12

Biofilms and Microbial Fouling

W. G. CHARACKLIS AND K. E. COOKSEY*

College of Engineering,
Montana State University,
Bozeman, Montana, and
**Division of Biology and Living Resources,*
Rosenstiel School of Marine and Atmospheric Science,
University of Miami,
Miami, Florida

I. Introduction 93
A. Definitions 93
B. Relevance and Application of Biofilms 94
C. Objectives 94
II. Biofilm Formation: A Process Analysis 97
A. Transport to the Wetted Surface 97
B. Adsorption of a "Conditioning" Film 101
C. Adhesion of Microbial Cells to the Wetted Surface 104
D. Reactions within the Biofilm 114
E. Detachment of Biofilm 118
III. Properties and Composition of Biofilms 119
A. Physical Properties 120
B. Chemical Properties 120
C. Cellular Densities 121
IV. Physiological Ecology and Biochemistry 122
A. Interactions between Biotic and Abiotic Components 122
B. Interactions between Biotic Components 126
V. Effects of Biofilms 129
A. Fluid Frictional Resistance 129
B. Heat Transfer Resistance 130
C. Corrosion 130
D. Water Quality in Natural Streams 131
VI. Areas of Research in Need of Further Study 131
VII. Summary 132
References 133

I. Introduction

A. Definitions

Microbial cells attach firmly to almost any surface submerged in an aquatic environment. The immobilized cells grow, reproduce, and produce extracellular polymer substances (EPS) that frequently extend from the cell, forming a tangled mass of fibers lending structure to the entire assemblage which shall be termed a *biofilm.* The term biofilm does not necessarily imply a surface accumulation that is uniform in time and/or space.

Biofilms serve beneficial purposes in natural environments and in some modulated systems. For example, biofilms are responsible for removing organic and inorganic "contaminants" from natural streams and in wastewater treatment processes (e.g., trickling filters and rotating biological contactors). However, the term *fouling* is generally reserved for those occasions when biofilms are a nuisance (Table I).

Fouling refers to the undesirable formation of inorganic and/or organic deposits on surfaces. These deposits can impede the flow of heat across the surface, increase the fluid frictional resistance at the surface, and increase the rate of corrosion at the surface. In any case, energy or material losses result.

Several types of fouling and their combination may occur: (1) crystalline or precipitation fouling, (2) corrosion fouling, (3) particulate fouling, (4) chemical reaction fouling, and (5) biological fouling or biofouling. Biological fouling results from (1) development of a biofilm consisting of microorganisms and their products (microbial fouling), (2) deposition and growth of macroorganisms such as macroalgae, barnacles and mussels (macrobial fouling), and/or (3) assorted detritus. This paper will address only microbial fouling.

Microbial fouling often accelerates other types of fouling. For example, microbial fouling can increase the deleterious effects of sedimentation (or particulate) fouling by providing a more "adsorbent" surface (Zelver *et al.*, 1982). Biofilms apparently can influence precipitation processes (Turakhia, unpublished results) and corrosion processes (Characklis *et al.*, 1983). Microbial fouling always precedes macrobial fouling although it may not be a necessary prerequisite (Wood, 1967; Miller *et al.*, 1948).

B. Relevance and Application of Biofilms

Table I lists some of the relevance of biofilms to various rate processes—both beneficial applications and nuisance conditions. Section V relates why and how biofilms affect some of these relevant processes.

C. Objectives

This article provides a framework for understanding the process of biofilm development on a surface and the consequences of the accumulation on the environment. We begin by describing biofilm development in terms of selected fundamental rate processes and environmental parameters influencing their rate and extent. The physical, chemical, and biochemical properties of the biofilm which determine the influence of the biofilm on its microenvironment are discussed. The properties of the biofilm and its microenvironment lead to a discussion of the microbial ecology within the

TABLE I

EFFECT AND RELEVANCE OF BIOFILMS ON VARIOUS RATE PROCESSES

Effects	Specific process and result	Concerns
Heat transfer reduction	Biofilm formation on condenser tubes and cooling tower fill material. *Energy losses*	Power industry Chemical process industry U.S. Navy Solar energy systems
Increase in fluid frictional resistance	Biofilm formation in water and wastewater conduits as well as condenser and heat exchange tubes. Causes increased power consumption for pumped systems or reduced capacity in gravity systems. *Energy losses*	Municipal utilities Power industry Chemical process industry Solar energy systems
	Biofilm formation on ship hulls causing increased fuel consumption. *Energy losses*	U.S. Navy Shipping industry
Mass transfer and chemical transformations	Accelerated corrosion due to processes in the lower layers of the biofilm. Results in *material deterioration* in metal condenser tubes, sewage conduits, and cooling tower fill	Power industry U.S. Navy Municipal utilities Chemical process industry
	Biofilm formation on remote sensors, submarine periscopes, sight glasses, etc., causing *reduced effectiveness*	U.S. Navy Water quality data collection

(*continued*)

TABLE I (*continued*)

Effects	Specific process and result	Concerns
	Detachment of microorganisms from biofilms in cooling towers. Releases *pathogenic organisms* (e.g., *Legionella* in aerosols)	Public health
	Biofilm formation and detachment in drinking water distribution systems. Changes *water quality* in distribution system	Municipal utilities Public health
	Biofilm formation on teeth. Causes *dental plaque and caries*	Dental health
	Attachment of microbial cells to animal tissue. Causes *disease* of lungs, intestinal tract, and urinary tract	Human health
	Extraction and oxidation of organic and inorganic compounds from water and wastewater (e.g., rotating biological contacters, biologically-aided carbon adsorption, and benthal stream activity). *Reduced pollutant load*	Wastewater treatment Water treatment Stream analysis
	Biofilm formation in industrial production processes *reduces product quality*	Pulp and paper industry
	Immobilized organisms or community of organisms for conducting *specific chemical transformations*	Chemical process industry
	Fouling biofilm accumulation *reduces effectiveness* of ion exchange and membrane processes used for high quality water treatment	Desalination Industrial water treatment

biofilm and the physiology of the organisms immobilized within it. Finally, the effects of the biofilm on its environment, both beneficial and detrimental, are presented. The literature reviewed was not restricted to that concerning the microbiology of biofilms. For example, studies of mammalian cell adhesion have much to teach us concerning the initial events in attachment of microbes to substrata. There are many questions and concerns stated in this article regarding biofilm processes. The last section presents our viewpoints on areas for future research concerning this topic.

II. Biofilm Formation: A Process Analysis

The physical, chemical, and biological transformations of interest in biofilm development are completed in a certain period of time. For biofilm development, a specified change may signal the shutdown of manufacturing operations and the beginning of cleaning operations. The time required for this specified change is inversely proportional to the *rate* at which the process occurs. Thus, the rate is the most important quantity in process analysis. If the overall process consists of a number of processes in series, the slowest step of the sequence exerts the greatest influence and *controls* the overall process rate. This step is called the "rate-determining step" or "rate-controlling step."

In this discussion, biofilm development will be considered to be the net result of the following physical, chemical, and biological processes (Fig. 1): (1) transport of organic molecules and microbial cells to the wetted surface; (2) adsorption of organic molecules to the wetted surface, resulting in a "conditioned" surface; (3) adhesion of microbial cells to the conditioned surface; (4) metabolism by the attached microbial cells, resulting in more attached cells and associated material; and (5) detachment of portions of the biofilm.

A. Transport to the Wetted Surface

When a clean surface is immersed in natural water, transport controls the initial rate of deposition. In very dilute suspensions of microbial cells and nutrients, transport of microbial cells to the surface may be the rate-controlling step for long periods of time. Biofilm development in open ocean waters or distilled water storage tanks may be illustrative of these cases. Transport of molecules and particles smaller than 0.01–0.1 μm is described satisfactorily in terms of diffusion. In turbulent flow, the diffusion equation must be modified to include turbulent eddy transport (an eddy is a current or bundle of fluid moving contrary to the main current). Transport of such small molecules and particles is relatively rapid compared to transport of larger parti-

cles. Consequently, adsorption of an organic film is reported to occur "instantaneously" in many cases.

Transport processes are also significant in later stages of biofilm development. For example, mass transfer and diffusion of nutrients can influence the growth rate of cells within the biofilm (see Section II,D,2).

Larger particles develop a sluggishness with respect to the surrounding fluid. As the particle approaches the wetted surface, eddy transport diminishes and the viscous sublayer exerts a greater influence. For soluble matter and small particles, diffusion can adequately describe transport in the viscous sublayer (Lister, 1979; Lin *et al.*, 1953; Wells and Chamberlain, 1967). For larger particles, other mechanisms must be considered to explain experimental observations.

Within a turbulent flow regime, larger particles suspended within the fluid are transported to the solid surface primarily by fluid dynamic forces. Particle flux to the surface increases with increasing particle concentratio However, particle flux is also strongly dependent on the physical properties of the particles (e.g., size, shape, and density) and is influenced by many other forces near the attachment surface.

Microbial cells (0.5–10.0 μm effective diameter) can be transported from the bulk fluid to the wetted surface by several mechanisms, including the following: diffusion (Brownian), gravity, thermophoresis, taxis, and fluid dynamic forces (inertia, lift, drag, drainage, and downsweeps).

1. Transport Mechanisms

Particles in turbulent flow are transported to within short distances of the surface by eddy diffusion. Particles are propelled into the viscous (or laminar) sublayer by their own momentum. Turbulent eddies supply the initial impetus and frictional drag slows down the particle as it penetrates the viscous sublayer (Friedlander and Johnstone, 1957; Beal, 1970). For microbial cells, the inertial forces are very small because of their small diameter and density (in relation to water).

If the particle is traveling faster than the fluid in the region of the wall, the *lift force* directs the particle toward the wall (Rouhiainen and Stachiewicz, 1970). This would normally be the case if particle density is greater than fluid density and the particle is moving toward the wall. *Frictional drag forces* can be significant, especially in the viscous sublayer region. The drag force slo down the particle as it approaches the surface and is proportional to the difference between particle velocity and fluid velocity.

If the mass density of the particle differs substantially from the fluid density the *gravity force* may be significant. For microbial cells in turbulent flow, the gravity force is generally negligible. *Thermophoresis* is only rele-

vant when particles are being transported through a temperature gradient (Lister, 1979). If the surface is hot and the bulk fluid is cold, the thermophoretic force will repel the particle from the surface. *Eddy diffusion* may be instrumental in dispersing particles in the turbulent core region, thus maintaining a relatively uniform concentration in that region. However, eddy diffusion will not be significant in transporting particles to the wall. *Brownian diffusion* contributes little to the transport of microbial cells (> 1.0 μm diameter) in turbulent flow. Certain microbes are capable of *motility* or *taxis* by way of their own internal energy, independent of fluid forces. Velocities as high as 50 μm/second have been observed. Taxis could possibly be a significant transport process in laminar flow or within the viscous sublayer. For particles in liquids, the *fluid drainage force* is significant (Lister, 1979). The drainage force describes the resistance the particle encounters near the wall due to the pressure in the draining fluid film between the two approaching surfaces. This force is quite large for a microbial cell as it approaches the wall.

Recent research on the structure of the viscous sublayer in turbulent flow indicates that *downsweeps* of fluid from the turbulent core penetrate all the way to the wall (Cleaver and Yates, 1975, 1976). Particles in the bulk fluid are transported all the way to the wall by these convective downsweeps. Aside from lift, this is the only fluid mechanic force directing the particle to the wall. Downsweeps are apparently quite important in terms of particle transport to the wall in turbulent flow.

For a tube 3 cm i.d. with a fluid velocity of 100 cm/second at a temperature of 20°C, the bursts resulting from the downsweeps have the following characteristics:

burst diameter	0.11 cm
average axial distance between bursts	0.50 cm
mean time between bursts	0.0006 seconds

Minimum transport rate of particles would be observed when particle diameter approximates 0.1×10^{-4} cm under constant fluid flow conditions. At this diameter, Brownian diffusion starts exerting a significant effect. Particle flux from the bulk fluid to the pipe wall for a bulk fluid particle concentration of 10^4 particles/cm^3 is approximately 0.1 particles/cm^2/second.

2. *Influence of Surface Roughness*

Surface roughness significantly influences *transport rate* and *microbial cell attachment* for several reasons, including the following: (1) it increases

convective mass transport (i.e., mass transport due to fluid motion) near the surface, (2) provides more "shelter" from shear forces for small particles, and (3) increases surface area for attachment.

If surface roughness elements are larger than the viscous sublayer, the roughness can be measured quantitatively by hydraulic methods. If surface roughness elements are smaller than the viscous sublayer (i.e., microroughness), measurements of roughness are difficult to quantify and interpret (Thomas, 1982). Browne (1974) reports that particle deposition from gases is very sensitive to surface roughness too small to affect fluid frictional resistance.

3. *Consequences of Transport Rates on Biofilm Development*

When a "clean" surface first contacts water containing biological activity, organic substances and microbial cells must be transported to the surface before biofilm development can begin. Consequently, the rate of transport of these components determines the length of the "induction" period, i.e., the initial period during which no macroscopic effects of the biofilm are evident. In very dilute solutions (e.g., open ocean), the rate of transport may control the overall rate of biofilm development for long periods. Rate of transport is proportional to the concentration difference between the bulk fluid and the surface (Bryers and Characklis, 1981). In dilute solution, this difference is small. The flow regime (zero, laminar, or turbulent flow) also significantly influences transport rates and should be defined carefully in any experimental system used for biofilm studies. Maintenance of surface characteristics is also critical in the reproducibility of the results and their application because as surface roughness increases so will transport and attachment rates. Which rate controls—that of transport or that of adhesion?

4. *Summary of Transport Processes*

So little is known about rate of transport of particles (e.g., bacterial cells) in water under fluid flow or quiescent conditions that the cell "striking" rate at a surface cannot be determined. Consequently, *net* attachment, adsorption, or adhesion is the quantity generally reported. Determination of cell transport rate would permit calculation of a cell sticking efficiency, a useful criterion for comparing performance of coatings, chemicals, and other antifouling treatments. Particulate transport research could also determine the dominant transport mechanisms under different conditions and lead to unique proposals for fouling prevention and/or control.

B. Adsorption of a "Conditioning" Film

Microorganisms select their habitats on the basis of many factors, including the nature of the wetted surface (material of construction and surface roughness). Adsorption of an organic monolayer occurs within minutes of exposure and changes the properties of the wetted surface. Investigations have shown that materials with diverse surface properties (e.g., wettability, surface tension, electrophoretic mobility) are rapidly conditioned by adsorbing organics when exposed to natural waters with low organic concentrations. These organic molecules frequently appear to be polysaccharides or glycoproteins.

