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BIOFOULING OF ENGINEERED MATERIALS AND SYSTEMS

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8.1 INTRODUCTION

The operational definition of "fouling" is the undesirable formation of deposits on surfaces of equipment, which results in compromised performance and/or reduced life of the equipment. Fouling occurs on a variety of wetted surfaces, including pipes, heat exchangers, membranes, and medical implants. Fouling can occur through several mechanisms, involving biological, corrosion, particulate, and precipitation reactions (Videla and Characklis, 1992).

Biofouling is the result of extensive growth of organisms on surfaces. Biofouling occurs rapidly in flowing systems where nutrients are supplied on a regular basis. Extensive growth of microorganisms and their secretion of extracellular polymers lead to the formation of visible slimy deposits on solid surfaces. In spite of intense research over the past 30 years to develop a surface that is resistant to biofouling, no viable candidate material has yet emerged. Consequently, biofilm control still depends on judicious monitoring and regularly scheduled equipment surface cleaning that minimizes biofilm accumulation in order to achieve acceptable equipment performance. In this chapter, the process of biofouling and its impact is described for a wide variety of engineered systems. In addition, the engineering aspects of biofouling are revisited in light of recent discoveries in biofilm behavior. Finally, advances in monitoring biofilm behavior are described, and the applications and limitations discussed.

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8.2 BIOFOULING OF STRUCTURES IN THE MARINE ENVIRONMENT

8.2.1 Oil and Gas Platforms

Man-made structures deployed in the offshore marine environment are subject to biofouling. Seawater contains the planktonic organisms that colonize submerged structures as well as the nutrients to support their growth and biofilm formation. Engineered structures of all types are prone to biofouling in seawater. In the southern North Sea the most severe fouling occurs in the tidal zone and on the first 20 m of structure below the water surface. Oil and gas platforms accumulate fouling organisms to an extent rarely observed in other industrial environments. Upon initial immersion, platform legs accumulate microscopic bacteria, including sulfur-reducing bacteria, algae, and fungi. Later, the microfouled surface becomes colonized by invertebrates such as mussels, barnacles, anemones, and seaweeds. In exceptional cases, fouling layers of 250 mm in thickness have been reported. Biofouling can develop to such an extent on platform legs that their structural properties change dramatically, exceeding the design allowance and accelerating fatigue. Costly, periodic cleaning, involving removal of the fouling layers by divers with hand tools, must be scheduled to avoid failure. Platform legs are so conducive to the growth of sessile organisms that they have been exploited for commercial shellfish rearing in some parts of the world.

The oil/water separation systems of an oil production operation provides a surface, an aqueous environment, and organic nutrients to support a rich anaerobic microbial biofilm that impedes the oil/water separation process, promotes corrosion, and plugs filters. Many bacteria in oil/water mixtures produce surfactants that act as emulsifiers. The emulsifiers form a separate phase that reduces the efficiency of the oil/water separation process. The biofilms formed in the oil/water separation systems periodically slough from the surfaces creating flocs in suspension that eventually become trapped in downstream filters. Plugging of the filters by the flocs also decreases the efficiency of the oil/water separation systems.

The seawater handling system of offshore oil production platforms treats, stores, and distributes water for various uses: to maintain pressure in the oil reservoir, fight fires, and provide water for heat exchangers and domestic services. The seawater handling system is a common site of biofouling. Biofouling of the water handling system contributes to injection well plugging, loss of pressure in fire water lines, and loss of heat exchange efficiency in heat exchangers.

Water produced with the oil during secondary production is often used as makeup water for reinjection into the oil reservoir. "Souring" of the oil reservoir occurs when sulfide-producing bacteria, slough from the anaerobic biofilms in the oil/water separation systems and are transported into the oil reservoir during water reinjection. Injection of raw seawater into the reservoir also promotes souring. Raw seawater contains relatively high concentrations of nutrients, electron donors, and sulfate, the electron acceptor used by sulfate-reducing bacteria (SRB) to form sulfide. Sour oil costs the industry in terms of corrosion and implementation of safety measures related to its toxicity at the production stage as well as in terms of air pollution mitigation during refining operations.

Biofilm bacteria sloughed into the makeup water also contribute to plugging at the site of water injection into the reservoir (Geesey et al., 1987). A thin but continuous biofilm develops at the interface of the well bore and porous reservoir sand, creating a back-pressure that requires more energy to pump the water into the reservoir. Eventually, the back-pressure caused by the membranelike biofilm becomes so great that the injection well must be taken off-line and treated with chemicals to remove the biofouling layer and restore reservoir permeability.

8.2.2 Biofouling of Ship Hulls

Fouling of ship hulls by marine organisms has been a problem for the maritime industry since shipbuilding began. Microbial biofilm formation is believed to precede macrofouling by multicellular algae and invertebrates. Among the macrofouling organisms, balanoid barnacles, mussels, polychaete worms, bryozoans, sponges, tunicates, and algae represent major fouling populations in the marine environment. The composition of the fouling community varies, however, depending on environmental conditions. The main macrofouling organisms off the Japanese coast in the East China Sea have been identified as *Balanus trigonus*, *Mytilus edulis*, and *Hormomya mutabilis* (Nakasono et al., 1993).

Indirect evidence suggests that surface growth of a wide diversity of bacteria and diatoms of the genera *Amphora*, *Amphiprora*, *Navicula*, and *Achnanthes* promote subsequent macrofouling (Cooksey and Wigglesworth-Cooksey, 1992). The accumulation of biomass on the hull increases turbulence at the hull surface, which in turn increases fluid frictional resistance and hydrodynamic drag to the ship as it moves through the water. It has been proposed that the nonrigid biofouling layer causes this increase in drag. Details of this phenomena can be found in Section 8.7.1.1. Microfouling alone has been shown in sea trials to increase drag by 5–15%. The drag-related fuel penalties have been estimated at $75–100 million per year (Alberte et al., 1992). The increased drag-related fuel costs incurred during the late 1970s forced the U.S. Navy to spend $360 million in dry dock costs for biofilm removal and prevention in 1981 (Alberte et al., 1992). Worldwide, the maritime industry spends an estimated $1 billion to control biofouling (Field, 1981).

The U.S. Navy applies antifouling coatings to hulls to reduce biofouling between scheduled hull overhaul dry-docking every 5–7 years. One coating developed by the Navy, RTV-11, now manufactured by General Electric Company, consists of a silicone-based polymer that presents a very unstable surface to colonizing marine organisms. The coating prevents ship hull biofouling as long as the ship is moving at moderate speed. The hull is subject to biofouling when it is anchored in port, however. Furthermore, the coating is easily abraided, resulting in exposure of the underlying hard surface to colonizing organisms, which can promote disbondment of the surrounding unabraided coating.

8.3 BIOFOULING OF HEAT EXCHANGERS

Heat exchangers are structures engineered to cool fluids. A schematic diagram of a typical tube-in-shell heat exchanger, such as that used to condense steam in the electric power industry is illustrated in Figure 8.1. As a heated fluid contacts a clean metal surface with high heat conductivity, the heat from the fluid is transferred to the metal. The metal then transfers the heat to another fluid, usually partitioned from the heated fluid by the metal itself. Copper-containing metal tubing often serves as the partition between the heated and cooled fluids in tubular heat exchangers. Heat transfer in fluid systems is controlled by (1) convective heat transfer through a gas, (2) conductive heat transfer from a fluid across the metal of the heat exchanger, and (3) advective heat transfer due to movement of a liquid across the metal surface (Marshall, 1992).

