

tannic acid and benzoic acid are strongly repellent for oyster larvae. It remains to be seen if alginic acid prevents fouling when placed in a marine coating.

Our data makes clear that attraction of larvae to the specific organic chemicals produced by the primary microbial film on a surface is important for invertebrate settlement and metamorphosis. Conversely, repulsion of the larvae by chemicals in the surface films can prevent settlement. This concept provides a useful means of preventing surface fouling by invertebrates, though a large number of chemicals must be screened in order to find a broad-spectrum larval repellent. In addition, long-range fouling control will necessitate the development of appropriate slow release matrices.

Our recent research showing the role of binding proteins

(lectins) in the settlement and metamorphosis of invertebrate larvae suggests another approach to fouling control. Since the lectins attach to specific polysaccharides produced by bacteria in the biofilms, larval attachment might be prevented by blocking the bacterial polysaccharides with a lectin analogue. A less specific approach would involve control of the formation of bacterial biofilms.

Acknowledgments

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Initial Events in Microbial Film Formation

By
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Abstract

The earliest phases of biological fouling of synthetic or engineering materials in the sea are dominated by deposits of high molecular weight polymeric substances that serve as "conditioning" films or interface conversion layers. The film-forming substances are contributed from two major sources: 1) spontaneous adsorption of biological macromolecules—or more often their oxidized end-products, such as humic matter and gelbstoffe—originally in solution or suspension in the seawater, and 2) bacterial secretion of protein-polysaccharide slimes by the pioneer attaching microbial organisms. Significant specificity seems to be shown for both these processes, in that the adsorbing macromolecules and earliest attached organisms are not necessarily the most abundant components present in the adjacent aqueous boundary layers. Conversely, a degree of universality is indicated by the observations 1) substratum changes do not evoke changes in the primary film compositions, and 2) initial fouling events at the surface are not strong functions of the flow velocity of the bulk fluid. The latter observation may be explained by noting the limitation of these early deposits to a zone considerably thinner than the laminar sublayer remaining even in highly turbulent flow. With increasing exposure time, these initial films can be modified by displacement, adsorption competition, desorption, digestion, and reentrainment—all of these processes showing some dependence on original substratum surface properties which might, therefore, be modified to achieve practical improvements in fouling resistance.

Introduction

The accumulation of both microfouling and macrofouling organisms known to be detrimental to energy efficiency in navigation and in heat exchange follows a complex and ordered process, but a process amenable to detailed investigation by methods already successful in other bioadhesion studies (Baier, 1970, 1975). Preceding the attachment and growth of macrofouling organisms is the formation of microfouling layers which, in turn, are supported by earlier biopolymeric (conditioning) films and bacterial exudates. Our research has sought to provide a basic understanding of the microfouling process by elucidating the biophysical properties of the spontaneously adsorbed conditioning films and how they relate to the properties of the original substrata and to the subsequent attachment and distribution of bacteria and other cellular or particulate matter, living or dead.

Surface chemical methodology has been most useful in this endeavor since the microfouling process involves the formation and/or destruction of several interfaces during the "growth" of boundary deposits. The solid/gas interface is first replaced by the solid/liquid surface, which quickly changes due to the preferential adsorption, exclusion, or

reorganization of inorganic ions, extracellular biopolymers that are omnipresent in natural seawater, and boundary water molecules themselves. Next, the solid/biopolymer interface is altered in surface charge, and perhaps surface energy and chemical composition, as the biopolymer is conformationally changed, replaced by other biopolymers, or masked by additional material. As arriving bacteria become enabled to adhere by the preexisting macromolecular film, the biopolymer/bacteria interface is formed. Through the secretion of the bacterial slime, and often digestive exudates as well, the biopolymer/slime and slime/bacteria interfaces are also made. Subsequent attachment and colonization of the microfouling layer by cyprids and larvae from macrofouling species introduce many new interfaces that dominate the macrofouling stages. Although interesting in themselves, the macrofouling attachments are beyond the scope of the current report.