1. Rate and Extent of Adsorption

Loeb and Neihof (1975) and DePalma *et al.* (1979) have measured adsorption rates of organic molecules in seawater, and Bryers (1979) has observed adsorption rates in a laboratory system. Rate and extent of adsorption in these investigations are presented elsewhere (Characklis, 1981). Rates as high as 0.45 nm/minute were observed but maximum accumulation from molecular fouling was always less than 0.1 μm. The rate of molecular fouling can be considered instantaneous because it is much greater than the rate of microbial fouling. Based on "thickness" measurements, molecular fouling can have no significant effect on fluid flow or heat transfer. Nevertheless, the surface properties resulting from adsorption of an organic film may affect the sequence of microbial events which follow.

A unique aspect of diatom adhesion is that at least one organism may not require surface conditioning films to be present on the substrata before adhesion takes place. K. E. Cooksey (1981) found that a washed culture of the diatom *Amphora coffeaeformis* adheres to glass surfaces in less than 5 minutes (see Fig. 1). In these experiments, preadsorbed macromolecular films could arise from the washing procedure for the glassware, the analytical grade simple salts used in the suspending fluid, or from the cells themselves. Preconditioning the substrata with media from previous experiments did not alter the kinetics of the diatom attachment (B. Cooksey, unpublished results).

Brash and Samak (1978) presented experimental evidence that significant turnover occurs in molecular (proteinaceous) fouling films on polyethylene. Protein molecules in the bulk fluid are continuously exchanging with adsorbed proteins. This suggests that dispersed microbial cells and their associated extracellular material may be continually exchanging with biofilm material at the wall.

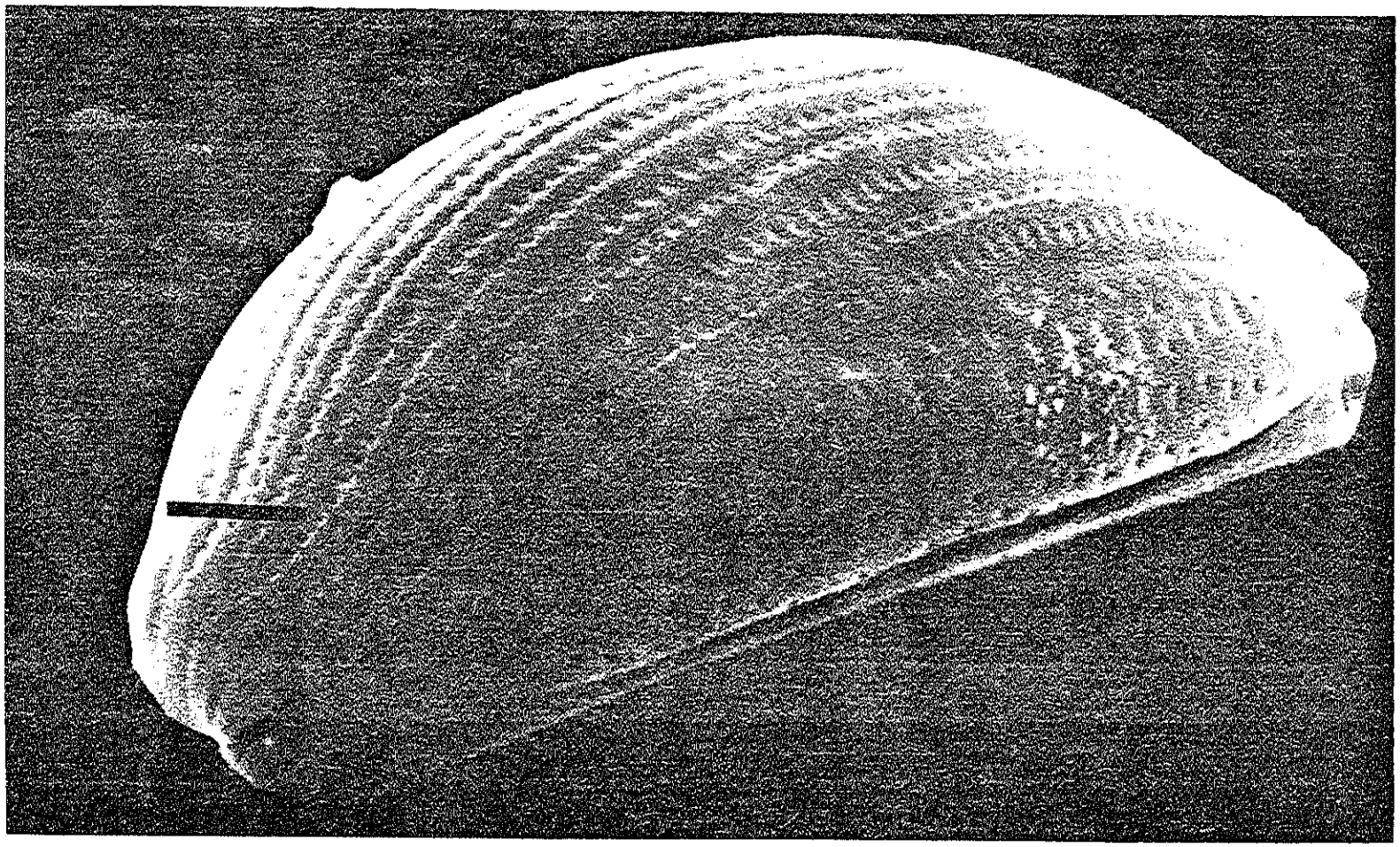


FIG. 1. Adhesion of the marine fouling diatom *Amphora coffeaeformis* to a glass surface. (Scanning electron micrograph by Daniel Webster, University of mi Medical School.) Bar is 1 μm.

2. *Characterization of the Conditioning Film*

These new, organically conditioned interfaces influence considerably the adhesion of microbes. These conditioning films have been investigated by various means. For instance, Loeb and Neihof (1975) found that the contact angle (Zisman, 1964) of the platinum–water or –methylene iodide interface increased considerably when the platinum had been exposed to natural sea water. Similar results have been obtained by DePalma *et al.* (1979) in natural systems. The phenomenon did not occur when the organic fraction of the sea water had been photooxidized with short-wave UV radiation. From this and some studies of the fluorescence of the adsorbed layer, Loeb and Neihof concluded that the film responsible for the decreased wetting of the platinum is organic and that humic acids may be implicated in its formation. Baier and various co-workers have characterized these acquired films as largely glycoprotein (Baier, 1980; Baier and Weiss, 1975; Marshall, 1979). These conclusions depend on the internal total reflectance infrared (IR) spectrophotometric analysis of the films adsorbed on pure germanium prisms. The technique has been described in detail by Harrick (1967). Marshall (1979), from results obtained in collaboration with Baier, implied that because the protein and polysaccharide IR absorption signals are detectable on the germanium prism before the onset of bacterial adherence, the formation of the conditioning film is an obligate first step in the attachment of organisms to surfaces. Baier (1980) made the statement more emphatically. This is probably so in all natural systems because of the universal presence of organic macromolecules in natural waters and because of the differential kinetics of the two processes. There appears to be no evidence, however, that microorganisms can *only* attach to conditioned surfaces. Indeed, some surfaces with adsorbed proteins inhibit bacterial adhesion. For example, Fletcher (1976) showed that the adhesion of a marine pseudomonad to polystyrene was inhibited by albumin, gelatin, fibrinogen, and pepsin. The influence of such compounds is certainly not clear-cut since Meadows (1971) found that although albumin was inhibiting in his system, casein and gelatin facilitated the process of adhesion. Adsorption of such molecules decreases the surface energy of clean, high-energy surfaces (70 dyn/cm) but has little effect on low-energy surfaces (20 dyn/cm) (Baier, 1980). The concept of surface energy is discussed further below. One would expect that surfaces of initially differing energies, after conditioning with an adsorbed layer of protein, would influence the adhesion of cells similarly. This appears not to be the case. Baier (1980) has shown that the spread areas of mammalian cells, a parameter related to the firmness of adhesion, is correlated with the initial energy of the surface, i.e., before conditioning has taken place. Thus, siliconized surfaces promoted adhesion of cells, even after

protein conditioning of those surfaces. The configuration of the conditioning film, therefore, must be influenced by the initial surface state of the substratum (Baier, 1980). However, alternative explanations are possible (see below). These subtle modifications of surfaces by organic macromolecules are reflected in changes of their surface charge. Neihof and Loeb (1972) showed the convergence of surface charge on various types of particles (by means of microelectophoresis experiments) when exposed to natural sea water. Thus, it seems that the role of conditioning films in the adhesion of cells to surfaces is not yet clear. One of the problems in drawing conclusions from the published investigations in this area of research is related to the use of divergent experimental designs. For instance, various workers have used different microbial types, substrata, and conditioning macromolecules. One further problem lies in the fact that the ability of cells in laboratory culture to adhere sometimes changes with time (Costerton *et al.*, 1981).

C. Adhesion of Microbial Cells to the Wetted Surface

Shortly following the conditioning of the substratum, bacterial adhesion begins. In most studies, adhesion has not been distinguished from colonization, which includes the effects of subsequent growth of bacteria. Thus, numbers of bacteria reported on substrata may represent an integration of both processes.

Previous research (Marshall *et al.*, 1971; Zobell, 1943) suggests the existence of a two-stage adhesion process: (1) reversible adhesion followed by (2) an irreversible adhesion. Reversible adhesion refers to an initially weak adhesion of a cell which can still exhibit Brownian motion and is readily removed by mild rinsing. Conversely, irreversible adhesion is a permanent bonding to the surface, usually aided by the production of EPS. Cells attached in this way can only be removed by rather severe mechanical or chemical treatment. The forces influencing both reversible and irreversible adhesion will be discussed below.

1. Hydrodynamic Effects

Most of the research on cell adhesion has been conducted at very low fluid shear stress or in quiescent conditions, which suggests sedimentation or diffusion may control the *rate of adhesion*. There is yet to be a demonstration of reversible adhesion in turbulent flow.

In turbulent flow, the *net* rate of adhesion is the quantity most easily measured. The net rate of cell adhesion is the difference between the rate of cell adhesion and rate of cell detachment. Powell and Slater (1983) clearly showed that any analysis that assumes that all cells contacting the surface

become irreversibly attached grossly overestimates the surface cell population. Cell detachment results from several forces, including the following: (1) fluid dynamic forces, (2) shear forces, (3) lift (upsweeps), and (4) taxis. Upsweeps are analogous to the downsweeps discussed in relation to transport. Downsweeps and upsweeps result in turbulent bursts which move to and away from the surface into the bulk flow. Upsweeps generate a lift force normal to the surface, which can influence detachment. Drag or viscous shear forces act in the direction of flow on attached cells and are approximately 1000 times greater than the lift forces acting on attached cells. Note that although viscous shear may dislodge a particle, unless a lift force is present, the particle will presumably roll along the surface until another surface adhesion site is found.

2. *Physicochemical Forces*

The forces that reversibly bind a cell to a surface have been reviewed at various levels of mathematical complexity (Pethica, 1961, 1980; Baier, 1980; Daniels, 1980; Dolowy, 1980; Fletcher, 1980; Gingell and Vince, 1980; Rutter, 1980; Rutter and Vincent, 1980). Despite the large number of reviews and a considerable amount of work, theory does not explain the natural phenomena very well.

There are basically two theories concerning the initial interactions of cells and substrata. In the first, the electrostatic properties of the system (DLVO theory) are considered, whereas the second considers interfacial free energy of the system ("wettability" theory).

a. DLVO Theory of Adhesion. The DLVO theory is named for Derjaguin and Landau (1941) and Verwey and Overbeek (1948). The positions of attraction have been called the primary minimum (at small separations) and the secondary minimum (at larger distances of separation). At a point between, repulsive forces are maximal. Problems with this approach reside in the values used for the charges on the surfaces, the different geometry at the attachment site, and the varying dielectric constant of the liquid as the two surfaces approach. In addition, Hamaker's constant cannot be measured in these types of systems (Rutter, 1980). The theory predicts that reversible adhesion can take place at the secondary minimum (about 5–10 nm). This at least appears true and has been described by Marshall *et al.* (1971). Time spent at this distance may be sufficient for other adhesive forces to become effective, e.g., polymer bridging. It is unlikely that cells are able to approach a substratum sufficiently closely (e.g., less than 1 nm) to overcome the repulsive peak which exists between the primary and secondary minima. For instance, it has been calculated that the energy developed by a pseudomonad swimming at 33 μm/second is insufficient to overcome this barrier

(Marshall *et al.*, 1971). The mathematical expression of DLVO theory of particle interaction includes the radius of the particles. As the radius of the particles decreases, the repulsive energy barrier decreases. Thus, when cells are able to reduce their effective radius, as in the production of filopodia (e.g., mammalian cells) or fimbriae (bacteria), they may overcome the repulsive maximum and adhere at the primary attraction minimum (Rogers, 1979; Weiss and Harlos, 1977). All of the results mentioned above have been obtained in systems with little or no fluid shear stress—a situation that rarely obtains in the natural environment.

b. Interfacial Free Energy and Adhesion. Theoretically, if the total free energy of a system containing a cell and an adjacent substratum is reduced by contact, then adhesion of the cell to the substratum will result. In many cases, adhesion of cells has been related to the critical wetting tension (mammalian tumor cells, Baier, 1980; bacteria, Dexter *et al.*, 1975; diatoms, Cooksey, Cooksey, and Baier, unpublished, see Fig. 2). This parameter is, in turn, related to the contact angle between model liquids and the substrata being studied (Zisman, 1964). Harper and Harper (1967) showed that diatom adhesion to glass was stronger than to plastic. The activity of the surfaces was not reported but the glass probably had the higher surface energy. Diatom adhesion to substrata, as judged by experiments with *A. coffeaeformis*, exhibits the same relationship with substratum surface energy as has been described for other organisms, including minimal adhesion at approximately 25 dyn/cm (Grinnell *et al.*, 1972; Dexter, 1979; Baier, 1970, 1973, 1975).

Pethica (1980) found the relationship between critical wetting tension and adhesion of particles (cells) to be qualitative at best. He reminds us that the Young equation demands that particles be homogeneous and the surface be insoluble in the wetting liquid used to measure the contact angle. In practice, none of these requirements is rigorously obtained. Experimental results do allow us, however, some confidence in the use of contact angles for ranking both particles (cells) and the substrata.