Deposits on the metal surface reduce heat transfer across the metal. Deposits may be either organic or inorganic or a combination of both. Calcium carbonate scales are an example of an inorganic deposit, whereas slimes or biofilms are an example of an organic deposit. It has been estimated that condenser tube failure through biological fouling is responsible for a 3.8% overall loss in availability of power plants generating 600MW or more in the United

States (Anson, 1977). The accumulation of a biological fouling layer on the metal surface decreases conductive heat transfer. The influence of a biological fouling layer on advective heat transfer, however, depends on the properties of the biofilm as described in Section 8.7.3.

8.3.1 Tubular Heat Exchangers

Fluid velocities in tube-in-shell heat exchangers such as that shown in Figure 8.1 generally range from 0.3–30 m/s and turbulent regime is the prevailing flow situation (Vieira et al., 1993). The buildup of a biofouling layer has several effects on hydrodynamics of the system. The buildup of a biofilm reduces the cross-sectional area of the water flow path, causing a pressure drop across the length of heat exchanger tubing. To force the same volume of water through a smaller cross-sectional area of tubing, one must increase flow velocity, which requires greater applied pressure. This results in increased turbulence at the tube wall, which can increase heat transfer efficiency. However, an additional expenditure of energy is required to force the water through the tubing, thus reducing overall operation efficiency. When the cross-sectional area of the water flow path is reduced by buildup of thicker fouling layers on the tube wall to a critical value that can no longer be compensated by increased pumping pressure, flow can change from turbulent to laminar regime. The reduction in the volume of water passing through the tubing per unit time and the change in flow regime will result in a reduction in heat transfer efficiency.

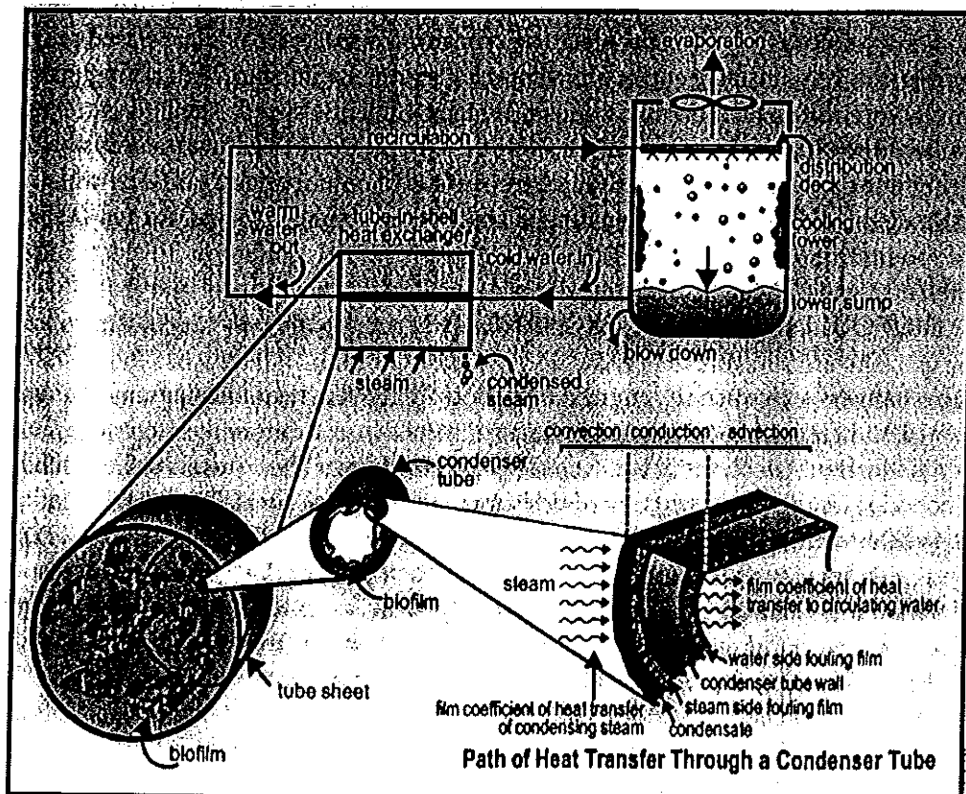

Figure 8.1 Schematic diagram of the tube-in-shell heat exchanger used in steam-generated power plant.

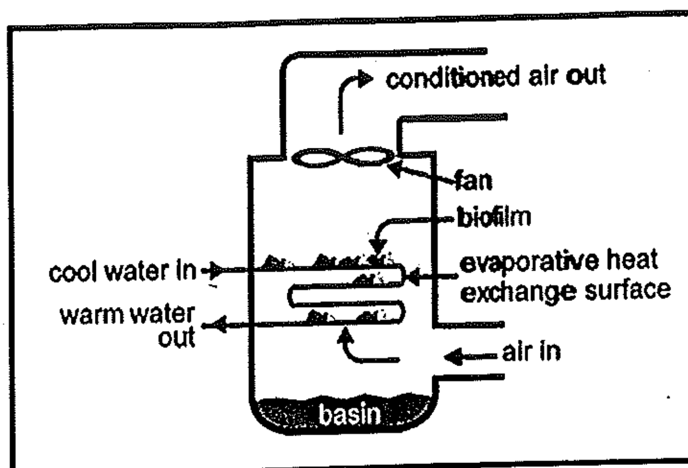

Figure 8.2 Schematic diagram of evaporative heat exchanger used in air-conditioning unit.

The presence of a biofouling layer on the surface of heat exchangers also alters the surface roughness. A biofilm layer converts the heat exchanger surface from a smooth rigid structure to one with a high degree of viscoelasticity, capable of deformation under the influence of the flowing fluid. This will increase surface roughness, which in turn increases fluid frictional resistance to flow near the tube wall. An increase in fluid frictional resistance at the heat exchanger surface can have different effects on heat transfer resistance. Fluid frictional resistance can decrease flow across the surface and thereby reduce heat transfer efficiency as described previously. Conversely, fluid frictional resistance increases turbulence at the heat transfer surface, thereby increasing heat transfer efficiency. The frictional effects of relatively thin biofilms (e.g., <100 μm thickness) are no larger than would be expected from the decrease in flow area. However, at some critical film thickness, frictional resistance increases sharply with thickness (Bott and Miller, 1983).

Characteristics of a heat exchanger system can influence the properties of the fouling layer. Within the range of fluid flow velocities encountered in tubular heat exchangers, hydrodynamics can significantly affect the physical and biological structure of the biofouling layer on the heat exchanger wall. The effect of fluid velocity on biofilm formation has been summarized as follows: higher fluid velocities (1) favor the transport of cells to the heat exchanger surface, (2) impede attachment of cells to the surface, (3) favor replication and production of extracellular polymeric substances (EPS) by the cells that do attach, (4) favor detachment of cells from the surface, and (5) favor denser, thinner, more cohesive biofilm structures with lower thermal resistance.

Internal diffusivity of nondegradable substances is lower in thinner, more compact biofilms. Diffusivity is therefore biofilm dependent and cannot be reduced to a common value (Vieira et al., 1993). Biofilm mass transfer coefficients can be interpreted as the quotient of the internal diffusivity and biofilm thickness. Mass transfer coefficients of biofilms formed over a range of fluid velocities typical of those in heat exchangers, do not differ significantly, however (Vieira et al., 1993).

Other system characteristics exert less of an influence on biofilm properties. Early studies demonstrated that substratum surface roughness has little influence on biofilm thickness (Harty and Bott, 1981). This is particularly relevant to weldments and the

long-standing belief that grinding and polishing of all welds are necessary to achieve a surface roughness of less than 1 μm in order to eliminate sites for biofilm development on food preparation surfaces (Anonymous, 1993a). However, at other scales (i.e., roughness values of tens of microns or greater) surface roughness may influence properties such as biofilm thickness.