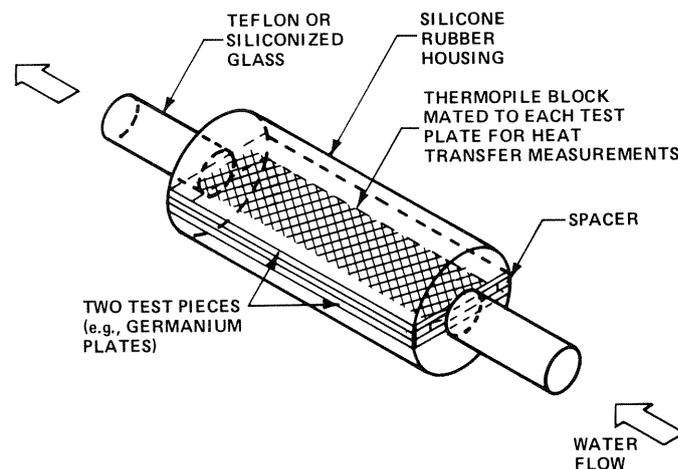
In an extensive series of maritime and freshwater evaluations of routinely used and newly developed materials, our universal finding was that the first discernible event at the engineering material/seawater interface was the spontaneous adsorption of a protein-dominated film, even in the presence of significant antifouling agents such as heavy metals, chlorine, and other poisons. Following this universal primary event were the universal secondary event of bacterial adhesion to the protein "conditioned" substrate and the tertiary event of bacterial exudation and further adhesion. Depending upon the response of the initially adherent bacteria to the "conditioned" surfaces, and also on the presence of toxic agents, bacterial aggregation with subsequently arriving bacterial cells occurred to trigger frank microfouling or, instead, the originally adherent bacteria detached from the "conditioned" surface to leave an apparently long-term fouling-resistant ablative protein layer on the material surface. These investigations during the past twelve years were mainly directed towards a more detailed understanding of the identity and properties of spontaneously formed protein interfacial films, often using surface chemical/physical methodology and purified protein solutions and synthetic polypeptides as experimental materials. The major tasks of these investigations included the formation and characterization of seawater interfacial protein films and their adhesive relationship to bacteria; the development of improved *in situ* evaluation techniques; and continued testing by surface characterization techniques of materials of special significance (e.g., coatings) as they became available.

Here, we provide only the briefest possible introduction to the investigative techniques employed, and to the most general findings by these methods for the initial stages of microbial film formation. Additional details are available in many supporting specialty publications (see Baier, 1972, 1981; Goupil, DePalma, and Baier, 1973, 1980; DePalma and Baier, 1978; Baier et al., 1981).

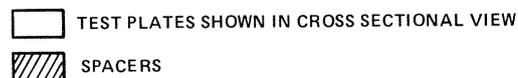
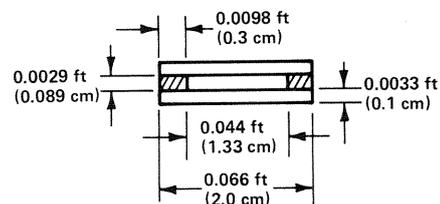
Methods and Materials

To acquire the initial biofouling films under dynamic conditions, variously treated and coated germanium prisms and other materials were mounted in flow cells, as illustrated in Figure 1. The flow cell housings were made of Sylgard 184 (Dow Corning, Midland, Michigan). This silicone housing material remained free of gross biofouling in our laboratory and field tests and has been shown not to influence the adsorbed conditioning films or microbial layers. Candidate test coatings were applied directly to germanium prisms following a glow discharge treatment procedure described earlier (Baier and DePalma, 1970).

Once assembled, the flow cells were connected to the feed lines from artificial seawater laboratory aquaria or natural ocean water piping installed in the Gulf of Mexico or off Key West, Florida. The flow cells were mounted in a vertical position and the usual flow (3 ft/sec) directed against the gravitational field. This procedure eliminated both inhomogeneous adsorption onto the test plates and entrapped air bubbles that might serve as nucleation sites for macromolecular denaturation. In the aquaria, water was sometimes pulled through the flow cells in order to eliminate the possibility of contamination from the pump. No evidence of pump-contributed contaminants was found



(a) THE FLOW CELL



(b) DIMENSIONS OF RECTANGULAR FLOW REGION

Figure 1. Schematic diagram of the Calspan flow cell.