Some of the objections related to the measurement of contact angles under one set of conditions, and their application in quite different experimental circumstances, have been overcome by Fletcher and Marshall (1982). They measured contact angles of experimental surfaces both in the "clean" and conditioned state in an aqueous system, using an air bubble contact method. They found that the relative adhesion of bacteria to plastic substrata became modified by the adsorption of various proteins and that these modifications were reflected in a change in measured contact angles.

c. Other Noncovalent Forces. Other forces that are responsible for cel-

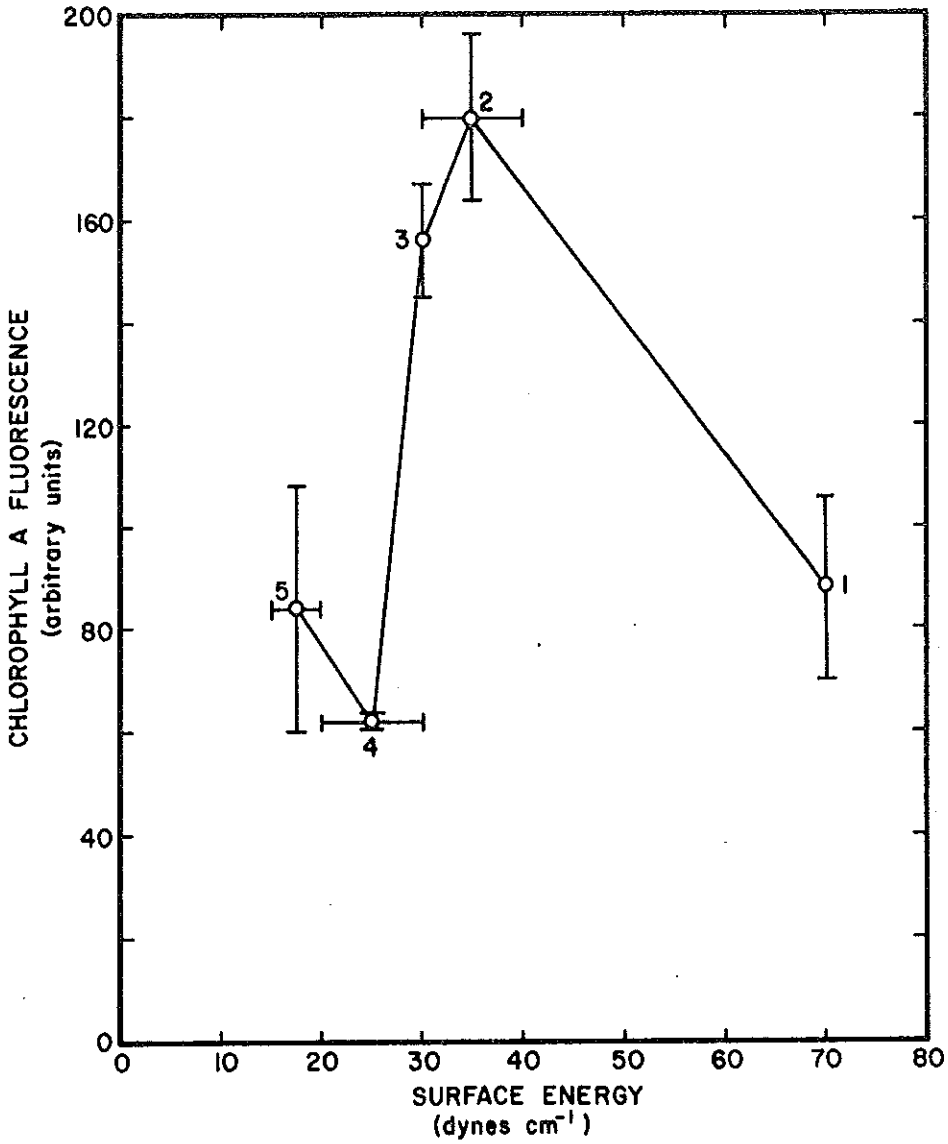


FIG. 2. Attachment of diatoms to chemically modified glass surfaces. Glass microscope slides were treated to obtain substrata of differing surface energies. Surface 1 was treated with a radio frequency glow discharge and stored in distilled water until use; surface 2 was the same as 1 but stored in air; surface 3 was treated with chloropropyl trichlorosilane; surface 4 with dichlorodimethylsilane and surface 5 with a perfluorinated silane (3-HEPT). Surface energies were measured by the contact angle method (Zisman, 1964). Attachment of diatoms to substrata was quantified after rinsing the slides and then measuring the chlorophyll *a* fluorescence of the remaining organisms (K. E. Cooksey, 1981).

3. *Extracellular Polymeric Substances (EPS)*

Marshall (Marshall *et al.*, 1971; Marshall, 1980) has interpreted the physicochemical theories above in practical terms. Initially, cells are held close to a surface in a state of reversible or temporary adhesion. Cells in this state are

undergoing gliding motility, although temporarily adhered, are not removed by this stress. If the cell resides at a surface for some critical time, it becomes irreversibly bound through the mediation of a cementing substance. This implies that such cells are no longer motile.

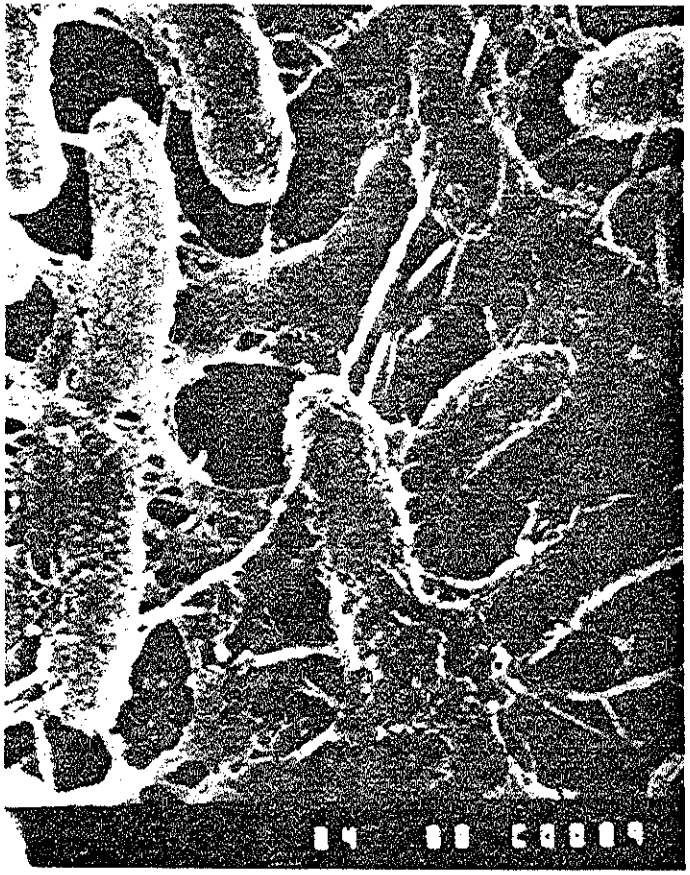
Zobell (1943), a pioneer in the field of microbial adhesion, suggested the participation of extracellular cementing substances in the adhesion of cells to substrata. Since then, considerable attention has been directed at these extracellular polymeric substances (EPS). However, much confusion exists over the terminology for the extracellular material intimately related to biofilms (Bowles and Marsh, 1982). Glycocalyx, slime, capsule, and sheath have all been used in referring to extracellular polymeric substances associated with individual cells, cell aggregates, or biofilms. EPS appears to be the least restrictive term. For example, glycocalyx is defined as "tangled fibers of polysaccharides or branching sugar molecules" (Costerton *et al.*, 1978). However, in biofilm processes and in microbial adhesion in general, other macromolecules besides polysaccharides and sugars are found within the organic matrix, including glycoproteins (Humphrey *et al.*, 1979), proteins, and nucleic acids (Nishikawa and Kuriyama, 1968). Therefore, unless extensive identification has been performed, components of the organic matrix will be referred to as EPS (Geesey, 1982). EPS can conceivably contribute to biofilm processes in many ways, including the following: they may (1) provide cohesive forces within the biofilm, (2) adsorb nutrients, (3) protect immobilized cells from rapid environmental changes, including the influence of biocides, (4) adsorb heavy metals from the environment, (5) adsorb particulate material and other detritus, (6) serve as a means of intercellular communication within the biofilm, (7) provide short-term energy storage via the cell membrane potential, and (8) enhance intercellular transfer of genetic material. EPS also significantly influences the physical properties of the biofilm, including the diffusibility, thermal conductivity, and rheological properties. Presumably, water activity and/or osmotic pressure are elevated in a dense aggregate of EPS.

a. Bacterial EPS. As yet, we have little information concerning the structural analysis of purified adhesive EPS in microbial systems. This is in contrast to the expansive literature on the structure of one of the adhesive EPS of mammalian cells, fibronectin (Olden *et al.*, 1980). For light microscopy, EPS can be stained with crystal violet, ruthenium red, and alcian yellow (or blue). Some of the stains have been used also for transmission electron microscopy (TEM). Conclusions concerning the chemical structure of EPS based on staining alone are tenuous (see below). In electron microscopic studies, especially where staining with ruthenium red or other dyes

fibrillar material with certain of the fibrils bridging to the substratum (e.g., Corpe *et al.*, 1976; Fletcher, 1980). The fibrillar nature of such polymers may be an artifact of fixation or drying in preparation for TEM examination, because in nature the EPS is highly hydrated (Geesey, 1982) (see Fig. 3).

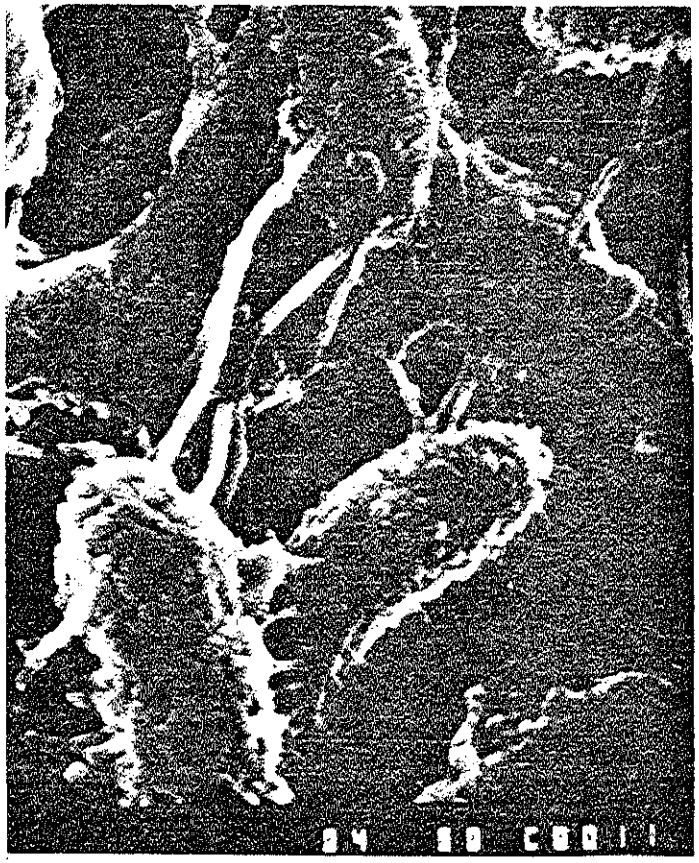
There are many qualitative analyses of bacterial EPS—usually considered to be carbohydrate with acidic groups (Corpe *et al.*, 1976; Fletcher and Floodgate, 1973), amino groups (Baier, 1975), and sometimes associated with proteins (Corpe *et al.*, 1976). In most qualitative analyses, however, the possibility of multiple polymers of different structures and composition is rarely considered. Thus, the various functional groups may reside on separate polymers, e.g., detection of protein and carbohydrate in an EPS does not imply the presence of a glycoprotein unless the polymer is known to be a single, covalently linked entity. This problem has been recognized by Fletcher (1980). Based on electron microscopic histochemical evidence, she earlier postulated (Fletcher and Floodgate, 1973) that the attachment polymer of a marine pseudomonad, NCMB 2021, was an acid polysaccharide. Hydrolysis of an extracellular carbohydrate fraction of these cells often shows the presence of neutral sugars (Sutherland, 1982) found in polymers of this type (glucose, mannose and galactose, glucosamine, rhamnose and ribose), but no uronic acids. Carboxylic acid groups detected in the polymer by IR spectroscopy were considered to be associated with protein because no uronic acids were detected after hydrolysis. Uronic acids were, however, detected in adhesive polymers from *Flexibacter* analyzed by Humphrey *et al.* (1979). This analysis is probably one of the most detailed for a substance known to be involved directly in bacterial adhesion. These workers found that a partially purified extracellular slime contained both protein and carbohydrate, with glucose, galactose, fucose and deoxysugars, besides uronic acids in the hydrolysate. Repeated attempts to remove the protein from the carbohydrate fraction were unsuccessful. Thus, it was concluded that the polymer could be glycoprotein. Calculations based on measurements and reasonable assumptions for the system showed that the force required to separate *Flexibacter* cells from surfaces was five times more than was needed for horizontal movement, i.e., the polymer really did possess Stefan adhesive properties. Polymers in EPS may well attach to substrata by ionic bonds (if they contain $-COOH^-$ groups) or hydrogen bonds. The possibility certainly exists that bacterial polymers could form heterocopolymers with surface-adsorbed materials, thus partially accounting for their adhesive nature (Rogers, 1979).