8.3.2 Evaporative Heat Exchangers

Tubing coils that circulate cooling water or other fluids may also have a surface in contact with a gas phase. Such evaporative heat exchangers are often used in air conditioning units as shown in Figure 8.2. The evaporative process results in the condensation of water from the air and the concentration of dissolved solids in the water. When this water condenses on the outer surface of the tubing, it promotes biofilm and scale formation on the tube surface. In addition to compromising heat transfer, biofouling of evaporative heat exchange surfaces poses a public health concern. Biofilms harbor infectious microorganisms such as *Legionella pneumophila*, which causes Legionnaires' disease, a type of pneumonia. The bacterium is infectious when inhaled as an aerosol. Evaporative cooling tower heat exchangers generate aerosols and thus create favorable conditions for the spread of infection, particularly among the aged and immuno-compromised human population. Dissemination of aerosol-borne opportunistic pathogens is likely to be widespread since many air-conditioning systems for residential and institutional buildings utilize this type of heat exchanger.

8.4 BIOFOULING AND BIOCONTAMINATION OF MEMBRANE SYSTEMS

Membrane systems are widely used in water purification. A number of membrane systems have been developed to remove particles, molecules, and ions from water streams. These include reverse osmosis (RO) membranes, ultrafiltration (UF) membranes, and microporous (MP) membranes, all of which are vulnerable to fouling (Fig. 8.3). In membrane

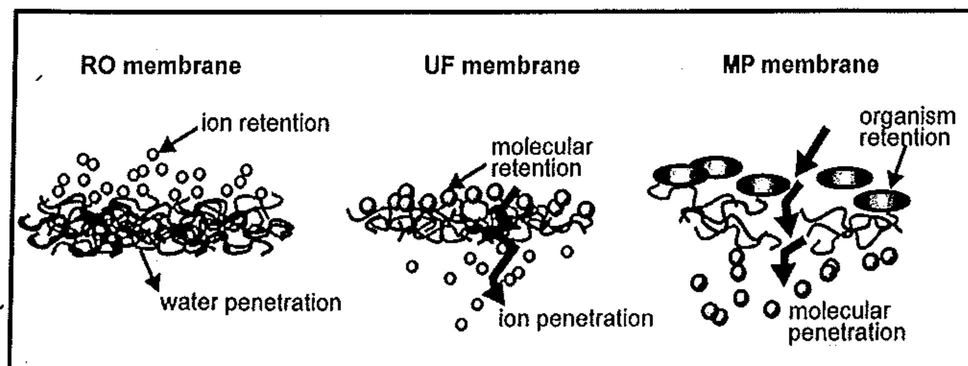

Figure 8.3 Schematic design of various types of membrane systems used to remove dissolved and particulate material from aqueous phase. RO, reverse osmosis; UF, ultrafiltration; MP, microporous membrane.

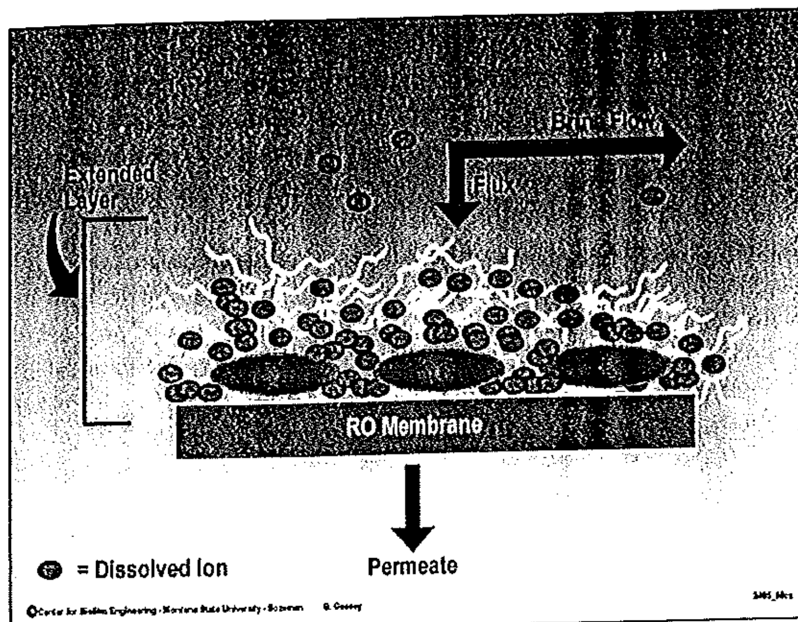

Figure 8.4 Schematic design of biofouling of reverse osmosis membrane. (Courtesy of Dr. Harry Ridgway.)

technology, the most important types of fouling are: crystalline fouling (deposition of inorganic minerals), organic fouling (deposition of humic substances, oils, and grease), particle and colloidal fouling, and microbiological fouling (accumulation of microorganisms as biofilms) (Flemming et al., 1994). Whereas the first three types of fouling can be controlled by reducing the foulant concentration in the water phase, biofouling of a membrane surface is not necessarily controlled by reducing the concentrations of microorganisms in the bulk water. Accumulation of a biofouling layer may occur by replication of the microorganisms adhering to the surface of the membrane. Bacterial attachment to and biofilm formation on membrane surfaces occur within the first hours of operation of a membrane unit if it is operated under nonsterile conditions. Although much effort has been directed to the development of membrane materials that resist bacterial adhesion, progress has been slow.

8.4.1 Reverse Osmosis Membranes

The presence of a biofilm on an RO membrane causes a transmembrane pressure drop ($\Delta p_{membrane}$ and a feed-brine pressure drop ($\Delta p_{feedbrine}$), which lead to a decline in flux of product water across the membrane (Fig. 8.4). The net result is a loss in membrane performance. The change in membrane performance is a gradual response to a gradually increasing accumulation of biofilm on the membrane surface.

The biofilm causes an increase in the concentration of ions or other dissolved substances on the raw water side of the RO membrane—a phenomenon referred to as "concentration polarization" (Fig. 8.4). This increases the pressure requirement for separation of the dissolved substances from the water. Concentration polarization also leads to precipitation of dissolved substances on the membrane as their solubility product is exceeded.

The RO membranes may accumulate surface biofilms ranging in thickness from 10–100 μm. The physical properties of the biofilm limit water transport across the membrane. The biofilm acts as a secondary membrane and impacts hydraulic resistance. When this hydraulic resistance leads to an unacceptable loss in permeability (membrane performance), it is referred to as "biofouling." Whereas water movement across a clean membrane is advection-controlled, a biofouled membrane is diffusion-controlled or advection-controlled, depending on the physical properties of the biofilm. Permeation across a biofouled membrane is controlled by the properties of the pore channels of the biofilm, not by the porosity of the membrane. The hydrodynamics of the water flowing across a biofouled RO membrane is no longer controlled by that of the bulk solution or the membrane spacers, but rather by the mass transport properties of the biofilm.

Flow porosity (p_f) is a term used to describe the movement of water through the biofilm matrix on a membrane (McDonogh et al., 1994). It refers to the available area for flow in an infinitesimal cross-section of the matrix, for example, percentage area of EPS. For any observed permeability, the resistance to water flow contributed by the biofilm matrix R_{bio} is a function of p_f.

$$R_{bio} = (p_f/\eta L_p) - R_{mem} \tag{8.1}$$

where L_p is the permeability of the filtration layer, R_{mem} is the resistance to flow by the membrane, and η is the viscosity of the flowing liquid.