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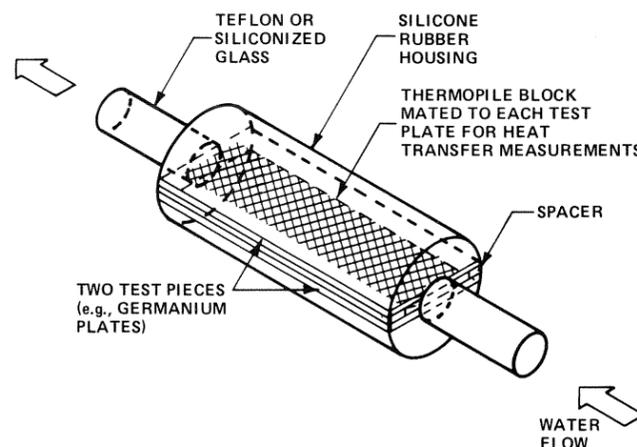
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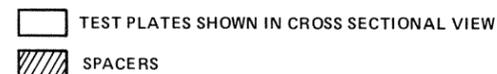
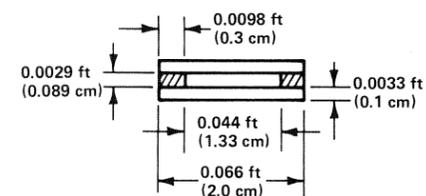
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Figure 1. Schematic diagram of the Calspan flow cell.

in any experiment, however, either in the laboratory or field trials.

The aquaria consisted of 50-gallon tanks that housed live, reproducing colonies of barnacles, algae, bryozoa, tubeworms, and tunicates as well as several types of bacteria and a wealth of macromolecular components. These colonies originated from Biscayne Bay, Miami Beach, Florida, where they had spontaneously attached to experimental panels under static test conditions. Thence, they were shipped to Calspan and placed in an environment of artificial seawater. The barnacles were fed a diet of freshly hatched brine shrimp on a regular basis.

Over a period of more than ten years, we have tested under either static or dynamic conditions a variety of metals (including copper-nickel alloy, aluminum, titanium, steel), toxic and nontoxic coatings (including silicones, fluorocarbons, polyamides, hydrophilic polymers, ablative layers, ionomers), and plastics (including nylon, polyvinylchloride, polyethylene, polytetrafluoroethylene, polyethylene terephthalate) in waters of Biscayne Bay, Florida; Monterey Bay, California; Chesapeake Bay, Maryland; the Gulf of Mexico; and off the Florida keys. The results in regard to the initial microfouling events were always similar, as obtained from the battery of tests illustrated in Figure 2. The large majority of our data was taken from germanium prisms and glass slides, either uncoated or serving as substrata for very thin films of surface-modifiers.

Typical Results

Figure 3 is a scanning electron micrograph of one of our test plates in a region deliberately scuffed to reveal the type of "conditioning" film always noted to deposit by spontaneous adsorption prior to successful attachment of particles of any sort. This initial microfouling event occurred for all substrata in all biological fluids under all

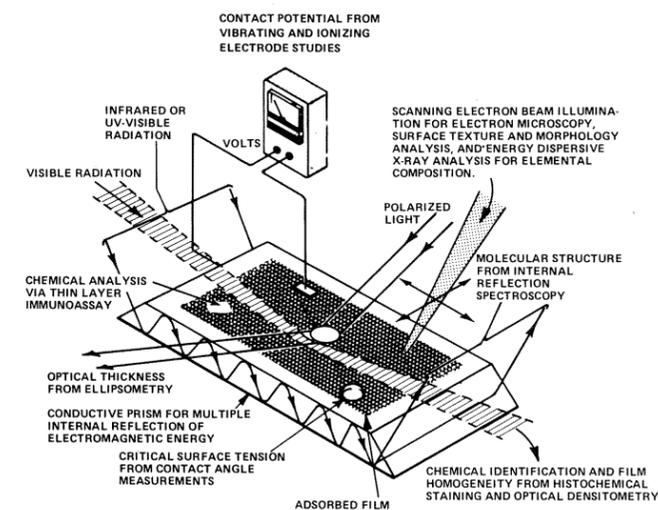


Figure 2. Schematic diagram of *in situ* analytical methods which are applicable in turn to the same surface deposit, with sensitivity at the sub-microgram level.