There is no clear picture concerning the participation of *fimbriae* in the formation of biofilms. Although the adhesion of *Escherichia coli* to mammalian epithelial cells involves fimbriae, they are not involved in its attachment to glass. *Actinomyces* and *Bacteroides* species inhabiting the human



a

1 µm



0.5 µm

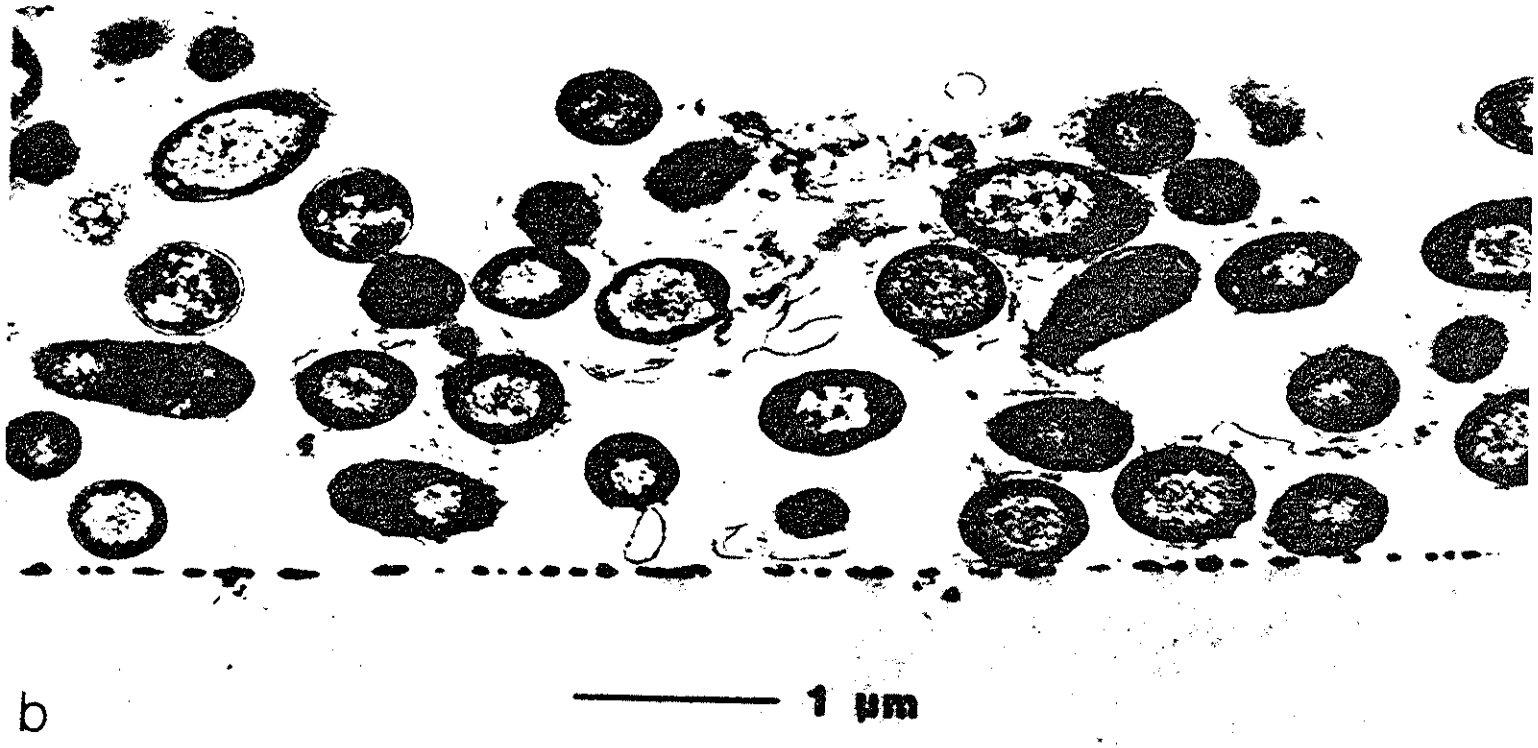


FIG. 3. *Pseudomonas aeruginosa* grown on a Millipore filter in glucose mineral salts medium. (a) Scanning electron micrograph. Note EPS anchors. (b) Transmission electron micrograph of film depicted in (a). Note condensed nature of EPS and intercellular space. Bar is 1 µm. Photomicrographs by Michael Trulear, Montana State University.

mouth are fimbriate and are involved in the formation of dental plaque, most certainly a form of biofilm (Slots and Gibbons, 1978). One particularly interesting aspect of adhesion mediated by fimbriae has been described by Rosenberg *et al.* (1982). These workers showed conclusively that fimbriae on the cell surface of *Acinetobacter calcoaceticus* were involved in its adhesion and subsequent growth on hexadecane at the hydrophobic liquid–liquid interfaces. Mutants lacking fimbriae were not adherent; revertants adhered and also acquired the ability to produce fimbriae. One aspect of adhesion mediated by fimbriae that does not seem to have been exploited in research on adhesion to inanimate objects is its specific inhibition by mannose and sometimes by 2-deoxyglucose.

There seems to be no evidence for the participation of bacterial flagella in biofilm adhesion. They may be concerned in propelling cells to the secondary minimum, but we have not been able to find examples of cells attached only by their flagella. Conclusions implicating flagella in adhesion that depend on results obtained with chemically fixed microbial cells should be treated with caution (Ward and Berkeley, 1980).

There are no documented cases of lectin mediation in adhesion of microorganisms to nonliving surfaces. However, if one regards the dental pellicle as nonliving, then bacteria possessing lectin-like ligands are known to attach with considerable specificity to receptors on its surface (Gibbons, 1980). Further examples of lectin-like interactions will likely be documented in the future as research workers study the specificity of adhesion both at the macro- and microbiological level.

b. Microalgal EPS. The most common microalgae to adhere to submerged substrata are diatoms. There are several methods by which these cells attach, as discussed at length by Chamberlain (1976). Light microscopy shows cells attached by mucilage pads, stalks, or in some cases inside mucilage tubes attached to a substratum, e.g., *Amphipleura rutilans*. A further method of attachment concerns the raphe canal. In these cases, light microscopy does not show the means of adhesion. The raphe system of a diatom is a single or double slit in the silica cell wall running along the long axis of the cell, allowing direct communication between the cytoplasmic membrane and the extracellular environment. The raphe is clearly involved in gliding motility and, therefore, adhesion (Harper, 1977). Daniel *et al.* (1980) have described a series of mucilage-containing intracellular vesicles in *A. veneta* that appear to arise from the cisternae of the dictyosome. These may be the same organelles described earlier by Hopkins and Drum (1966) as crystalloid bodies and postulated by the authors as the source of diatom trail substance, and thus implicated in both adhesion and motility. We have electron micrographic evidence that indeed these vesicles are secreted into

the raphe canal (Webster *et al.*, 1982), and thus may be the source of the cellular adhesive. Because tunicamycin (1 μg/ml), which inhibits the formation of the linkage between carbohydrate and protein in fibronectin, inhibits diatom adhesion, it might be speculatively implied that the vesicles contain glycoprotein (Cooksey and Cooksey, unpublished).

Several workers have made attempts at analysis of attachment polymers of diatoms. By means of histochemical tests, Chamberlain (1976) showed that the polar mucilage pads, investing mucilages, the polymers of the tube-dwelling diatoms, and mucilagenous stalks were acidic polysaccharides. As mentioned previously (Section II,C,3,a), analytical results based on staining reactions of unpurified materials must be regarded as properties of a mixture. Some hydrolyses have been performed, but in no case has an attempt been made to show that the polymeric material was a single molecular species. Thus, the capsule of *Navicula pelliculosa* contains glucuronic acid (J. C. Lewin, 1955), that of the oval cells of *Phaeodactylum tricornutum* contains xylose, mannose, fructose, and galactose (J. C. Lewin *et al.*, 1958), the mucilage tubes of *Amphipleura rutilans* contain xylose, mannose, possibly rhamnose, and some proteins (R. A. Lewin, 1958), and the stalks of *Gomphonema olivaceum* are composed of a β-linked sulfated polymer containing galactose and xylose (Huntsman and Slonecker, 1971). All the polymers analyzed so far are different from the diatom storage polymer chrysolaminarin, a β 1-3-linked glucan. Even less is known concerning the trail substance referred to earlier, probably because it appears to differ in physical properties from the enveloping polymers (capsules, etc.) described above. Trail substance is slowly water-soluble (unpublished results quoted in Edgar and Pickett-Heaps, 1982; Webster *et al.*, 1982). In a detailed histochemical study where temporary and permanent adhesive polymers of *Amphipleura veneta* were not distinguished, Daniel *et al.* (1980) showed that the extracellular polymer contains uronic acids and sulfate groups, but no protein or lipid.

4. Cations

Roux (1894) reported the necessity for divalent cations, notably Ca^{2+}, in cellular adhesion. Calcium has been shown to be necessary for adhesion of aquatic bacteria (Marshall *et al.*, 1971; Fletcher and Floodgate, 1973) and marine diatoms (K. E. Cooksey, 1981), although in this report it was found that strontium could substitute poorly for Ca^{2+}. The role of these cations in adhesion is presently unknown. It has been suggested that divalent ions, especially Ca^{2+}, can form bridges between negatively charged substrata and microorganisms, stabilize the structure of EPS (Fletcher and Floodgate, 1973), or cause precipitation of EPS in the space between a cell and substratum (Rutter, 1980). Fletcher and Floodgate (1973) noted that lanthanum

decreases bacterial adhesion and postulated an EPS-denaturing action on the part of this ion. Lanthanum is known to inhibit Ca^{2+} transport into cells and to displace calcium from cellular membranes (Weiss, 1974), so that the effect noted above may have been related to a diminution of the flux of Ca^{2+} to the intracellular space. Lanthanum inhibits diatom adhesion, a process which is Ca dependent. Further evidence for the involvement of Ca^{2+} in the adhesive process comes from the use of complexing agents. Attachment of a marine bacterium was inhibited by EDTA, but the same agent did not remove cells already attached to substrata (Fletcher, 1980). B. Cooksey and K. E. Cooksey (1980) were able to remove the marine diatom *Amphora coffeaeformis* from glass with a more specific Ca^{2+} chelant, EGTA. Attached diatom cells treated with this substance left behind substratum-attached material in the exact shape of the raphe canals. The material, which stained with acridine orange, was certainly involved in the attachment of the organism. Similar, more detailed experiments by Culp and his co-workers illustrate the dangers of adopting simplistic explanations of adhesion (Culp and Black, 1972; Terry and Culp, 1974; Rosen and Culp, 1977). These workers found that EGTA removed tissue culture cells from culture vessels but left behind "substrate-attached material" (SAM). Based on this finding, it was thought that mammalian tissue cells attached to this substratum by ionic bridges. Papers by the same group (Culp *et al.*, 1979) have shown that the situation regarding attachment, detachment, and the formation of SAM is considerably more complex. SAM certainly exists, but not only does it contain glycoprotein, it also contains certain elements of the cytoskeleton such as actin. SEM studies suggest EGTA causes minimal changes in the adhesive "footpads" which give rise to the SAM. This causes the cells to "round up" and, in doing so, fibers of cellular material between the cells and the substratum are formed. The cytoskeleton within these fibers reorganizes, the fibers break, and the cells are liberated, leaving the footpads behind as SAM. There are no studies at this level of detail with microbiological biofilms.

D. Reactions within the Biofilm

1. *Fundamental and Observed Rate Processes*

Biofilm and biofouling studies thus far have relied on a relatively unstructured approach to analysis of the biomass component. The biotic component is generally characterized only in terms of cell numbers or cell mass with little attention given to the physiological state of the organisms, although there have been some limited attempts at providing more structure (Trulear, 1982; Bakke, 1982). Within the restrictions of unstructured microbial pro-

cess models, four *fundamental rate processes* can be identified: growth, product formation, maintenance and/or endogenous decay, and death and/or lysis.

Any or all of these processes may be occurring in a biofilm at any time. Growth refers to cell growth and multiplication. The cells also form products, some of which are retained in the biofilm (e.g., EPS) and some of which diffuse out into the bulk fluid. The cells also have to maintain their internal structure, another energy-consuming process. If nutrients are depleted or toxic substances are present, death and/or lysis ensues.

The rates of the fundamental microbial processes are difficult to measure directly and are generally inferred from more easily *observed rate processes.* The more familiar observed rate processes include the following: substrate consumption, electron acceptor consumption, biomass production, and product formation.

TABLE II
A MATRIX REPRESENTATION FOR THE FUNDAMENTAL MICROBIAL RATE PROCESSES[a]

Process rate		Stoichiometry							
		Reactants			Products				
Fundamental process					Biomass		Product		Metabolite
Process	Rate	Substrate (s)	Nutrient (z)	Electron acceptor (e)	x_T	x_d	p_e	p_i	(a)
Growth	μ	−	−	−	+		+	(+)	+
Maintenance									
Exogenous	m	−	−	−			+		+
Endogenous	k_e		+	−	−	(+)	+	−	+
Product formation	k_p	−	−	−			+	+	+
Death									
Loss of viability	k_d				−	+			
Lysis	k_L	(+)	(+)		−	(+)	+		
Observed rate		q_s	q_z	q_e	μ_n		q_p		q_a

[a] q = specific production or removal rate (t^{-1}).
μ = specific growth rate or specific biomass production rate (t^{-1}).
x_T = total biomass concentration (ml^{-3}).
x_d = inert solids concentration (ml^{-3}).
p_e = extracellular microbial product concentration (ml^{-3}).
p_i = intracellular microbial product concentration (ml^{-3}).
s = substrate concentration (ml^{-3}).
z = nutrient concentration (ml^{-3}).
e = electron acceptor concentration (ml^{-3}).
μ_n = net solids production rate (t^{-1}).

Table II presents the relationship between fundamental and observed process rates. The stoichiometry of the process is qualitatively represented by each row in the matrix (− refers to reactants and + refers to products). The columns of the matrix indicate the fundamental rate processes that may contribute to the observed rates (last row in the matrix). For example, substrate removal (column 1) is the net result of growth, maintenance, and product formation.

Trulear and Characklis (1982), Bryers and Characklis (1982), and Trulear (1983) have used process analysis techniques in experimental biofilm reactors to quantify the fundamental rate processes within a biofilm. Their results suggest the following:

1. The growth rate of cells in the biofilm can be estimated from their growth rate in chemostats when substrate concentration in the microenvironment of the cell is equal.
2. Product formation by biofilm cells is the same as that for dispersed cells and depends on substrate loading rates as well as substrate concentration. Product may be the dominant component of the biofilm.
3. Maintenance requirements are essentially negligible until the biofilm becomes very thick. Even then, the results of anaerobic metabolism deep within the biofilm may be mistaken for energy maintenance requirements.

Process analysis techniques may be useful in determining whether attached cells are more active than dispersed cells. Substrate removal rate alone is not a sufficient criterion for comparing their activity because substrate removal is the net result of several fundamental processes. Removing the cells from the surface obviates any relevance in subsequent measurements that purportedly describe the activity of attached cells.

2. *Mass Transfer and Diffusion*

Analysis of biofilm process rates and stoichiometry is frequently complicated by significant mass transfer resistances in the liquid or diffusional resistances within the biofilm. Trulear and Characklis (1982) have observed that the substrate removal rate increases in proportion to biofilm thickness up to a critical thickness, beyond which removal rate remains constant. The critical or "active" thickness was observed to increase with increasing substrate concentration. This behavior has been observed by others (Mueller *et al.*, 1966; Baillod and Boyle, 1970; Williamson and McCarty, 1976; Matson and Characklis, 1976) and attributed to diffusional resistance within the biofilm. Once the biofilm thickness exceeds the depth of substrate penetra-

tion into the biofilm, the removal rate is unaffected by further biofilm accumulation.

The biofilm process rates may also be controlled by mass transfer limitations in the bulk fluid phase (Trulear and Characklis, 1982). For example, the substrate removal rate is dependent on fluid velocity past the biofilm. At low fluid velocities, a relatively thick mass transfer boundary layer can cause a fluid phase mass transfer resistance that decreases substrate concentration at the fluid–biofilm interface, thereby decreasing the substrate removal rate. Two factors may result in low mass transfer rates from the bulk fluid to the biofilm: low fluid velocities, and the transport of dilute liquid phase concentrations of the material.

Much biofilm fouling research has been conducted at relatively low flows or under quiescent conditions. Mass transfer may be the rate-controlling step for the overall process in these studies and, without further analysis, may be confused with the rates of more fundamental processes such as growth rates, adsorption rates, etc. In highly turbulent systems, mass transfer in the liquid phase is rarely a significant factor.

3. *Summary of Biofilm Reactions*

The microbial processes occurring in a biofilm are more complex than suggested by the four fundamental processes defined above. However, this classification has been useful in determining, to some extent, the flow of substrate energy through the biofilm. Mathematical description of the kinetic expressions has also been accomplished (Trulear, 1983). Further structuring of biofilm processes may await more sophisticated methods for observing the processes within the biofilm (as opposed to the influence of the processes on the overlying liquid phase) and more specific identification of the products being formed.