The acids and exoenzymes excreted by biofilm microbial populations are concentrated in the EPS matrix at the membrane surface and can promote degradation of the membrane. The RO membranes fabricated of cellulose acetate reportedly are attacked and hydrolyzed by microbial products (Sinclair, 1982). Kutz et al. (1986) found evidence of bacteria of the genus *Selibera* in severely degraded areas of cellulose acetate membranes. In summary, biofouling contributes to a decline in membrane flux, a decrease in mineral rejection, an increase in transmembrane pressure, and possibly, membrane degradation.

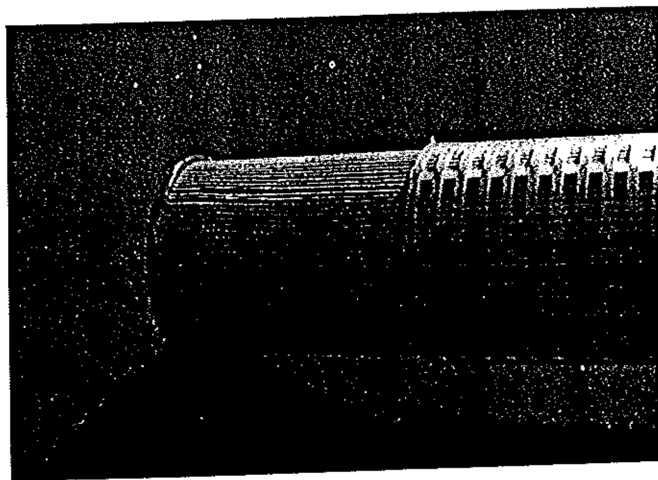

Figure 8.5 Ten-inch cellulose acetate cartridge filter (nominal pore size 0.2 μm) with plastic support removed from end of cartridge to reveal folded cellulose acetate media. (Courtesy of Marc Mittelman.)

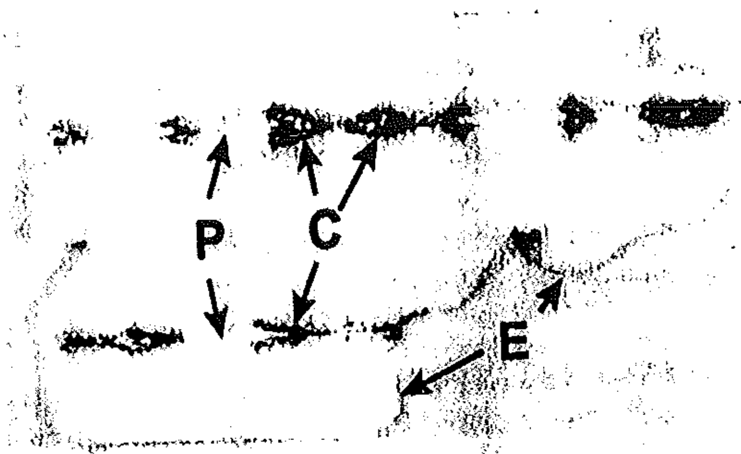

Figure 8.6 Cellulose acetate membrane media from cartridge filter similar to that shown in Figure 8.5 contaminated by bacteria. C is contaminate areas of cartridge localized at pleats (P). E shows edge of cut section of filter. (Courtesy of Marc Mittelman.)

8.4.2 Microporous Membranes

Although microporous membrane filters are designed to reduce or eliminate microorganisms in the bulk aqueous phase, their service life is intimately linked to the microbial biofilms, which develop on upstream surfaces. The sloughing and erosion of biofilm microorganisms into the bulk fluid phase is most often responsible for fouling of membrane surfaces downstream and the resultant transmembrane pressure increases that compromise system operating efficiency both in terms of increased energy demands and in membrane fatigue and failure. Perforation of membranes as a result of excess back-pressure due to biofouling leads to downstream contamination—a condition the membrane was intended to prevent.

8.4.3 Cartridge Filters

Cartridge filters are one of the most common types of filters used for solid/liquid separation (Fig. 8.5). Biofilm growth on cartridge filter surfaces also leads to filter deterioration (Fig. 8.6). Biofouling of these filters is a persistent problem in many industrial applications. The seawater handling systems for the offshore oil industry described earlier in this chapter, represent an important application of cartridge filters. Seawater filtration is usually carried out in two stages: coarse filtration, using 80–150 μm porosity filters and fine filtration using 2–10 μm porosity filters (Williams and Edyvean, 1995). The most important groups of organisms that contribute to blockage of filters used for seawater filtration are copepods, diatoms, dinoflagellates, and bacteria. Among the latter, iron- and sulfur-depositing bacteria are particularly problematic. Adhesive organic material, derived from the EPS of microbial biofilms, contribute to the blockage of filter elements. Cartridge filters used to filter seawater from the North Sea for oil field reservoir injection generally have a life span of anywhere from 3–40 days (Williams and Edyvean, 1995). The relative importance of particle loading and organic fouling in filter element blockage is an important question that has yet to be resolved for many of the applications of these types of filters.

8.5 ELECTRONICS-GRADE WATER SYSTEMS

Particulates constitute an important source of contamination in semiconductor device manufacturing deionized (DI) water lines. Particulates deposited on a chip surface during rinse procedures cause short-circuits and product rejection. With ever-increasing device densities, allowable levels of particulates must be reduced to enable manufacturing to maintain acceptable product yields. While all types of particulates cause this problem, biological particulates are the only ones that are self-replicating within the system. Whereas removal of nonliving particles from the water anywhere in the system reduce particle loads downstream, this is not necessarily the case with viable bacteria living on distribution line walls.

Conventional wisdom is that viable bacteria do not exist in DI water systems due to the lack of nutrients. Even in 18 megaohm water, where nutrient concentrations are extremely low or undetectable, bacteria propagate in biofilms on conduit, value, and various filter surfaces. Fluid flow velocities in these systems do not prevent bacterial biofilms from accumulating on these surfaces. In fact, the moving fluid promotes displacement of cells from the biofilm and their dissemination into the bulk liquid. The cells displace into the bulk liquid can serve as an inoculum for the colonization of other downstream surfaces, and ultimately the product (Fig. 8.7). Bacterial loading of the system is therefore a nonpoint source contamination phenomenon. The oligotrophic conditions associated with purified water, originally thought to prohibit bacterial growth and replication, actually promote bacterial attachment and colonization of surfaces, favoring the biofilm mode of growth that is so efficient in scavenging the trace amounts of nutrients present in the bulk aqueous phase flowing by.

Bacteria and other particles present in the source water are generally removed before the water is introduced to the DI water system. Because it is a closed system, the biofilm, which

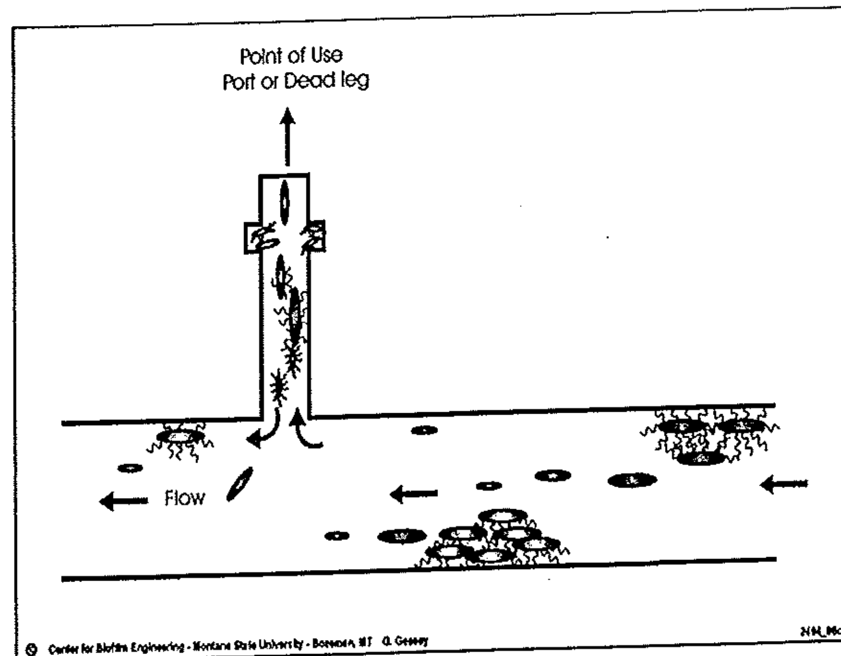

Figure 8.7 Schematic diagram of microbial contamination of a purified water distribution system via erosion of bacterial cells from a surface-associated biofilm.