Figure 3. Scanning electron photomicrograph of a scuffed region of a metal plate after having acquired an organic "conditioning" film by spontaneous adsorption from flowing seawater.

conditions of flow, although the rate of film buildup obviously depended on the actual prevailing flow and solute concentrations of adsorbable matter.

Figure 4 provides a collection of infrared spectra, obtained by the multiple, attenuated, internal reflection technique for such films still in place—as adsorbed—on their substrata, revealing the dominant film composition to be the proteinaceous materials so ubiquitously present in natural aqueous systems. With continued exposure time in simulated or field environments, bacterial colonization, exudation, growth, and recruitment of attaching organisms thickened the initial fouling layer quite rapidly and altered its composition in the direction of increasing complexity. The lowermost spectral trace in Figure 4 typifies the transition microbial fouling film: just past the "induction" period and about to enter the phase of exponential growth.

Figure 5 is a scanning electron micrograph of such a microbial fouling layer just near the end of the induction period. Flagellated bacteria had by this time replaced the originally adherent rod-shaped microorganisms and begun to exude a polysaccharide-dominated "slime" that spread over and coupled to the preadsorbed glycoproteinaceous, cell-free conditioning film. Interestingly, polishing scratches or other surface irregularities did not have any major influence on the initial film formation or on microbial colonization events. Quickly following the attachment and secretory phase of initial microbial fouling (illustrated in Fig. 5) was the proliferation by both division and growth of filamentous forms. The resulting fibrous surface mats entrapped particulate debris and were excellent new sub-

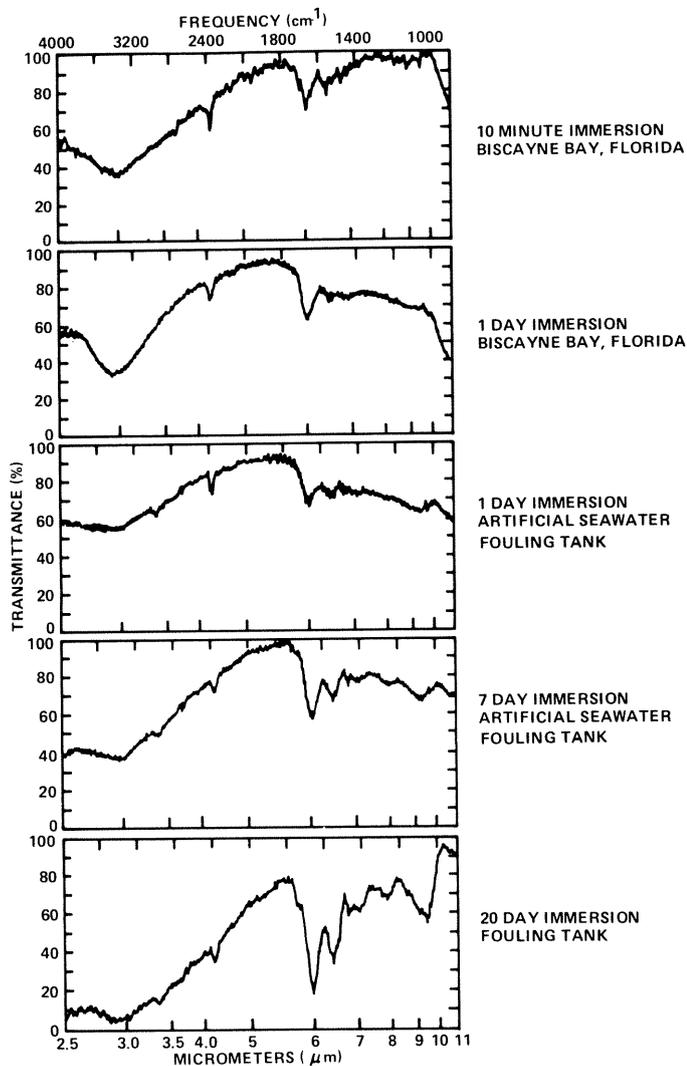


Figure 4. Characteristic infrared spectra of the spontaneously accumulated "conditioning" films on metallic slabs (germanium) statically immersed in fouling-prone seawater.

strata for attachment of diatoms. Without the use of toxic agents, such as leachable heavy metals in the coatings or active chlorine in the flowing stream, this filamentous growth and biological recruitment phase continued unabated for most materials. The induction period, during which no or sometimes negative changes in heat-transfer resistance and/or friction factor were noted, ended abruptly as the microfouling film extended beyond the laminar boundary layer to deteriorate operating efficiency significantly, as measured by increasing drag, friction, pressure loss, or heat-exchange retardation.