Bakke (1983) has observed a remarkable biofilm phenomenon which demands more attention. He increased the supply of growth substrate stepwise to a biofilm and observed the following: (1) biofilm material immediately detached, (2) biofilm cell numbers remained constant, and (3) specific substrate removal rate and product formation rate increased instantaneously.

These observations cannot be described with unstructured models but suggest that the biofilm organisms may slough their EPS in response to the "shock." In addition, the attached cells seem to possess a "reaction potential" which is expressed in response to an instantaneous increase in substrate loading. The experiments clearly indicate the need to observe microbial physiology while the organisms are attached in their growth environment (*in situ*). More attention must be directed to transients because of their relevance to natural and technological phenomena.

E. Detachment of Biofilm

Detachment of microbial cells and related biofilm material occurs from the moment of initial attachment (see Section II,D,1). However, the macroscopic observation of biofilm detachment is easier as the biofilm becomes thicker.

Detachment phenomena can be arbitrarily categorized as "shearing" or "sloughing." Shearing refers to continuous removal of small portions of the biofilm, which is highly dependent on fluid dynamic conditions. Under these circumstances, rate of detachment increases with increasing biofilm thickness and fluid shear stress at the biofilm–fluid interface (Trulear and Characklis, 1982). Sloughing refers to a random, massive removal of biofilm generally attributed to nutrient or oxygen depletion deep within the biofilm (Howell and Atkinson, 1976) or some dramatic change in the immediate environment of the biofilm (see previous section). Sloughing is more frequently witnessed with thicker biofilms developed in nutrient-rich environments. Shearing probably occurs under the same conditions under which sloughing occurs, but no direct measurements have been attempted.

1. Hydrodynamic Influences

Both Powell and Slater (1982) and Timperley (1981) conducted studies to determine the influence of fluid dynamics on detachment. The investigators observed an increase in detachment with an increase in Reynolds number, i.e., fluid velocity. Timperley also considered different tube sizes and, within that context, concluded that mean fluid velocity was more significant in determining cleaning effectiveness than Reynolds number.

As fluid velocity increases, the viscous sublayer thickness decreases. Consequently, the region near the tube wall subject to relatively low shear forces (i.e., the viscous sublayer) is reduced. As a result, there may be some upper limit to the effectiveness of any cleaning operation based on fluid shear stress. The viscous sublayer may provide a valuable a priori criterion for predicting the maximum effectiveness (the minimum thickness attainable) of any cleaning technique dependent on fluid dynamic forces.

Detachment processes are also significant in the processes of cell turnover in the biofilm. As a biofilm develops, succession in species is observed (see Section IV,A,2). Trulear (1983) developed a biofilm of *Pseudomonas aeruginosa* under conditions of relatively high shear stress and then challenged it with *Sphaerotilus natans*. *Sphaerotilus* quickly became the dominant species within the biofilm. Detachment, influenced strongly by fluid shear stress, may serve to "wash out" organisms from the biofilm.

2. *Chemical Treatment*

Detachment may occur for reasons other than hydrodynamic forces. Bakke (1983) has observed massive detachment when substrate loading to the biofilm was instantaneously doubled (see Section II,D,3). He hypothesizes that cell membrane potential plays a key role in the phenomenon. Turakhia (unpublished results) and Characklis (1980) have observed dramatically increased detachment upon the addition of chelants (EGTA and EDTA, respectively) suggesting the importance of calcium to the cohesiveness of the biofilm. Many other chemical treatments have been used to detach biofilm material with varying success, including chlorine (Characklis *et al.*, 1980; Characklis and Dydek, 1976; Norrman *et al.*, 1977), bromine chloride (Bongers *et al.*, 1977), bromochlorodimethylhydantoin (Matson and Characklis, 1983), and surfactants.

3. *Summary of Detachment Processes*

Detachment processes play a major role in the ecology of the biofilm. Microorganism detachment from and absorption into the biofilm provides the means for interaction between dispersed (planktonic) organisms and the biofilm. Detachment of biofilm is the major objective of many antifouling additives used in manufacturing processes.

Very little is known regarding the kinetics of detachment and the factors affecting the removal. Such kinetic expressions would be useful for modeling purposes and for serving as comparative criteria in testing of antifouling treatments.

III. Properties and Composition of Biofilms

Microorganisms, primarily bacteria, adhere to surfaces ranging from the human tooth and intestine to the metal surfaces of condenser tubes exposed to turbulent flow of water. The microorganisms "stick" by means of extracellular polymeric fibers, fabricated and oriented by the cell, that extend from the cell surface to form a tangled matrix of extracellular polymer substances (EPS). The fibers may conserve and concentrate extracellular enzymes necessary for preparing substrate molecules for ingestion, especially high-molecular-weight or particulate substrate frequently found in natural waters.

The biofilm surface is highly adsorptive, partially due to its polyelectrolyte nature, and can collect significant quantities of silt, clay, and other detritus in natural waters.

Physical, chemical, and biological properties of a biofilm are dependent on the environment to which the attachment surface is exposed. The physical

and chemical components of the microenvironment combine to select the prevalent microorganisms which, in turn, modify the microenvironment of the surface. As colonization proceeds and a biofilm develops, gradients develop within the biofilm and average biofilm properties change. Changes in biofilm properties that occur during biofilm development must be considered when attempting to predict the influence of biofilms on the immediate environment. These changes have been largely ignored in past studies.

A. Physical Properties

Relevant *thermodynamic properties* of biofilm are volume (thickness) and mass. In turbulent flow systems, wet biofilm thickness seldom exceeds 1000 μm (Picologlou *et al.*, 1980). The biofilm dry mass density can be determined from the wet biofilm thickness if the biofilm mass and the wetted surface area are known. The dry mass density reflects the attached dry mass per unit wet biofilm volume and measured values in turbulent flow systems range from 10–50 mg/cm^3. Biofilm density increases with increasing turbulence and increasing substrate loading (Picologlou *et al.*, 1980; Trulear and Characklis, 1982). The increase in biofilm density with increasing turbulence may be caused by one of the following phenomena: (1) selective attachment of only certain microbial species from the available population, (2) microbial metabolic response to environmental stress, or (3) fluid pressure forces "squeezing" loosely bound water from the biofilm.

The relatively low biofilm mass densities compare well with observed water content of biofilm (Characklis, 1973, 1980).

The *transport properties* of biofilm are of critical importance in quantifying effects of biofilms on mass, heat, and momentum transfer. Diffusion coefficients for various compounds through microbial aggregates have been reported in the literature (Matson and Characklis, 1976), mostly for floc particles. Matson and Characklis (1976) reported variation in the diffusion coefficient for glucose and oxygen with the growth rate and the carbon-to-nitrogen ratio. In biofilms, the diffusion coefficient is most probably related to biofilm density. *In situ* rheological measurements indicate that the biofilm is viscoelastic with a relatively high viscous modulus (Characklis, 1980). Reported biofilm thermal conductivities are not significantly different from that of water (Characklis *et al.*, 1981).

B. Chemical Properties

1. *Elemental Composition*

Inorganic composition of biofilms undoubtedly varies with the chemical composition of the bulk water and probably affects the physical and biolog-

ical structure of the film. Calcium, magnesium, and iron probably affect intermolecular bonding of biofilm polymers that are primarily responsible for the structural integrity of the deposit. In fact, chelants are effective in detaching biofilm (Characklis, 1980; Turakhia, unpublished results). In heat exchangers, corrosion products and inert suspended solids can adsorb to the biofilm matrix and influence its chemical composition. Characklis (1981) reports a range of inorganic compositions observed in selected biofilms.

2. *Macromolecular Composition*

The organic composition of the biofilm is closely related to the energy and carbon sources available for metabolism. Classical papers (Herbert, 1961; Schaecter *et al.*, 1958) have demonstrated the effect of environment and microbial growth rate on the composition of the cells and their extracellular products. For example, nitrogen limitation can result in production of copious quantities of extracellular microbial polysaccharides. Characklis (1981) presents data on the composition of biofilms developed in the field and in the laboratory. In terms of macromolecular composition, Bryers (1979) has measured protein-to-polysaccharide mass ratios ranging from 0 to 10 (polysaccharide concentration in terms of glucose and protein concentration based on casein) with increasing biofilm accumulation. Other chemical analyses of biofilm have been reported by Bryers and Characklis (1979).

C. Cellular Densities

The organisms which colonize the attachment surface will strongly influence biofilm development rate and biofilm chemical and physical properties. However, organism–organism and organism–environment interactions undoubtedly shift population distributions during biofilm accumulation. Several investigators have observed succession (see Section IV,A,2) during biofouling. The first visible signs of microbial activity on a surface are usually small colonies of cells distributed randomly on the surface. As biofilm development continues, the colonies sometimes grow together, forming a relatively uniform biofilm. The viable cell numbers are relatively low in relation to the biofilm volume (10^4–10^8/cm^3 biofilm), occupying only from 1 to 10% of the biofilm in dilute nutrient solutions (Characklis, 1980; Trulear, 1983). Jones *et al.* (1969) presented photomicrographs which corroborate these data. Areal densities have been observed as dense as 10^{13} cells/m^2 (Zelver *et al.*, 1982). Many surfaces, presumably clean but untreated in any rigorous way, contain as many as 10^4 cells/m^2 (Zelver, unpublished results). Sometimes it is not obvious that the precautions described by DiSalvo (1973) have been taken when withdrawing substrata from the aqueous phase through the air–water interface. This interface, which is rich in bacteria, will contami-

nate surfaces drawn through it and lead to overestimations of the numbers of bacteria attached on experimental substrata.

In many cases, filamentous forms emerge as the biofilm develops further. *Hyphomicrobium, Sphaerotilus* (Trulear and Characklis, 1982), *Caulobacter* (Corpe, 1970), *Saprospira* (Lewin, 1965), and *Beggiatoa* (Heukelekian, 1956) are frequently identified. The filamentous forms may gain an ecological advantage as the biofilm develops because their cells can extend into the flow to obtain needed nutrients or oxygen which may be depleted in the deeper portions. Obtaining representative cell numbers from filamentous biofilms is very difficult.

IV. Physiological Ecology and Biochemistry

A. Interactions between Biotic and Abiotic Components

1. Why Go to the Surface?

The ways in which cells reach surfaces have been discussed, but not the reasons for remaining. This section will outline some apparent advantages of sessile existence and initial metabolic events in the response of a cell to a surface. Marshall has described the conditioned surface rather poetically as a "relatively nutrient-rich haven in an otherwise low nutrient environment" (Marshall, 1979). Adsorption of molecules from the bulk water by substrata in all types of aquatic systems is well accepted. The degree to which these molecules can function as microbial nutrients is largely unknown. Fletcher and Marshall (1982) have compared the situations that promote the attachment of oligotrophic and copiotrophic bacteria on such substrata. Copiotrophs are organisms that depend on a relatively rich medium for growth, whereas oligotrophs are able to grow in nutrient-poor situations (Poindexter, 1979, 1981; Hirsch, 1979). There is no obligate nature implied in this definition of the oligotroph, but it may not be able to respond to short-term nutrient excess. Because surfaces are nutrient rich compared to the bulk water, copiotrophs will have a reason to stay there when they are transported to a surface. Oligotrophs present concomitantly at the same surface will not be able to compete with the faster growing copiotrophs and will not grow during the early stages of colonization of a substratum. Fletcher an Marshall (1982) described these events as typical during the first 24 hours of immersion of a substratum (e.g., Corpe, 1973). Pioneer organisms are copiotrophs, often pseudomonads. The secondary colonizers will then be oligotrophs, because according to Fletcher and Marshall, the copiotrophs will have reduced the surface nutrient concentration to a level at which the

oligotroph has a competitive advantage. Although this sequence is observed in field observations (Corpe, 1973), the advantage to the oligotroph is unclear at this point. The oligotroph comes from a nutrient-poor environment only to attach to a surface that is in a similar state. The explanation must be that the surface is not quite so hostile as the bulk water, even after nutrient depletion by copiotrophs. If it attaches, the oligotroph can then grow and at the same time keep the concentration of nutrients from reaching levels that will attract copiotrophs.

One cannot make these heterotrophic physiological comparisons for microalgae growing on surfaces. Although these organisms are photoautotrophs primarily, some, especially diatoms, are capable of heterotrophic growth (Hellebust and Lewin, 1977). There is no information concerning the frequency with which heterotrophic microalgae can be isolated directly from surfaces compared to their frequency of isolation from the water column. However, the literature shows that most heterotrophic diatoms are pennate rather than centric forms (Hellebust and Lewin, 1977) and it is the pennate organisms that are found most frequently on surfaces. In a group of nine morphologically distinct diatoms isolated from glass or stainless steel substrata, none was found to be heterotrophic but seven showed growth increases in the presence of organic substrates when photosynthesis was limited by light (i.e., mixotrophy, B. Cooksey *et al.*, 1980; Miller and Cooksey, unpublished observations). The reduced diffusion of small molecules and shading of subsurface layers that takes place in a biofilm may favor this type of physiology.

Many bacteria are chemotactic (Adler, 1975; Koshland, 1980) so that they are able to detect nutrient sources on a surface (Chet and Mitchell, 1976a). It would seem also that they are inherently sticky and adhere temporarily to a surface without depending on a metabolic output. Fletcher (1980) has reported the inhibition of bacterial adhesion by metabolic poisons such as uncouplers. From a description of her methods, we conclude that she was measuring inhibition of permanent adhesion. Thus, conversion of temporary to permanent adhesion requires metabolic work. Diatom adhesion is somewhat different in its requirement for energy. Even temporary adhesion requires energy expenditure by the cell (K. E. Cooksey, 1981) because it is inhibited by uncouplers of electron transport. The stimulus that informs an organism that it is on a substratum and that the environment is not hostile is unknown. If it is to convert temporary to permanent adhesion, the bacterial cell must synthesize EPS. In contrast, the diatom must synthesize its extracellular adhesive and secrete it through its raphe canal in order to adhere even temporarily, before indulging in gliding motility. In each case, a signal arising as a result of the proximity of a substratum must be passed to the metabolic machinery of the cell in order for it to synthesize the required

macromolecules. Nothing appears to be known of this phenomenon. The avoidance reaction in the ciliate *Paramecium* is somewhat similar, however. Eckert *et al.* (1976) have investigated the process whereby a paramecium contacts a surface, reverses, turns at an angle to the original angle of movement, and then continues swimming in the new direction. They have shown that it does this by reversing its ciliary beat in response to a depolarization of the anterior membrane and a subsequent calcium flux, triggered by the surface contact. Research along these lines may give some clues to the means of controlling EPS synthesis on surfaces by bacteria and the initiation of adhesion and gliding motility in diatoms. Rees *et al.* (1978) have proposed a model for the recognition of surfaces by fibroblast cells, but as yet, our knowledge of the process in bacteria or microalgae is insufficient for us to compare their model with these types of cells.