develops on the surface of system components, represents the only significant source of particle contamination in the system (Patterson et al., 1991). Once deposited on the product surface, cell viability is not an issue. Cell viability is only an issue in terms of the bacteria being able to replicate on the walls of the system and inoculate the water. Controlling this phenomenon to limit system loading can be a daunting challenge. A major device manufacturer has established a limit of 35 bacteria per liter for rinse water for the 4-megabyte (Mb) DRAM chip and 8 bacteria per liter for rinse water for the 16-Mb product. Monitoring such low concentrations of total bacteria has become another challenge to the industry.

The diversity of bacteria recovered from DI water system biofilms is surprisingly broad considering the paucity of available nutrients. Although the vast majority appears to display the gram-negative cell wall structure, numerous gram-positive bacteria have been isolated as well. Using classical microbiological identification methods and phospholipid fatty acid profiles from isolates recovered by culture techniques, 12 broad groups of bacteria have been identified including *Pseudomonas*, *Moraxella*, *Morganella*, *Flexibacter*, *Caulobacter* and other stalked forms, gram-positive pleomorphic rods, and *Sphaerotilus*-like organisms (Patterson et al., 1991). Many of the isolates, however, do not match any of the known organisms in the existing libraries. Since the detection techniques are biased toward those bacteria that are cultivable on the enrichment and isolation media employed, much of the microbial diversity responsible for biofilm behavior remains to be determined in these water distribution systems.

8.6 BIOFOULING AND HUMAN HEALTH

8.6.1 Biofouling of Food Preparation Surfaces

Initially, food contamination was thought to be the result of entrapment of microorganisms in the food, which proper rinsing could control. However, a portion of bacteria attached to meat and poultry carcasses cannot be removed by spray washing. Today, microbial adhesion to and biofilm development on product or product contact surfaces in food processing plants is considered to be another potential process leading to food contamination. The important problem that arises from surface biofouling is the resultant repeated contamination of food during processing (Notermans, 1994). There is a growing awareness within the food, beverage, and dairy industries that biofilms on food preparation and other contact surfaces cause serious hygiene problems or economic losses due to organoleptic alterations (spoilage) of the product. The reader is referred to Chapter 10 in this volume for details on this topic.

Biofouling of surfaces in the food industry is of particular concern when pathogenic microorganisms are associated with the biofilms. Pathogenic microorganisms such as *Staphylococcus aureus* and *Salmonella* spp. are common inhabitants of poultry processing lines, *Listeria monocytogenes* is common in both meat and dairy plants, and *Bacillus cereus* is common in milking equipment on dairy farms. Of the environmental contamination routes in food processing, surface biofilms are probably the most important route of infection both directly via product contact surfaces or indirectly as reservoirs of infection for product contamination by way of other routes (Timperley et al., 1992). The principal factors controlling contamination of food during processing are believed to be: (1) the total number of microbial cells on the surfaces of contact, and (2) the proportion of the total population associated with the surface that become detached and is picked up by the product (Dunsmore et al., 1981). The formation of biofilms and the consequences in the general

food processing environment have been the subjects of several excellent reviews (Notermans et al., 1991; Mattila-Sandholm and Wirtanen, 1992; Carpentier and Cerf, 1993; Zottola and Sashara, 1994).

In the United States, the National Sanitation Foundation has developed a number of guidelines for the dairy industry and food service equipment. These include (1) materials for product contact, (2) surface finish, (3) joints, (4) fasteners, (5) drainage, (6) internal angles and corners, (7) dead spaces, (8) bearings and shaft seals, and (9) instrumentation (Timperley et al., 1992). In the case of fasteners, exposed screw threads must not be used at the product side. Where screwed connections are unavoidable, a metal-backed elastomer gasket should be used (Anonymous, 1993b).

Stainless steel is the material of choice for fabrication of equipment used to process dairy products as well as other food and beverage products because it is durable, corrosion resistant, and easily cleaned. The most commonly used material in the New Zealand dairy industry is unpolished 304L stainless steel with a 2B finish (Flint et al., 1997). In the United States, a No. 4 surface finish provides a surface roughness (R_a) of 1 μm or less. Such a finish is recommended based on the understanding that smoothness is directly related to resistance to bacterial colonization and cleanability. For this reason, welded seams must currently be ground and polished. Although surface roughness is likely to exert some influence on these factors, its significance at circa the 1 μm range is questionable (Tide et al., 1999).

In addition to surface roughness, a number of other environmental factors influence biofilm formation on food preparation surfaces. Bulk liquid phase ionic strength, pH, nutrient content, and temperature all exert an effect on surface biofilm development. Unlike many habitats, food-processing surfaces are repeatedly exposed to food products, which are nutritionally rich and support rapid, balanced microbial growth. Food-derived compounds also adsorb to food processing surfaces during contact to create a conditioning film that influences the development of bacterial biofilms.

The process of bioadhesion on stainless steel surfaces in the food processing industry has been reviewed by Boulange-Petermann (1996) and is presented in Chapter 10 of this volume. Predominant microorganisms that colonize stainless steel surfaces in meat processing plants are *Pseudomonas* spp. and *Klebsiella* spp. (Hood and Zotolla, 1992). Because *Listeria* spp. are ubiquitous in the food preparation environment, have been found in biofilms, and are considered a human pathogen, they have received considerable attention as a contaminant of foods and dairy products. In some cases, adhesion may involve specific interactions between bacterial cell surface proteins and the food. The adhesion of *Salmonella* serotypes to chicken muscle fascia was correlated with the ability to be agglutinated by hyaluronan, a structural polysaccharide component of tissue (Sanderson et al., 1991).

8.6.1.1 Dairy Industry. Biofouling in the dairy industry has been reviewed by Criado et al. (1994) and Flint et al. (1997). Biofouling in the dairy industry can cause serious obstruction, corrosion, and health problems. In spite of the technological advances in the design of milking rooms and machinery, the increased effectiveness of detergents and disinfectants, and the introduction of on-site refrigeration, biofilms still persist in these environments. Biofilms develop rapidly in the food-processing environment, achieving 10^6 bacteria/cm^2 within 12 h under refrigeration conditions (Bouman et al., 1982). However, a period of 48 h is usually necessary for detection of a biofilm on product processing equipment, although this depends on the sensitivity of the detection method (Wirtanen and Mattila-Sandholm, 1992).

A single species often dominates process biofilms in dairy manufacturing plants (Flint et al., 1997). Lewis and Gilmour (1987) found that rubber and stainless steel surfaces exposed to raw milk directly from the udder were colonized by essentially a monoculture bacterial population, which differed from the populations present in the milk. Heat pasteurization may contribute to monoculture biofilms by killing sensitive gram-negative species, allowing thermoduric, gram-positive species such as *Streptococcus thermophilus* to dominate other biofilm populations. In some cases, psychrotrophic microflora have simply replaced mesotrophic bacteria as important agents of contamination. *L. monocytogenes* reportedly displays some degree of heat-resistance when associated with dairy products (Lee and Frank, 1990), so this species may not be as susceptible to the steam rinses applied during surface cleaning as other biofilm populations.

