1))**.5)/2.
        AM2=(V1-(V1**2+(4.*A1))**.5)/2.
        F=(SO+(B1/A1))/(1.-(AM2/AM1))
        S=(F*EXP(AM2*Z))-(B1/A1)
        DX=ALD*((DE*TH)**BE)/(U*H)
        CALL PRNTF(PI,PF,NF,Z,TH,S,X,AM2,DX)
        GO TO (13,5),NF
C       INTEGRATION***
13      CALL INTI(Z,DZ,IO)
        CALL INT(X,DX)
        GO TO 4
        END
```

```
15:10 MAR 03 '82 DECAY4.RCMAA

C       DECAY----NON-STEADYSTATE USING CHE.ENG.LIB.
        DIMENSION S(0:100),DS(0:100),RB(0:100),RBT(0:100)
        OUTPUT 'ENTER Z,...'
        INPUT Z,DZ,DT,PI,PF,IO
        OUTPUT 'ENTER SO,...'
2       INPUT SO,BG,V,Y,H,TEMP,ALD,DE,DL
C       INITIATION **
        A=0.0126;B=0.0
        T=0.0
        M=Z/DZ
        OUTPUT 'READING THRU 106'
        READ(106,1000)S
1000    FORMAT(25X,F8.4,40X)
        OUTPUT 'FINISHED WITH 106'
C       BOUNDARY CONDITIONS***
4       T1=1.+T/3.
        S(0)=8.26-1.48*COS(2.*3.14*T1/9.)
        S(M)=S(M-1)
        DO 11 N=1,M-1
        RB(N)=(A*S(N))+B
        RBT(N)=RB(N)*1.098**(TEMP-20.)
        DS(N)=(DL/DZ**2.)*(S(N+1)-2.*S(N)+S(N-1))
       /-(V/(2.*DZ))*(S(N+1)-S(N-1))-(RBT(N)/(Y*H))
11      CONTINUE
        CALL PRNTF(PI,PF,NF,T,S(0),S(M/5.),S(2*M/5.),
       /S(3*M/5.),S(M))
        GO TO (7,2),NF
7       CALL INTI(T,DT,IO)
        DO 9 N=1,M-1
        CALL INT(S(N),DS(N))
9       CONTINUE
        GO TO 4
        END
```

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LITERATURE CITED

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