Our work has shown that adjustment of the critical surface tension of the original substrata to values between 20 and 30 mN/m correlates with minimal interface binding strengths at the level of the primary conditioning film, holding out the promise that effective engineering control of surface fouling within the induction period may be obtained by methods completely free of environmental or ecological hazards.

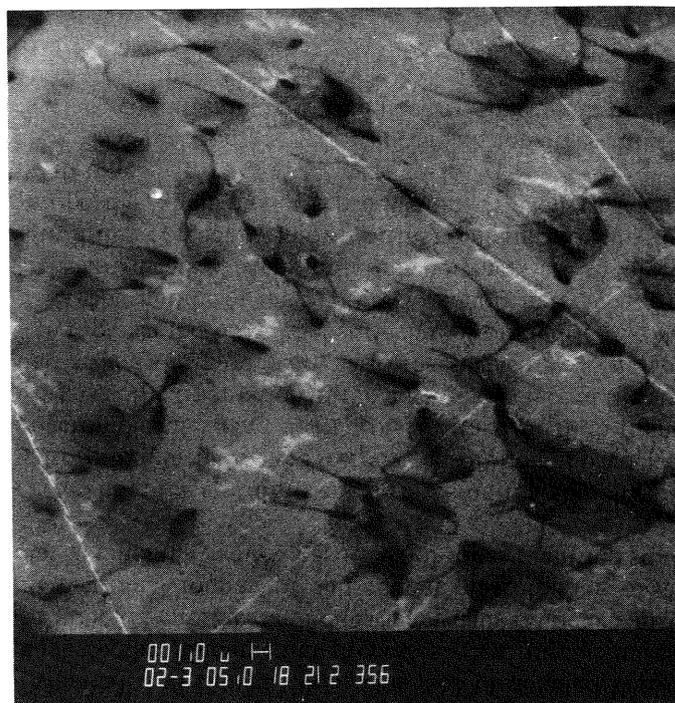


Figure 5. Scanning electron photomicrograph of a metal plate bearing a preadsorbed organic "conditioning" film, on top of which colonizing marine bacteria are in the process of secreting additional "slime-forming" material during the early process of microbial fouling in flowing seawater.

Discussion: Priorities for Future Research and Relationship to Ongoing Investigations

As our typical results show, adsorbed macromolecular layers and pioneer attaching microorganisms dominate the initial stages of biological fouling in all the circumstances so far evaluated. There exists a serious need, therefore, to develop an improved understanding of the adsorption of macromolecules and of microorganisms at surfaces (Marshall and Baier, 1981). Our lack of detailed knowledge of the fundamental processes occurring at diverse solid-liquid interfaces militates against any rational control of microbial fouling or subsequent macrofouling on an environmentally sound basis. Major areas requiring study are:

1. The effects of different surfaces on macromolecular adsorption, including the selective adsorption of particular macromolecules, the degree of coverage of the original surfaces, the orientation of the macromolecules at the surfaces, and the modifications to the surface free energies (as represented by critical surface tension data);
2. The nature of the microbial exopolymers anchoring cells to surfaces. Many different polymers are involved, but little is presently known of their chemical or physical properties, their mode of production, or their variability with time;
3. The reactions between microbial polymers and the surface-conditioning macromolecular films that provide