The dairy industry has invested heavily into research on not only the nutritional status of microorganisms that contaminate dairy products but also on the factors that control their adhesion to equipment surfaces where the products become contaminated. When grown as a monoculture, cells of *Listeria monocytogenes* do not attach well to glass and stainless steel surfaces (Sasahara and Zottola, 1993). However, *L. monocytogenes* is often found with other bacteria such as staphylococci and lactobacilli when present in biofilms (Frank and Koffi, 1990). Under some conditions, the presence of other bacteria on the surface does not appear to enhance surface attachment of *L. monocytogenes* (Frank and Koffi, 1990). However, when grown in co-culture with the extracellular polymer-producing bacterium, *Pseudomonas fragi*, *L. monocytogenes* readily colonizes these surfaces (Sasahara and Zottola, 1993). *P. fragi* appears to be the primary surface colonizer, forming an exopolymeric matrix that traps *L. monocytogenes* and other debris in the biofouling layer. The production of exopolymer appears to be more important than hydrophobicity, surface charge, or motility in the attachment of *L. monocytogenes* to inert surface in flowing systems. The success of bacteria such as *L. monocytogenes* in colonizing surfaces is ultimately a function of their competitiveness against other species present on the surface.

Some dairy products reportedly inhibit bacterial attachment to surfaces. Components of whole milk inhibit short-term colonization of surfaces by some bacteria. Adsorbed milk proteins significantly reduce adhesion of *Salmonella typhimurium* and *L. monocytogenes* to steel compared to surfaces without proteins (Helke et al., 1993).

Some spoilage organisms are thought to be transferred to dairy products as a result of their contact with surfaces containing biofilms. Contamination attributed to product contact with equipment surface biofilms has been reported in the manufacturing of cheese, whey, milk powder, and in general milk processing. The psychrotrophs *Acinetobacter*, *Pseudomonas*, and *Flavobacteria*, when found in pasteurized milk, are believed to have been introduced after pasteurization as a result of contact with improperly cleaned surfaces. Table 8.1 lists the different types of microorganisms that form biofilms in various dairy manufacturing processes. A summary of the types of microorganisms that reside on different dairy product surfaces has been prepared by Mucchetti (1995).

A number of questions related to biofouling in the food industry have been raised in a scientific status summary of the Institute of Food Technologists' Expert Panel on Food Safety and Nutrition (Zotolla, 1994). These include:

- Are the biofilms in nonfood systems different from those in food processing systems?
- How long does it take for attached microbes to form biofilms in food systems?
- Does biofilm formation, even after removal from a surface, promote subsequent surface biofouling?

- Does renewal of a food contact surface by regular cleaning and sanitizing encourage or discourage biofilm formation?

The answers to these questions depend on the specific conditions being considered or compared. Our severely limited understanding of biofilms in the food, beverage, and dairy industries precludes drawing many general conclusions except that biofilms are likely to be as unique as the environments in which they are found. For more details on this subject, the reader is directed to Chapter 10.

8.6.2 Biofouling of Drinking Water Systems

Public health standards consider water safe for human consumption when it contains a maximum of 500 CFU/ml, when it is free of coliforms, and when its nephelometric turbidity is less than 2 (Geldreich, 1986). Drinking water standards are established to eliminate frank pathogens–not all microorganisms from potable water. The cost of treatment to eliminate all potential pathogens from public potable water supplies would be unaffordable to most customers.

Microorganisms accumulate on the surfaces of water distribution mains and suspended particular matter in drinking water (Ridgway and Olson, 1981). The community of microorganisms attached to the wall of the pipeline represents the major fraction of biomass in a drinking water distribution system. These biofilm populations contribute to the continuous contamination of the moving water phase through surface growth and subsequent displacement of cells from the pipe surface as a result of fluid shear (Fig. 8.8). Bacteria are not the only microbes present in biological fouling layers on the pipe wall surface. Fungi and protozoa are also present. *Hartmannela vermiformis*, *Vannella mira*, *Cochliopodium minutum*, and *Naegleria* sp. have been identified in an industrial pilot-scale drinking water system operated with treated surface waters at temperatures of 24°C (Block et al., 1993).

A quasi-steady state biofilm is typically achieved within a few weeks of putting a system on-line. Several parameters govern biofilm accumulation including hydraulic regime, flux of nutrients and organisms, and substratum properties, and in the case of drinking water distribution systems, disinfectant concentration and protozoan grazing of attached bacteria. Assimilable organic carbon (AOC) is a term often used to describe the readily degraded fraction of dissolved organic carbon that is measured by the number of organisms that grow in the water. Biodegradable dissolved organic carbon (BDOC) is that quantity of the total organic carbon that has been consumed after supporting all the microbial growth

TABLE 8.1. Biofilms Associated with Dairy Product Processing Equipment

Environment	Organism	Reference
Ultrafiltration membrane, whey processing	*Bacillus subtilis*	Flint and Hartley, 1993
Stainless steel surfaces, milk powder processing	*Bacillus stearothermophilus*	Standhouders et al., 1982
Milk processing equipment	*Thermus thermophilus*	Klijn et al., 1992
Lines handling raw milk	Gram-negative psychrotrophic bacteria	Lewis and Gilmour, 1987

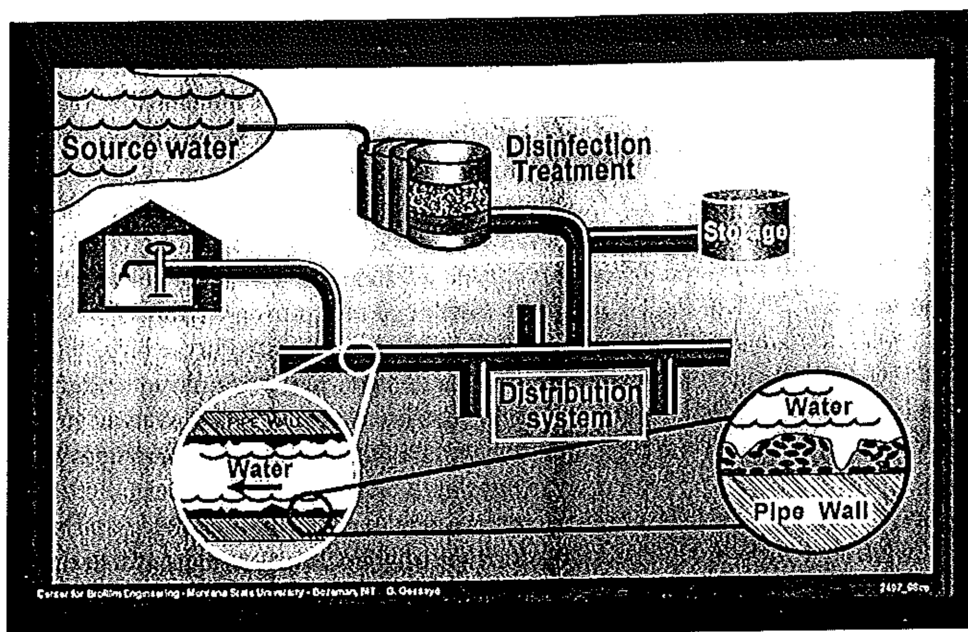

Figure 8.8 Schematic diagram of a public drinking water treatment and distribution system. Expanded views reveal microbial biofilm on pipe wall surface.

in the system (Camper, 1996). Regardless of how it is defined, it represents the nutrients that control biofilm growth in drinking water distribution systems. Although it consists of a complex mixture of compounds, it provides a useful indication of the potential for biofouling in many systems. In a constant hydrodynamic regime, nutrient flux and disinfectant concentration control microbial growth in a biofilm, which in turn controls protozoan grazing on attached bacterial cells and flux of cells into the water phase. The relationship between AOC flux and disinfectant concentration on biofilm growth is complex. Corrosion product generation and accumulation on the pipe wall increases the surface area for biofilm accumulation. Furthermore, corrosion products, along with AOC and biofilm biomass, consume disinfectant.