2. *Effects of the Surface on Cells*

In recent years, a further and quite different effect of the solid–liquid interface on bacteria has been described. It appears that the so-called dwarf bacteria (Dawson *et al.*, 1981) and ultramicrobacteria (Torella and Morita, 1981) that have been observed recently in open ocean water samples are miniature forms of normally sized bacteria. (Dawson *et al.*, 1981; Tabor *et al.*, 1981; review Morita, 1982). The change to an abnormally small size is a response to nutrient limitation and appears to be a strategy for survival. The miniature forms arise more rapidly when the organisms are at a solid–liquid interface than when they are in the bulk liquid. For instance, these workers found that of 15 rod-shaped organisms investigated, 12 hydrophilic forms decreased in size more rapidly at the interface than in the liquid phase. The remaining three hydrophobic organisms did the reverse (Humphrey *et al.*, 1983). Kjelleberg *et al.* (1982) believe the effect of the surface to be very important in the process of miniaturization and refer to its "triggering effect" as causing the phenomenon. The physiological basis for the effect is unknown but can be considered as yet another surface recognition phenomenon. Interfaces rich (rather than poor) in nutrients allow these small cells to grow and quickly resume their normal size.

The rate of increase of cells on the submerged surfaces is substratum dependent (Sechler and Gundersen, 1973). Similarly, Marszalek *et al.* (1979) found the kinetics of increase in bacterial cell numbers on surfaces of glass, stainless steel, copper–nickel alloys, and brass to be related to the surface composition. Glass and stainless steel were indistinguishable over the short term, but the corrosion products of the metal had an effect after about 5 weeks immersion. The copper-containing alloys fouled more slowly but eventually the numbers of cells found were similar to those on the less

less on the more toxic copper-containing surfaces. Dempsey (1981), comparing relatively nontoxic paint matrices with antifouling paints, found similar results. Early colonization of the toxic surfaces may be partially inhibited by the toxicant. With time, less sensitive, mucilage-forming species colonized the substratum, giving rise to a relatively nontoxic surface that was then colonized by the more sensitive forms. Few studies emphasizing the fungal colonization of substrata in the aquatic environment have been performed. Studies involving the degradation of such surfaces as wood are, however, quite common. Marszalek *et al.* (1979) and Gerchakov *et al.* (1977a,b) showed that fungi were early (2 days) arrivals on glass and stainless steel in the marine subtropics. They found that there was differential settlement with regard to species on glass and metal substrata and that several fungi (yeasts), although common in the water column, were rarely isolated from the metal surfaces. When a two-tier microbial fouling community developed after about 5 weeks immersion, fungi were found in both layers.

Diatoms and other microalgae also form part of the initial fouling community if the substratum is illuminated. Although diatoms have been found inside heat exchanger tubes, it is most unlikely that these organisms proliferate in these circumstances, even though some species are capable of heterotrophic growth (Hellebust and Lewin, 1977). Gerchakov *et al.* (1977a) found diatoms to be common on stainless steel and glass surfaces exposed in Biscayne Bay, Miami, Florida after about 2 weeks immersion. They were found much less frequently and in lower numbers on copper-containing alloys. This time scale for diatom fouling was confirmed by Sechler and Gundersen (1973), Chamberlain (1976), and Chet and Mitchell (1976b). Other workers (Wood, 1950; Skerman, 1956; Bishop *et al.*, 1974; B. Cooksey *et al.*, 1980) have shown diatom colonization to take place concomitantly with the pioneer bacteria and have mentioned diatoms as the most numerically significant members of the fouling layer. Bishop *et al.* (1974) suggested that between latitudes 40°S and 40°N, diatoms (and other microalgae) dominate illuminated surfaces, whereas outside these limits, bacteria are more prevalent. Diatoms found on surfaces are usually of the pennate type and many workers mention the genera *Nitzschia* and *Navicula* as being common on nontoxic surfaces. However, diatoms of the genus *Amphora* are often the only organisms on copper-containing antifouling paints (Bishop *et al.*, 1974; Daniel *et al.*, 1980), whereas *Achnanthes* often dominates organotin paints (Callow and Evans, 1981). Initial diatom colonization occurs when fouling films are rarely more than 1–2 μm in thickness. Diatoms increase this thickness by an order of magnitude. For instance, diatom films 500 μm thick have been measured on supertanker hulls (Bishop *et al.*, 1974).

Most successional investigations of microfouling in natural waters have mentioned the presence of microalgae other than diatoms, e.g., blue–green

algae/cyanobacteria (Paerl, 1980; Rastetter and Cooke, 1979; Evans, 1981); filamentous algae of no specific type (Gerchakov *et al.*, 1976a). Bacteriovorous protozoa are also very common on submerged surfaces. We have found only passing reference to the role of these organisms in the dynamics of the biofilm.

3. *Synthesis of Extracellular Polymeric Substances*

There are certain environmental conditions that, in general, promote the synthesis of bacterial EPS. For instance, growth limitation by nitrogen usually promotes polymer synthesis, but such cells as the riverine bacteria investigated by Brown *et al.* (1977) were not adhesive; on the other hand, under carbon limitation little polymer was formed by the same culture, but the cells were able to attach (Brown *et al.*, 1977). There is no reason, of course, to believe that identical polymers were synthesized in each case. However, it was established that the mixed culture used in each of these experiments was predominantly (>75%) an *Aeromonas* sp. Similarly, Fletcher (1980) reported that log phase cells of *Pseudomonas* NCMB2021 were more likely to attach to surfaces than stationary phase cells. In many cases, stationary phase cells are noted for extracellular polymer synthesis, not log phase cells. Sutherland (1980) makes the point that his studies of bacterial exopolymers were undertaken to learn something about those materials that *may* (his italics) be involved in adhesion. The polymers he investigated were not specifically concerned with attachment. Thus, we do not consider it fruitful at this time to consider the biochemistry of the synthesis of EPS purported to be involved in cellular adhesion. Sutherland (1982) has reviewed the information on the biosynthesis of microbial exopolysaccharides *in general.* He has stressed the importance of working with pure polymers. Sutherland (1982) also points out that we do not know the mechanism of polymer secretion through the cytoplasmic membrane even for polymers whose biosynthesis has been described.

The situation for microalgae is similar to that for bacteria. Allan *et al.* (1972) analyzed by chromatography some polymers separated from diatom cultures by differential extraction techniques, but it is not possible to assign a role in adhesion to any of them, because the diatom cells were grown in suspension culture.

B. Interactions between Biotic Components

1. *Between Species*

This section will discuss primarily the interactions between autotrophs (producers) and heterotrophs (consumers) as a result of the excretion of

trophs. In most cases, the examples concern SOC production by diatoms and SOC consumption by heterotrophic bacteria. The remarks have general applicability to other heterotrophs such as fungi, or autotrophs such as cyanobacteria. There is a large body of literature on the interaction of phytoplankton and bacterioplankton (Derenbach and Williams, 1974; Williams and Yentsch, 1976; Wiebe and Smith, 1977; Bell, 1980; Wotter, 1982). Sharp (1977), however, has questioned the validity of much of the work on algal excretion on technical grounds because techniques used in these studies usually employ some sort of traumatic treatment of the algal cells, such as filtration or centrifugation. Algal excretion of organic materials is probably much more significant in a biofilm than in the water column. In an attached community, interorganism distance (consumer–producer) is much shorter, which in turn leads to much higher local concentrations of potentially useful algal metabolic products. In other words, the algal–bacterial transfer of SOC is much more likely to occur. Bauld and Brock (1974) recognized the importance of algal–bacterial interaction in hot spring microbial mats. Haack and McFeters (1982a,b) used a differential filtration method to show the interrelationship between autotrophs and heterotrophs in an epilithic mat community in a fast-flowing, oligotrophic stream. These workers tried to minimize artifacts resulting from algal leaking of labeled materials by fixing the biofilm components with formaldehyde before homogenization and differential filtration. No experimental evidence was offered to justify the assumption that manipulations of the biofilm did not cause the algal component to leak, rather than excrete, SOC. In our laboratory, we have grown diatom cells attached to the surface of culture vessels in the presence of $NaH^{14}CO_3$. Decantation of the medium did not traumatize the cells in any obvious way. Yet labeled organic materials, assimilable by marine fouling bacteria, were detected in the algal medium. Given the difficulties of working with organisms in a film and the potential for artifact caused by the methodology used to separate consuming and producing cells, it is not surprising that so few studies of attached communities have been made. So far in this discussion, interaction of bacteria and algal cells in films has been regarded as an incidental property of the system. Paerl (1980), on the other hand, makes the argument that the interaction may be essential. It is possible that toxic levels of photoproduced O_2 may build up in films, particularly where diffusion is restricted by EPS. Removal of oxygen by bacterial heterotrophic utilization may be necessary to protect the algae concerned in its production. Concomitant production of CO_2 may also contribute to the economy of the algal cells. Escher and Characklis (1982) have examined this system mathematically and have proposed a model to explain the interaction but do not consider the potentially toxic nature of the O_2. Because environmental O_2 levels control the oxygenase or carboxylase function of ribulose bisphosphate carboxylase

where O_2 diffusion is restricted could be regarded as extracellular metabolic modulators of algal photosynthesis. Similarly, Paerl (1980) has shown the apparent enhancement of nitrogen fixation (an anaerobic process) by cyanobacteria after attachment of bacteria to the cyanobacterial heterocysts. Bacterial chemosensing of the cyanobacterial heterocyst exudates was further demonstrated by Paerl. Other workers have also noticed the chemotactic response of bacteria to algal products (Bell and Mitchell, 1972), which could provide the means for the establishment of the symbiotic system. Once more, microbial sensing seems to play a central role in the formation of biofilms.

2. Between Microenvironments

A biofilm can contain several distinctly different microenvironments as experienced by a microbial cell. For example, diffusional resistances within the biofilm influence the nutritional conditions as biofilm depth increases. Schaftel (1982) observed the development of anaerobic conditions within a biofilm in contact with media containing measurable amounts of dissolved oxygen. The combined aerobic/anaerobic biofilm resulted in considerably higher yields of soluble organic products being excreted into the reactor fluid. The products apparently are produced in the anaerobic microenvironment and diffuse through the aerobic biofilm into the reactor fluid. A fraction of the products may be consumed by organisms in the aerobic biofilm.

Nitrification and denitrification can occur within the same aerobic reactor. Such observations have been explained by presuming an anaerobic region within a microbial aggregate (biofilm or biofloc) in which nitrate is converted to nitrogen gas. The existence of sulfate-reducing bacteria (SRB) in apparently aerobic environments can also be explained in this way. SRB have been implicated in accelerated corrosion of metals in cooling waters where dissolved oxygen concentrations are relatively high (Miller and Tiller, 1970).

Biofilms are not necessarily uniform in thickness or areal density over the entire substratum. Cracks, crevices, and microroughness may contribute to biofilm "patchiness" as do chemical characteristics of the surface. The resulting patchiness may result in microenvironments that are different but which interact in a fluid-flow system through the process of detachment (see II,B,4) and reattachment on another microcolony downstream. Schrader (1982) has indicated that these "islands" of biofilm may be important in elucidating a mechanism for microbial cell adhesion as well as microbially mediated corrosion.

3. Interaction between Dispersed and Attached Microorganisms

attached at the surface, especially in a fluid-flow system. Brash and Samak (1978) presented experimental evidence that significant turnover occurs in molecular (proteinaceous) fouling films on polyethylene. Protein molecules in suspension are continuously exchanging with adsorbed proteins. Trulear (1983) developed a biofilm consisting of *Pseudomonas aeruginosa* in a continuous flow reactor. *P. aeruginosa* was fed continuously throughout the experiment. After a mature biofilm developed, the reactor was challenged with a simultaneous feed of *Sphaerotilus natans* at a cell concentration approximately equal to that of the *P. aeruginosa*. Within a relatively short time, *S. natans* displaced the *P. aeruginosa* in the biofilm.

Microbial succession in the biofilm must be influenced by the microorganisms dispersed in the fluid phase and their relative concentrations. The succession rate is most probably influenced by fluid shear stress at the biofilm–fluid interface because detachment undoubtedly affects the interactions at the interface. The rate and mode of succession could determine how SRB become established in biofilms which are continuously bathed in aerobic media. Are the SRB present on the surface from time zero or do they enter the established biofilm by detachment–reattachment processes? We have no answer at present.

V. Effects of Biofilms

A. Fluid Frictional Resistance

Thin biofilms develop on wetted surfaces in tubes and pipes and dramatically increase fluid frictional resistance (and turbulent intensity) to flow even in very large-diameter conduits (Characklis, 1973). Biofilms affect flow in at least three ways (Picologlou *et al.*, 1980): they (1) reduce the cross-sectional area available for flow, (2) increase the roughness of the surface, and (3) increase the drag by virtue of their viscoelastic properties.

Generally, the biggest contributing factor is the increased roughness. The roughness effect is magnified by filamentous organisms that become established in the biofilm (Picologlou *et al.*, 1980; McCoy and Costerton, 1982).

As the biofilm develops in a fluid-flow conduit, one of the following two responses will be observed: (1) at constant fluid velocity, pressure drop will increase and (2) at constant pressure drop, fluid velocity will decrease.

Constant fluid velocity can be maintained in many pumped systems even though pressure drop is increasing. However, pumping costs increase. Constant pressure drop (pressure drop is directly proportional to fluid shear stress at the conduit wall) is characteristic of gravity flow systems. As fouling biofilms develop, flow rate decreases and causes serious problems for water supply and power plant operations (Characklis, 1973). Loeb *et al.* (1983)

have demonstrated the negative influence of biofouling on drag on ship hulls.

B. Heat Transfer Resistance

Biofilms develop on heat transfer surfaces (tubes) and generally impede the flow of heat across the interface. Heat transfer occurs through two mechanisms and biofilms influence both of them (Characklis *et al.*, 1981): (1) conductive heat transfer and (2) convective heat transfer.

Conductive heat transfer occurs through the tube surface and is dependent on the tube thickness and tube thermal conductivity. Biofilm accumulates on the tube and serves as an insulator, thereby reducing heat transfer from the water. Conductive heat transfer will also depend on the biofilm thickness and biofilm thermal conductivity.

Convective heat transfer depends on turbulent intensity that, in turn, depends on tube roughness and fluid velocity. Convective heat transfer reflects the transport of heat away from the tube wall by fluid motion. As a biofilm develops, tube roughness increases (see Section V,A) and convective heat transfer increases, a positive effect. In most cases, however, the increase in convective heat transfer is far outweighed by the decrease in conductive heat transfer (Characklis *et al.*, 1981).