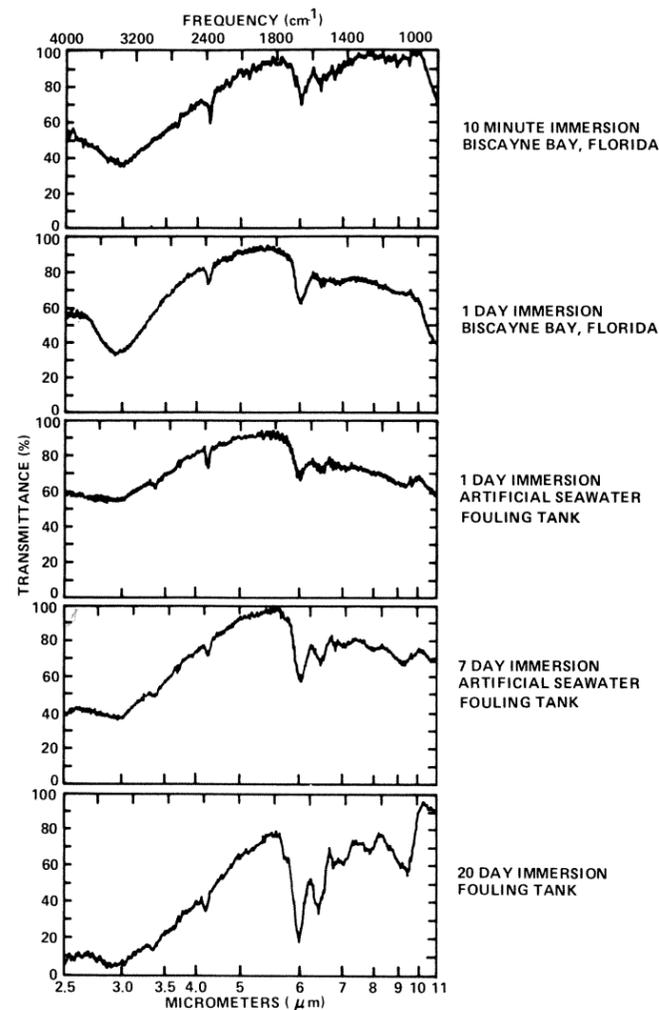


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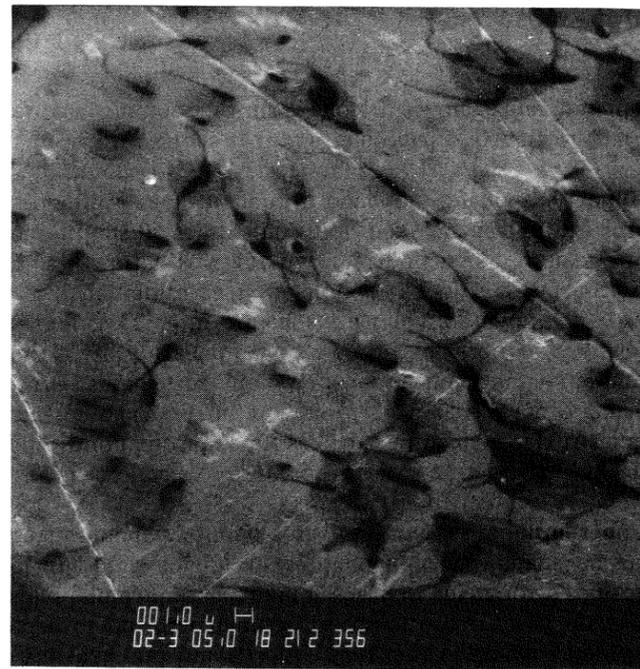


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ultimate binding of the microorganisms to the surfaces; and

4. The strengths of adhesion between the microbial polymers and the macromolecular films attached to surfaces of differing properties. This study could provide evidence of some weak links in microbial adhesion processes.

An important point to be stressed is that it may be unnecessary to prevent microbial fouling completely since we can produce effective engineering solutions, in many cases, by controlling the rate and reliability of reentrainment once the required fundamental data are in hand.

The fundamental interfacial data already acquired, with more to be sought, are supportive of, but not coincident with, the data currently being sought by other investigators who direct their attention mainly to the first attached organisms. K. C. Marshall (1981a) in Australia, W. Corpe (1980) in New York, T. Tosteson (Jimenez Velez et al., 1976) in Puerto Rico, G. Loeb (1980) in Washington, DC, S. Dexter (1978a) in Delaware, and R. Mitchell (1978) in Massachusetts are leading researchers of primary microbial fouling who have made sustained contributions to this field. More recently, in the context of U.S. Navy programs assisting the Ocean Thermal Energy Conversion program of the Department of Energy, B. Little (Little and Lavoie, 1979) of Mississippi has made substantial contributions to these studies, as have D. White (Bobbie, White, and Benson, 1980) and S. Gerchakov (Gerchakov et al., 1978) of Florida in the context of bioassay and corrosion assay development. Yet, we still must seek critical information that will improve our basic understanding of the phenomena assessed by the current investigative groups; namely, the properties of the various bacteria colonizing immersed surfaces, including their responses to nutrients accumulated at surfaces, their relative hydrophobicities, and their abilities to produce suitable bridging polymers.