The mild steel surfaces of water mains provide an excellent habitat for bacterial colonization, including coliforms. Mild steel supports 10 times more heterotrophs and coliforms than more inert surfaces such as polycarbonate (Camper, 1996). Mild steel surfaces also exhibit a higher chlorine demand than more inert surfaces. The high cell densities per unit area and high chlorine demand are believed to be related to an increase in surface area and chlorine reaction afforded by steel corrosion products that accumulate on the pipe surface.

The growth rate of cells on a cement substratum in a pilot-scale drinking water system without chlorine has been reported to be $\mu = 0.0017\ h^{-1}$ yielding a cell generation time of 17 d (Block et al., 1993). Such high surface-associated growth rates can result in a doubling of bacteria in the water phase along the length of pipe due to sloughing of cells from the biofilm. Whereas approximately 1% of the cultivable bacteria in the water phase are viable, as much as 6% of the bacteria in the biofilm are viable, suggesting that the most active population in these nutrient-depleted systems is the biofilm population.

In the presence of chlorine, the doubling time of attached cells is significantly increased more than 100 d). This slow growth rate represents a very stable community,

which is relatively resistant to further inactivation by disinfectant (Paquin et al., 1992). Whereas water quality just downstream from the water treatment plant reflects the efficiency of the plant operation, water quality at the extremity of the distribution system more closely reflects the activity of the biofouling layer on the upstream pipe wall.

The dominant biofilm populations in a drinking water system of one household form a separate cluster of closely related bacteria within the beta 1 subclass of the *Proteobacteria* (Kalmbach et al., 1997). They display less than 97.7% sequence similarity to their closest known relatives. An *Aquabacterium* sp., capable of anaerobic growth on nitrate, has been found to represent 75% of the bacteria in a biofilm formed during daytime flows. A population of *Acinetobacter* sp. in the gamma subdivision was favored in the biofilm at night when the water remained stagnant. The species composition of drinking water biofilm populations also varies from season to season at any one location in the system. It remains to be determined whether the population changes in the system are due primarily to regrowth in the system or to population changes in the raw water supply or in the treatment process.

In situ probing with highly-specific 16S rRNA oligonucleotides suggests that the dominant bacterial species were cultivable on R2A medium and that two-thirds of the autochthonous drinking water population are in a viable but nonculturable state (VBNC) (Kalmbach et al., 1997). VBNC has been demonstrated for a wide variety of bacterial pathogens of concern to the drinking water industry including: *E. coli*, *Vibrio cholerae*, *Vibrio vulnificus*, *Salmonella enteritidis*, *Shigella sonnei*, *Shigella flexneri*, and *Campylobacter jejuni* (Barer et al., 1993; Byrd et al., 1991).

Numerous studies have been conducted to determine the influence of biofilms on coliform survival and growth in drinking water distribution systems (Lechevallier, 1990). Both coliform and heterotroph levels in biofilms and the water tend to correlate positively with AOC concentration. However, coliform, but not heterotroph densities increase in biofilms when both AOC and chlorine residual are maintained (Camper, 1996).

Regrowth of coliforms in a drinking water system occurs as a result of their detachment from biofilms and transport into the bulk water. Regrowth is more likely to occur from slow-growing populations introduced from low nutrient sources than from fast-growing populations originating from fecal material (Camper, 1996). Coliforms transported to the bulk water from biofilms can recolonize surfaces downstream in the presence of a free chlorine residual. Coliforms derived from biofilm populations appear to be less susceptible to chlorine disinfection. Thus, biofilms on walls of the distribution system piping appear to negate efforts directed toward their removal from the water at the drinking water treatment plant.

Biofilms have been implicated as a reservoir for pathogenic bacteria in drinking water distribution pipelines (Rogers et al., 1994; Wireman et al., 1993). A mild steel surface, maintained under conditions that simulated a potable water system, developed a biofilm containing 10^6 total bacteria/cm^2. Such a biofilm supported a surface-associated population of *L. pneumophila* to a density of 10^4 cells/cm^2 and a planktonic *L. pneumophila* population density of 5×10^3 CFU/ml in the bulk aqueous phase. *L. pneumophila* has been detected in biofilms of complex microbial consortia by gas chromatography-mass spectrometric analysis of genus-specific hydroxy fatty acids (Walker et al., 1993).

Campylobacter jejuni, an important agent of human bacteria gastroenteritis, has been found to persist for long periods of time in biofilms of autochthonous drinking water microflora propagated in laboratory model drinking water distribution systems when detection is based on nonculturable methods (Buswell et al., 1998).

Morin et al. (1996) demonstrated that carbon fines from the treatment process transported viable cells of *K. pneumoniae* to mixed-population biofilms in drinking water systems. In this habitat, a portion of the *K. pneumoniae* population established in the biofilm was able to resist inactivation by chlorine disinfection.

Mycobacterium spp. have also been recovered from biofilms formed in warm potable water systems (Schulze-Robbecke and Fischeder, 1989). Large numbers of *Mycobacterium kansasii* and *M. flavescens* are distributed as dense aggregations of acid-fast cells within these biofilms. Reporter genes have also been used to monitor the biofilm-induced persistence of *Mycobacterium* in drinking water (Arrage and White, 1997). The resistance of *Mycobacterium* spp. to disinfectants makes them a particular threat to immunocompromised individuals who use the water from *Mycobacterium*-contaminated distribution systems.

Many bacterial pathogens have difficulty competing with other biofilm populations under the oligotrophic conditions characteristic of drinking water distribution systems. Unless protected by other means, the less competititve pathogens will eventually be displaced from the biofilm. One form of protection that has been demonstrated involves protozoan grazers. Bacteria are capable of intracellular survival and replication within protozoa. Ingestion of *Salmonella typhimurium*, *Yersinia enterocolitica*, *Shigella sonnei*, *Legionella gormanii*, and *Campylobacter jejuni* by the protozoans *Acanthamoeba castellanni* and *Tetrahymena pyriformis* not only protects these bacteria from competitors, but also increases their resistance to chlorine (King et al., 1988). Thus, biofilms offer various forms of protection to pathogens that find their way into drinking water distribution systems.

8.6.3 Biofouling of Purified Water Systems

8.6.3.1 Pharmaceutical Water. Most bacteria in purified water systems are gram-negative, nonfermentative bacilli. These bacteria contain a fever-causing substance in their cell wall, often referred to as endotoxin or pyrogen. The presence of these bacteria and their associated endotoxins in pharmaceutical waters poses the single greatest threat to product quality (Mittelman, 1995). The large surface area created by the various components of a purified water system provides space for surface bacterial colonization. Although purified water systems typically contain very low levels of dissolved solids, sufficient quantities of growth-promoting inorganic and organic substances exist in the flowing bulk aqueous phase or as constituents of the "inert" substratum to promote surface bacterial growth and biofilm development. Despite the dearth of information on the physiology and ecology of microorganisms inhabiting purified water systems, studies in other nutrient-poor environments have repeatedly demonstrated the tendency of "starved" cells to attach to surfaces (Mittelman et al., 1987). The EPS excreted by bacteria attached to surfaces possess a structure and chemistry that enhances nutrient scavenging from the bulk water. EPS can constitute a significant portion of the total surface biomass (bioburden). As a result of its reactivity with oxidizing biocides, EPS can consume a significant portion of a biocide added to inactivate the cells buried in the EPS matrix of a biofouling layer on an industrial surface (Characklis, 1980). The inherent nature of the system therefore selects for EPS-producing, surface biofilm populations. It is not surprising then that most microorganisms in pharmaceutical water systems are associated with surface biofilms.