C. Corrosion

The influence of biofilms on corrosion is determined by the activity at the anodic and cathodic corrosion sites. Some of the ways biofilms may influence corrosion processes include the following:

1. EPS produced by the biofilm is, essentially, polyelectrolyte material. As a consequence, EPS may serve as an electron sink at the cathode.
2. Differential concentration cells may form as a result of biofilm patchiness because different locations on the surface will contain varying amounts of biofilm.
3. Acid is produced within the biofilm, especially in anaerobic microenvironments.
4. Sulfate-reducing bacteria may influence corrosion by depolarizing the cathode or through other processes resulting from sulfide production.
5. The biofilm may serve as a molecular sieve that alters ion mobility near the metal surface.

The important role of biofilm and attached organisms on corrosion processes is only now being defined. Much more research is necessary before a

D. Water Quality in Natural Streams

Algae and bacteria in fast-moving, relatively oligotrophic waters, are primarily found attached to surfaces in the stream bed. Their photosynthetic and respirometric activity contributes to diurnal, as well as long-term, changes in water quality. Photosynthetic biofilms in streams can contribute to organic carbon (Escher and Characklis, 1982), bacterial growth (Haack and McFeters, 1982a,b), changes in alkalinity, dissolved oxygen concentration, and pH (Escher, 1983). The interaction between bacteria and algae within these biofilms is rather remarkable and may have a significant effect on the oxygen dynamics of a stream (Escher and Characklis, 1982).

The wide variation in pH (relatively high during the day) may significantly influence the role of the biofilms as an environmental sink for refractory organics and heavy metals.

Organisms attached to suspended particulates may also contribute significantly to biological activity in natural waters (Jannasch and Pritchard, 1972). Certainly, this is true of open ocean water.

VI. Areas of Research in Need of Further Study

Certain aspects of research concerning biofilm processes are lacking in detail and are pertinent to the progress of the field. Our recommendations for further study are listed below. The order does not represent any form of priority.

1. Many aspects of biofilm research mention EPS. Nowhere is there a definitive study of the polymer(s) concerned; i.e., purification, analysis of monomer components, and structural determination.
2. Research on EPS typically leads to its mode of synthesis. What is the biochemistry, where is it synthesized, and how is it exported across the cell membrane?
3. What are the necessary physical properties of EPS that relate to its role as an adhesive molecule?
4. How is a surface sensed by the cell? "Sensing" may very well lead to synthesis of EPS. Is sensing the initial step in synthesis of adhesive? (Perhaps one should first ask—*are* surfaces sensed by these organisms?)
5. What types of compounds or conditions lead to inhibition of EPS synthesis?
6. Are pioneer organisms important in directing the course of succession? Does prevention of early biofouling really alter the course of macrofouling? Until all experiments can be performed under controlled microbiological conditions (including those with invertebrate larvae), we will not be able to answer the question with assurance.

pounds are concerned, which are producers, and which are consumers? What is its significance in the economy of the film?

8. Does the close proximity of organisms in the biofilm facilitate genetic exchange?

9. The physicochemical theories proposed to explain adhesion are not altogether satisfactory. Are alternative theories available? For example, Edelman (1983) considers current physicochemical theories of cell–cell adhesion to be untenable. The logical design of new surface-protective coatings for immersed manmade structures may depend heavily on this research.

10. The task of comparing one worker's results in this field with those of another is difficult. It is said that biochemists, in general, work on two microorganisms. One is *Escherichia coli*, the other is not! Although we do not advocate the abandonment of all organisms but one, it would make a lot of sense to reduce the number of organisms being studied and to attempt to standardize some experimental procedures, such as those for the enumeration of attached cells. Obviously, where a particular manufacturing process is being studied, it makes sense to use those organisms known to cause the fouling problems. But for fundamental studies of the adhesive process, some rationalization would be useful.

11. Little is known about biofilm activity toward particulate substrates frequently found in natural and technological application. Most laboratory studies use soluble substrates.

12. Do biofilms serve as a sink for heavy metals in natural waters?

13. As a final comment, consider an opposing viewpoint to the one that has pervaded this article. Perhaps the surface, as opposed to the bulk liquid, is not an ideal place for an organism to be. The surface may be an inhospitable environment where the organism becomes trapped, and as a means of survival produces an extracellular polymer. The EPS effectively insulates the cell from the stresses of the environment. This viewpoint has rarely, if at all, been considered in the design of experiments on biofilm biology.

VII. Summary

Biofilms play important roles, beneficial and detrimental, in many natural and technological processes. Methods to effectively inhibit or exploit biofilm processes will require considerably more insight than is available at present. This article has established a framework within which to interpret new results and observations regarding biofilms, their activities, and their effects.

ACKNOWLEDGMENTS

The authors wish to thank the United States Office of Naval Research (Oceanic Biology and

Characklis also acknowledges partial research support from the National Science Foundation, Chemical and Biochemical Systems Program, Division of Chemical and Process Engineering, and Calgon Corporation.

References

Adler, J. (1975). *Annu. Rev. Biochem.* **44**, 341–356.

Allan, G. G., Lewin, J., and Johnson, P. G. (1972). *Bot. Mar.* **15**, 102–108.

Baier, R. E. (1970). *In* "Adhesion in Biological Systems" (R. S. Manley, ed.), pp. 15–48. Academic Press, New York.

Baier, R. E. (1973). *In* "Proceedings of the Third International Congress on Marine Corrosion and Fouling" (R. F. Acker, B. F. Brown, J. R. DePalman, and W. P. Iverson, eds.), pp. 633–539. Northwestern Univ. Press, Evanston, Illinois.

Baier, R. E. (1975). *In* "Applied Chemistry at Protein Interfaces" (R. E. Baier, ed.), pp. 1–25, Advances in Chemistry Series, 145. Amer. Chem. Soc., Washington, D. C.

Baier, R. E. (1980). *In* "Adsorption of Microorganisms to Surfaces" (G. Bitton and K. C. Marshall, eds.), pp. 59–104. Wiley, New York.

Baier, R. E., and Weiss, L. (1975). *In* "Applied Chemistry at Protein Interfaces" (R. E. Baier, ed.), pp. 300–307, Advances in Chemistry Series, 145. Amer. Chem. Soc., Washington, D. C.

Baillod, R. D., and Boyle, W. C. (1970). *J. Sanit. Eng. Div. Am. Soc. Civ. Eng.* **96**, 525–545.

Bakke, R. (1983). M.S. Thesis, Montana State University, Bozeman, Montana.

Bauld, J., and Brock, T. D. (1974). *J. Phycol.* **10**, 101–106.

Beal, S. K. (1970). *Nucl. Sci. Eng.* **40**, 1–11.

Bell, W. C. (1980). *Limnol. Oceanogr.* **25**, 1007–1020.

Bell, W. C., and Mitchell, R. (1972). *Biol. Bull.* **143**, 265–277.

Bishop, J. H., Silva, S. R., and Silva, V. M. (1974). *J. Oil Chem. Assoc.* **57**, 30–35.

Bongers, L. H., O'Connor, T. P., and Burton, D. T. (1977). United States Environmental Protection Agency, EPA-600/7-77-053. Office of Research and Development, Washington, D.C.

Bowles, J. A., and Marsh, D. H. (1982). *Am. Soc. Microbiol. News* **48**, 295.

Brash, J. L., and Samak, Q. M. (1978). *J. Colloid Interface Sci.* **65**, 495–504.

Brown, C. M., Ellwood, D. C., and Hunter, J. R. (1977). *FEMS Microbiol. Lett.* **1**, 163–166.

Browne, L. W. B. (1974). *Atmos. Environ.* **8**, 801.

Bryers, J. D. (1979). Ph.D. Thesis, Rice University, Houston, Texas.

Bryers, J. D., and Characklis, W. G. (1979). *In* "Condenser Biofouling Control" (J. F. Garey, ed.), pp. 169–184. Ann Arbor Science, Ann Arbor, Michigan.

Bryers, J. D., and Characklis, W. G. (1981). *Water Res.* **15**, 483–491.

Bryers, J. D., and Characklis, W. G. (1982). *Biotech. Bioeng.* **24**, 2451–2476.

Callow, M. E., and Evans, L. V. (1981). *Bot. Mar.* **24**, 201–205.

Chamberlain, A. H. L. (1976). *In* "Proceedings of the Third International Biodegradation Symposium" (J. M. Sharpley and A. M. Kaplan, eds.), pp. 417–432. Applied Science, London.

Characklis, W. G. (1973). *Water Res.* **7**, 1249–1258.

Characklis, W. G. (1980). Final Report, Electric Power Research Institute, RP 902-1. Palo Alto, California.

Characklis, W. G. (1981). *Biotech. Bioeng.* **23**, 1923–1960.

Characklis, W. G., and Dydek, S. T. (1976). *Water Res.* **10**, 515–522.

Characklis, W. G., Trulear, M. G., Stathopoulos, N., and Chang, L. C. (1980). *In* "Water

Chlorination" (R. L. Jolkey *et al.*, eds.), pp. 349–368. Ann Arbor Science, Ann Arbor, Michigan.
Characklis, W. G., Nimmons, M. J., Picologlou, B. F. (1981). *J. Heat Transfer Eng.* **3**, 23–37.
Characklis, W. G., Zelver, N., Nelson, C. H., Lewis, R. O., Dobb, D. E., and Pagenkopf, G. K. (1983). Paper presented at Symp. Corros., 1983, Anaheim, California.
Chet, I., and Mitchell, R. (1976a). *Annu. Rev. Microbiol.* **30**, 221–240.
Chet, I., and Mitchell, R. (1976b). *In* "Proc. Int. Biodegradation Symp." (J. M. Sharpley and A. M. Kaplan, eds.), 3rd, pp. 515–521. Applied Science, London.
Cleaver, J. W., and Yates, B. (1975). *Chem. Eng. Sci.* **30**, 983–992.
Cleaver, J. W., and Yates, B. (1976). *Chem. Eng. Sci.* **31**, 147–151.
Cooksey, B., and Cooksey, K. E. (1980). *Plant Physiol.* **65**, 129–131.
Cooksey, B., Cooksey, K. E., Miller, C. M., and Paul, J. H. (1980). *In* "Microbial Adhesion to Surfaces" (R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent, eds.), pp. 526–528. Ellis-Horwood, Chichester.
Cooksey, K. E. (1981). *Appl. Environ. Microbiol.* **41**, 1378–1382.
Corpe, W. A. (1970). *In* "Adhesion in Biological Systems" (R. S. Manly, ed.), pp. 73–87. Academic Press, New York.
Corpe, W. A. (1973). "Proceedings of the Third International Congress on Marine Corrosion and Fouling," pp. 598–609. Gaithersburg, Maryland.
Corpe, W. A. (1978). *In* "Proceedings on the Microbiology of Power Plant Effluents" (R. M. Gerhold, ed.), pp. 57–66. University of Iowa, Iowa City, Iowa.
Corpe, W. A. (1980). *In* "Adsorption of Microorganisms to Surfaces" (G. Bitton and K. C. Marshall, eds.), pp. 105–144. Wiley, New York.
Corpe, W. A., Matsuuchi, L., and Armbuster, B. (1976). *In* "Proceedings of the Third International Biodegradation Symposium" (J. M. Sharpley and A. M. Kaplan, eds.), pp. 433–442. Applied Science, London.
Costerton, J. W., Geesey, G. G., and Cheng, K. J. (1978). *Sci. Am.* **238**, 86–95.
Costerton, J. W., Irwin, R. T., and Cheng, K. J. (1981). *Annu. Rev. Microbiol.* **35**, 299–324.
Culp, L. A., and Black, P. H. (1972). *Biochemistry* **11**, 2161–2172.
Culp, L. A., Murray, B. A., and Rollins, B. J. (1979). *J. Supramol. Struct.* **11**, 401–427.
Daniel, G. F., Chamberlain, A. H. L., and Jones, E. B. G. (1980). *Helgol. Wiss. Meeresunters.* **34**, 123–149.
Daniels, S. L. (1980). *In* "Adsorption of Microorganisms to Surfaces" (G. Bitton and K.C. Marshall, eds.), pp. 8–58. Wiley, New York.
Dawson, M. D., Humphrey, B. A., and Marshall, K. C. (1981). *Curr. Microbiol.* **6**, 195–198.
Dempsey, M. J. (1981). *Bot. Mar.* **24**, 185–191.
DePalma, V. A., Goupil, D. W., and Akers, C. K. (1979). Paper presented at Offshore Therm. Energy Convers. Conf. 6th, June 19–22. Washington, D.C.
Derenbach, J. B., and Williams, P. J. LeB. (1974). *Mar. Biol.* **25**, 263–269.
Derjaguin, B. V., and Landau, L. (1941). *Acta Physicochim. URSS* **14**, 633–662.
Dexter, S. C. (1979). *J. Colloid Interface Sci.* **70**, 346–354.
Dexter, S. C., Sullivan, J. D., Williams, J., and Watson, S. W. (1975). *Appl. Microbiol.* **30**, 298–308.
DiSalvo, L. H. (1973). *Limnol. Oceanogr.* **18**, 165–168.
Dolowy, K. (1980). *In* "Cell Adhesion and Motility" (A. S. G. Curtis and J. D. Pitts, eds.), pp. 39–63. Cambridge Univ. Press, London and New York.
Eckert, R., Naitoh, Y., and Machemer, H. (1976). *In* "Calcium in Biological Systems: Society for Experimental Biology Symposium" (C. J. Duncan, ed.), Vol. 30, pp. 233–255. Cambridge Univ. Press, London and New York.
Edelman, G. M. (1983). *Science* **219**, 450–457.