Although a great deal of work has been done on the fouling of heat transfer surfaces, direct comparisons of most parameters have been impossible since differing experimental and test methods have been employed. It is vital that some uniformity in testing methods be evolved so that meaningful comparisons of different materials in different heat transfer systems can be made. Small, standardized field units—such as the flow cell shown in Figure 1—are now being developed to identify flow rates, shear forces, nutrient conditions, and so on. Such units can provide fouling indices with comparable meanings under a variety of conditions and at different times and sites. We expect that regular use of these inexpensive, standardized test units will provide a continually expanding data base concerning the effects of water quality, surface properties, and such like on rates of fouling under different physical, chemical, and biological conditions. Microbial films attaching to surfaces in such units will be preserved by appropriate fixation for subsequent laboratory examination to increase the information gained on the dominant organisms involved in film formation and growth.

The fundamental investigations proposed here are also critical to other aspects of the microbial fouling process that are of equally urgent concern and that cannot be divorced from the events occurring in the initial attachment period (Marshall and Baier, 1981). Transport processes in bulk aqueous phases may be rate limiting in microbial fouling. In addition, the compositions, adhesive strengths, cohesive strengths, and compactness of microbial films as well as the geometry and hydrodynamic properties of specific systems influence heat transfer characteristics and dictate features of turbulent flow and tendencies for deposit reentrainment. Studies of some of these factors are already in progress.

In Montana, W. Characklis (1981b) has emphasized that changes in heat-transfer resistance arise from the combined modifications of conductive and convective heat transfer paths. Conductive heat transfer can be limited by the increasing microbial film thickness and its diminished effective thermal conductivity. Convective heat transfer, resulting from fluid mixing, can be limited by the increasing frictional resistance induced by biofilm development. As long as the biofilm thickness is less than the thickness of the viscous sublayer (20–40 μm) existing in the fluid near the surface, however, changes in convective heat transfer are not accompanied by changes in friction factor. When filaments of the microbial layer project beyond the viscous sublayer and into the turbulent zone, turbulent wakes form behind each filament and result in an increase in friction factor and in turbulent heat-transfer rates. The increase in convective heat-transfer rate due to the higher friction factor is usually not sufficient to overcome the decrease in conductive heat transfer due to microbial film accumulation.

The repeated failure of direct engineering approaches to the control of such gross microbial fouling emphasizes the urgent need to examine the fundamental processes involved in the induction period so as to evolve a sound basis for future control measures. In view of the general lack of suitable nontoxic antifouling materials, the kinds of research described in this report seem most important for providing a sound conceptual basis for the development of antifouling or minimally-fouling surface materials that do not, themselves, pose secondary environmental or biological hazards.

Acknowledgments

I am grateful for the long and productive relationship, extending over the past decade, I have had with my professional colleagues in these studies: Dr. V. A. DePalma, Dr. D. W. Goupil, and Mr. R. W. King. The flow-cell concept reviewed here evolved from original concepts of V. A. DePalma as reported first in his Ph.D. dissertation, "Correlation of Surface Electrical Properties with Initial Events in Bioadhesion," State University of New York at Buffalo, 1976. Extensions of this work are now being pursued by M. Fornalik and H. Gucinski in their graduate

research programs under my direction in the Department of Biophysics, State University of New York at Buffalo. At Calspan Corporation, the project engineer for these continuing studies is Ms. A. E. Meyer, with able assistance from E. Gasiecki, G. Zigrossi, and J. Blickenstaff. Critical early contributions to this program were made by our colleagues Dr. R. Ziegler, S. Perlmutter, and R. Zontek.

I am also most appreciative of the sustained technical advice and criticism I have received over the past ten years

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