Edgar, L. A., and Pickett-Heaps, J. D. (1982). *Protoplasma* 113, 10–22.
Escher, A. (1983). M.S. Thesis, Montana State University, Bozeman, Montana.
Escher, A., and Characklis, W. G. (1982). *Biotech. Bioeng.* 24, 2283–2290.
Evans, L. V. (1981). *Bot. Mar.* 24, 167–174.
Fletcher, M. (1976). *J. Gen. Microbiol.* 94, 400–404.
Fletcher, M. (1980). *In* "Bacterial Adherence" (E. H. Beachey, ed.), pp. 347–374. Chapman & Hall, London.
Fletcher, M., and Floodgate, G. D. (1973). *J. Gen. Microbiol.* 74, 325–334.
Fletcher, M., and Marshall, K. C. (1982). *Appl. Environ. Microbiol.* 44, 184–192.
Friedlander, S. K., and Johnstone, H. F. (1957). *Ind. Eng. Chem.* 49, 1151–1156.
Geesey, G. G. (1982). *Am. Soc. Microbiol. News* 48, 9–14.
Gerchakov, S. M., Marszalek, D. S., Roth, F. J., and Udey, L. R. (1977a). *In* "Proceedings of the Fourth International Congress on Marine Corrosion and Fouling" (V. Romanovsky, ed.), pp. 203–211.
Gerchakov, S. M., Marszalek, D. S., Roth, F. J., Gallman, B., and Udey, L. R. (1977b). *In* "Proceedings of the OTEC Biofouling and Corrosion Symposium" (R. H. Gray, ed.), pp. 63–75. Pacific Northwest Laboratories, Richland, Washington.
Gibbons, R. J. (1980). *In* "Microbial Adhesion to Surfaces" (R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent, eds.), pp. 351–388. Ellis-Horwood, Chichester.
Gingell, D., and Vince, S. (1980). *In* "Cell Adhesion and Motility" (A. S. G. Curtis and L. D. Pitts, eds.), pp. 1–37. Cambridge Univ. Press, London and New York.
Grinnell, F., Milam, M., and Srere, P. A. (1972). *Arch. Biochem. Biophys.* 153, 193–198.
Haack, T. K., and McFeters, G. A. (1982a). *Microbiol. Ecol.* 8, 115–126.
Haack, T. K., and McFeters, G. A. (1982b). *Appl. Environ. Microbiol.* 43, 702–707.
Harper, M. A. (1977). *In* "The Biology of the Diatoms" (D. Werner, ed.), pp. 224–249. Univ. of California Press, Berkeley.
Harper, M. A., and Harper, J. F. (1967). *Br. Phycol. Bull.* 3, 195–207.
Harrick, N. J. (1967). "Internal Reflection Spectroscopy." Wiley (Interscience), New York.
Hellebust, J. A., and Lewin, J. (1977). *In* "The Biology of Diatoms," pp. 169–197. Univ. of California Press, Berkeley.
Hendey, N. I. (1951). *J. R. Microsc. Soc.* 71, 1–86.
Herbert, D. (1961). *Symp. Soc. Gen. Microbiol.* 11, 391–416.
Heukelekian, H. (1956). *Sewage Ind. Wastes* 28, 206–210.
Hirsch, P. (1979). *In* "Proceedings of the Dahlem workshop on Strategies of Microbial Life in Extreme Environments" (M. Shilo, ed.), pp. 357–372. Dahlem Konferenen Chemie Verlag, Berlin.
Hopkins, J. T. (1967). *J. Quekett Microsc. Club* 30, 209–217.
Hopkins, J. T., and Drum, R. W. (1966). *Br. Phycol. Bull.* 3, 63–67.
Howell, J. A., and Atkinson, B. (1976). *Water Res.* 18, 307–315.
Humphrey, B. A., Dickson, M. R., and Marshall, K. C. (1979). *Arch. Microbiol.* 120, 231–238.
Humphrey, B. A., Kjelleberg, S., and Marshall, K. C. (1983). *Appl. Environ. Microbiol.* 45, 43–47.
Huntsman, S. A., and Slonecker, H. H. (1971). *J. Phycol.* 7, 261–264.
Jannasch, H. W., and Pritchard, P. H. (1972). *Detritus Its Role Aquat. Ecosyst. Proc. IBP-UNESCO Symp.*
Jones, H. C., Roth, I. L., and Sanders, W. M. (1969). *J. Bacteriol.* 99, 316–325.
Karaeva, N. I. (1961) (translation 1968). *In* "Marine Fouling and Borers" (I. V. Starostin, ed.), pp. 109–119. US Dept. of Commerce and National Science Foundation, Washington, D.C.

Kjelleberg, S., Humphrey, B. A., and Marshall, K. C. (1982). *Appl. Environ. Microbiol.* **43**, 1166–1172.

Koshland, D. E., Jr. (1980). *In* "Bacterial Sensing as a Model Behavioral System," p. 193. Raven, New York.

Lewin, J. C. (1955). *J. Gen. Microbiol.* **13**, 162–169.

Lewin, J. C., Lewin, R. A., and Philpott, D. E. (1958). *J. Gen. Microbiol.* **18**, 418–426.

Lewin, R. A. (1958). *Limnol. Oceanogr.* **3**, 111–113.

Lewin, R. A. (1965). *Can. J. Microbiol.* **11**, 77–86.

Lin, C. S., Moulton, R. W., and Putnam, G. L. (1953). *Ind. Eng. Chem.* **45**, 636–640.

Lister, D. H. (1979). *In* "Fouling of Heat Transfer Equipment" (E. F. S. Somerscales and J. G. Knudsen, eds.), pp. 135–200. Hemisphere, Washington, D.C.

Loeb, G. I., and Neihof, R. A. (1975). *In* "Applied Chemistry at Protein Interfaces" pp. 319–335. Advances in Chemistry Series, 145. Amer. Chem. Soc. Washington, D.C.

Loeb, G. I., Laster, D., and Gracik, T. (1983). *In* "Interdisciplinary Research on Marine Biodeterioration" (R. C. Tipper and J. D. Costlow, eds.). Naval Institute Press, Annapolis, Maryland. in press.

McCoy, W. F., and Costerton, J. W. (1982). *Appl. Environ. Microbiol.* **43**, 1490–1494.

McFeters, G. A., Stuart, S. A., and Olson, S. B. (1978). *Appl. Environ. Microbiol.* 383–391.

Mackie, E. B., Brown, K. N., Lam, J., and Costerton, J. W. (1979). *J. Bacteriol.* **138**, 609–617.

Marshall, K. C. (1976). *In* "Interfaces in Microbial Ecology," p. 156. Harvard Univ. Press, Cambridge, Massachusetts.

Marshall, K. C. (1979). *In* "Strategies of Microbial Life in Extreme Environments" (M. Shilo, ed.), pp. 281–290, Dahlem Konferenzen Life Sciences Research Report 13. Verlag Chemie, Weinheim.

Marshall, K. C. (1980). *In* "Adsorption of Microorganisms to Surfaces" (G. Bitton and K. C. Marshall, eds.), pp. 1–5. Wiley, New York.

Marshall, K. C., Stout, R., and Mitchell, R. (1971). *J. Gen. Microbiol.* **68**, 337–348.

Marszalek, D. S., Gerchakov, S. M., and Udey, L. R. (1979). *Appl. Environ. Microbiol.* **38**, 987–995.

Matson, J. V., and Characklis, W. G. (1976). *Water Res.* **10**, 877–885.

Matson, J. V., and Characklis, W. G. (1983). *J. Cooling Tower Inst.* **4**, 27–32.

Meadows, P. S. (1971). *Arch. Mikrobiol.* **75**, 374–381.

Miller, J. D. A., and Tiller, A. K. (1970). *In* "Microbial Aspects of Metallurgy" (J. D. A. Miller, ed.), pp. 61–128. Amer. Elsevier, New York.

Miller, M. A., Rapean, J. C., and Whedon, W. F. (1948). *Biol. Bull.* **94**, 143–147.

Morita, R. Y. (1982). *Adv. Microb. Ecol.* **6**, 171–198.

Mueller, J. A., *et al.* (1966). *Proc. Annu. Purdue Ind. Waste Conf., 21st*, pp. 962–995.

Neihof, R. A., and Loeb, G. I. (1972). *Limnol. Oceanogr.* **17**, 7–16.

Nishikawa, S., and Kuriyama, M. (1968). *Water Res.* **2**, 811–812.

Norrman, G., Characklis, W. G., and Bryers, J. D. (1977). *Dev. Ind. Microbiol.* **88**, 581–590.

Olden, K., Hahn, L-H. E., and Yamada, K. M. (1980). *In* "Cell Adhesion and Motility" (A. S. G. Curtis and J. D. Pitts, eds.), pp. 357–387. Cambridge Univ. Press, London and New York.

O'Neill, T. B., and Wilcox, G. L. (1971). *Pac. Sci.* **25**, 1–12.

Paerl, H. W. (1980). *In* "Adsorption of Microorganisms to Surfaces" (G. Bitton and K. C. Marshall, eds.), pp. 375–402. Wiley (Interscience), New York.

Pethica, B. A. (1961). *Exp. Cell Res.* (Suppl. 8), 123–140.

Pethica, B. A. (1980). *In* "Microbial Adhesion to Surfaces" (R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent, eds.), pp. 19–45. Ellis-Horwood, Chichester.

Picologlou, B. F., Zelver, N., and Characklis, W. G. (1980). *J. Hydraul. Div. Am. Soc. Civ. Eng.* **106**, 733–746.

Poindexter, J. S. (1979). *In* "Proceedings of the Dahlem Workshop on Strategies of Microbial Life in Extreme Environments" (M. Shilo, ed.), pp. 341–356. Dahlem Konferenzen, Verlag Chemie, Weinheim.

Poindexter, J. S. (1981). *In* "Advances in Microbial Ecology" (M. Alexander, ed.), Vol. 5, pp. 63–89. Plenum, New York.

Powell, M. S., and Slater, N. K. H. (1982). *Biotech. Bioeng.* **24**, 2527–2537.

Powell, M. S., and Slater, N. K. H. (1983). *Biotech. Bioeng.* in press.

Rastetter, E. B., and Cooke, W. J. (1979). *Mar. Biol.* **53**, 271–280.

Rees, D. A., Badley, R. A., Lloyd, C. W., Thom, D., and Smith, C. G. (1978). *In* "Cell-Cell Recognition; Society for Experimental Biology" (A. S. G. Curtis, ed.), Vol. 32, pp. 241–260. Cambridge Univ. Press, London and New York.

Rogers, H. J. (1979). *In* "Adhesion of Microorganisms to Surfaces" (D. C. Ellwood and S. Melling, eds.), pp. 29–55. Academic Press, New York.

Rosen, J. J., and Culp, L. A. (1977). *Exp. Cell Res.* **107**, 139–149.

Rosenberg, M., Bayer, E. A., Delarea, J., and Rosenberg, E. (1982). *Appl. Environ. Microbiol.* **44**, 929–937.

Rouhiainen, P. O., and Stachiewicz, J. W. (1970). *J. Heat Transfer* (Trans. ASME) **92**, 169–177.

Roux, W. (1894). *Arch. Entwicklungsmech. Org.* **1**, 43–68.

Rutter, P. R. (1980). *In* "Cell Adhesion and Motility" (A. S. G. Curtis and J. D. Pitts, eds.), pp. 103–135. Cambridge Univ. Press, London and New York.

Rutter, P. R., and Vincent, B. (1980). *In* "Microbial Adhesion to Surfaces" (R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent, eds.), pp. 79–92. Ellis-Horwood, Chichester.

Schaechter, M., Maaløe, O., and Kjeldgaard, N. O. (1958). *J. Gen. Microbiol.* **19**, 592–606.

Schaftel, S. O. (1982). M.S. Thesis, Montana State University, Bozeman, Montana.

Schrader, M. E. (1982). *J. Colloid Interface Sci.* **88**, 296–297.

Sechler, G. E., and Gundersen, K. (1973). *In* "Proceedings of the Third International Congress on Marine Corrosion and Fouling" (R. F. Acker, B. F. Brown, J. R. DePalmar, and W. P. Iverson, eds.), 3rd, pp. 610–616. Northwestern Univ. Press, Evanston, Illinois.

Sharp, J. H. (1977). *Limnol. Oceanogr.* **22**, 381–399.

Skerman, T. M. (1956). *N. Z. J. Sci. Technol. Sect. B* **38**, 44–57.

Slots, J., and Gibbons, R. J. (1978). *Infect. Immun.* **19**, 254–264.

Sutherland, I. W. (1980). *In* "Microbial Adhesion to Surfaces" (R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent, eds.), pp. 329–338. Ellis-Horwood, Chichester.

Sutherland, I. W. (1982). *In* "Advances in Microbial Physiology" (A. H. Rose and J. G. Morris, eds.), Vol. 23, pp. 79–150. Academic Press, New York.

Tabor, P. S., Ohwada, K., and Colwell, R. R. (1981). *Microb. Ecol.* **7**, 67–83.

Terry, A. H., and Culp, L. A. (1974). *Biochemistry* **13**, 414–425.

Thomas, T. R. (ed.) (1982). "Rough Surfaces." Longman Group Limited, London.

Timperley, D. (1981). *In* "Fundamentals and Applications of Surface Phenomena Associated with Fouling and Cleaning in Food Processing" (B. Hallstrom, D. B. Lund, and Ch. Tragardh, eds.), pp. 402–412. Dept. of Food Science, Univ. of Wisconsin, Madison, Wisconsin.

Torella, F., and Morita, R. Y. (1981). *Appl. Environ. Microbiol.* **41**, 518–527.

Trulear, M. G. (1983). Ph.D. Dissertation. Montana State University, Bozeman, Montana.

Trulear, M. G., and Characklis, W. G. (1982). *J. Water Pollut. Control Fed.* **54**, 1288–1301.

Verwey, E. J. W., and Overbeek, J. Th.G. (1948). "Theory of the Stability of Lyophobic Colloids." Elsevier, Amsterdam.

Ward, J. B., and Berkeley, R. C. W. (1980). *In* "Microbial Adhesion to Surfaces" (R. C. W. Berkeley, J. M. Lynch, J. Melling, P. R. Rutter, and B. Vincent, eds.), pp. 47–66. Ellis-Horwood, Chichester.

Webster, D. R., Cooksey, K. E., and Rubin, R. W. (1982). *Am. Soc. Cell Biol.* (abstracts of annual meeting). Baltimore, Maryland.

Weiss, G. B. (1974). *Annu. Rev. Pharmacol.* 14, 343–354.

Weiss, L., and Harlos, J. P. (1977). *In* "Intercellular Communications" (W. C. deMello, ed.), pp. 33–59. Plenum, New York.

Wells, A. C., and Chamberlain, A. C. (1967). *Br. J. Appl. Physiol.* 18, 1793–1799.

Wiebe, W. J., and Smith, D. F. (1977). *Mar. Biol.* 42, 213–225.

Williams, P. J. LeB., and Yentsch, C. S. (1976). *Mar. Biol.* 34, 31–40.

Williamson, K. J., and McCarty, P. L. (1976). *J. Water Pollut. Control Fed.* 48, 281–296.

Wood, E. J. F. (1950). *Aust. J. Mar. Freshwater Res.* 1, 85–91.

Wood, E. J. F. (1967). "Microbiology of Oceans and Estuaries," Series 3. Elsevier, Amsterdam.

Wotter, K. (1982). *Mar. Ecol.* 7, 287–295.

Zelver, N., Flandreau, J. R., Spataro, W. H., Chapple, K. R., Characklis, W. G., and Roe, F. L. (1982). Paper presented at Am. Soc. Mech. Eng. Joint Power Generation Conference, Denver, Colorado.

Zisman, W. A. (1964). *In* "Contact Angle Wettability and Adhesion" (F. M. Fowkes, ed.), pp. 1–51. Advances in Chemistry Series, 43. Am. Chem. Soc., Washington, D.C.

Zobell, C. E. (1943). *J. Bacteriol.* 46, 39–59.

Zobell, C. E., and Allen, E. C. (1935). *J. Bacteriol.* 29, 239–251.