Sloughing or erosion of biofilm microorganisms contributes the majority of microbes in the bulk water of the fluid handling systems. McFeters et al. (1993) detected 10^2–10^3 CFU/mL in a model laboratory water purifier containing many of the components of a pharmaceutical grade water system. These cell concentrations likely represent a minimum,

as many of the cells released from biofilms exist in aggregates containing many cells held together by EPS, which give rise to a single colony-forming unit (CFU). These aggregates confer an infectious dose in a very small volume of water.

Because sloughing is episodic and unpredictable, these aggregates often go undetected during periodic sampling and analysis of the bulk water. Significantly, it is the EPS that affords biofilms protection from chemical treatments and possibly steam/hot water treatment implemented to eradicate bacteria from purified water systems. While both flowing steam and hot water (80°C) appear to be effective at inactivating biofilm bacteria in pharmaceutical waters, neither treatment effectively removes biomass from surfaces, including EPS. Surface residues of this nature facilitate subsequent surface recolonization and biofilm regrowth.

Currently there are no biofilm samplers commercially available for the pharmaceutical industry. Side-stream pipe sections containing a modified Robbins device have been used for recovery of biofilm bacteria in purified water systems, but this type of sampler is cumbersome to manipulate and the analyses are time consuming. A number of on-line biofilm detection systems are being developed, including Fourier transform infrared spectroscopy, fluorimetry, and quartz crystal microbalance.

8.6.3.2 Hemodialysis Units. Improperly maintained purified water systems have led to a number of endotoxin-related problems in hemodialysis facilities (Laurence and Lapierre, 1995; Murphy et al., 1987). These pyrogenic substances are likely released from the gram-negative bacterial biofilms growing on components of the purified water system or hemodialysis units and transported via the dialysis fluid into the body of the patients receiving treatment (Vincent et al., 1989). Once released and disseminated, endotoxins are extremely difficult to remove from surfaces with which they come in contact.

8.6.3.3 Dental Units. Every dental unit is equipped with small-bore flexible tubing to deliver water to different handpieces such as the air/water syringe, the ultrasonic descaler, and the high-speed drill. The water supply for dental units may be the potable municipal water plumbed in a building, or distilled or sterile water reservoirs housed in the dental office. The wall of small-bore plastic tubing used to deliver cooling water for these dental handpieces provides an ideal environment for the attachment of bacterial cells and the proliferation of biofilms. The high surface area-to-volume ratio afforded by such tubing ensures a high concentration of bacteria in the water due to sloughing of cells from the biofilm growing on the tubing wall. Since sloughing often involves the release of aggregations of cells, large infectious doses of biofilm-derived cells may be delivered to the patient's oral cavity. Contamination levels in dental treatment water may exceed 10^5 CFU/ml, with many of the colonies arising from not one cell but rather a clump of cells which alone can contain 10^5 cells (Williams et al., 1993). Heating the rinse or irrigation water to body temperature for patient comfort selects for populations of biofilm-forming microorganisms that are well adapted to grow in the body.

Biofouling of dental treatment units has been implicated as a source of infectious agents to dental patients (Schulze-Robbecke et al., 1995; Shearer, 1996). Despite the high levels of microorganisms often reported in dental treatment units, no outbreaks of disease and relatively few clinical case reports have been associated with contamination of dental water lines. Nevertheless, potentially pathogenic bacteria such as *Pseudomonas* spp., nontuberculous *Mycobacteria* and *Legionella* spp. have been isolated from dental treatment water. These same bacteria are opportunistic pathogens that cause nosocomial infections and infections in immuno-compromised patients.

Potable water from public water supplies may be a source of opportunistic pathogens associated with dental treatment units. *Sphyngomonas paucimobilis*, *Acinetobacter calcoaceticus*, *Methylobacterium mesophilicum*, and *Pseudomonas aeruginosa* have been reported as dominant isolates from dental unit water lines (Barbeau et al., 1996). Interestingly, *P. aeruginosa* was never isolated in tap water remote from or near the contaminated dental unit water lines. The source of this organism is likely the biofilm that accumulates in the water lines.

While many opportunistic pathogens are killed by the disinfection treatments applied at the water treatment plant, they are not necessarily eliminated from the source water for the dental treatment units. The providers of potable water are only required to guarantee quality to the point at which the customers connect to the mains—not to the tap or in-house equipment. The building owner, consumer or business operator is responsible for the quality of their water once it leaves the mains of a public drinking water system. Use of sterile deionized water, a costly alternative, offers a source of contamination-free water. However, one must recognize that once this pure source of water comes in contact with any nonsterile component of the dental unit system, it can become contaminated by microorganisms residing on that component's surface as a biofilm. Conscientious use of a separate water reservoir system with periodic or continuous chemical treatment can improve water quality to the extent that it meets the American Dental Association's recommendation of no more than 200 CFU/ml for acceptable water quality.

Retraction of dental treatment water from the mouth of an infected patient into the water lines and subsequent discharge into another patient's mouth has been proposed as a means of the spread of disease. Many dental units today flush the water lines for 20–30 sec after each use and are equipped with check valves or antiretraction valves, which are designed to minimize water line contamination. However, every additional safeguard of this nature offers yet another surface for colonization and biofilm development. Flushing is ineffective due to the adherent nature of the bacteria. Santiago et al. (1994) found that the numbers of bacteria in water of dental units were not reduced by flushing. Nothing short of sterilizing the entire dental treatment unit, which is virtually impossible with today's units, will guarantee a biofilm-free system.

Control of biofouling, presented in Chapter 9, involves measures specific to the type of equipment under consideration. In the case of dental treatment equipment, control depends on technical factors, effective training of personnel using the equipment, and establishing and following validated operating procedures. Judicious compliance with cleaning and treatment protocols appears to be critically important for long-term success in avoiding disease transmission via dental treatment units.

8.6.4 Biofouling of Implanted Prostheses

Implanted devices such as arterial grafts, hip joints, and dental implants have been designed and approved for use in the human body on the basis of host tissue biocompatibility and functional characteristics. Criteria for materials used in the fabrication of the total artificial heart are based on their blood compatibility and structural properties. Lack of tissue integration and associated infections are two of the major factors leading to premature failure of devices implanted in soft tissue and transcutaneous devices, including catheters. Biofouling of implant surfaces by invasive microorganisms is the single most important deterrent to expansion of implant applications and a primary reason for failure of those in use. Infection rates are as high as 100% for certain urinary catheters.

Biofilm formation on devices implanted in the human body can lead to an intractable infection of the patient by pathogenic microbes and/or malfunction of the implanted device (Gristina, 1987). Implanted materials, which have become the locus for bacterial infection, if not replaced, lead to chronic illness (Dickinson and Bisno, 1989). *Staphylococcus epidermidis* and other coagulase-negative staphylococci, and gram-negative bacteria are frequently encountered biofilm-forming microbes on implanted prostheses.

Biofouling of an implanted device provides a source of infectious cells that can be disseminated to other parts of the body via the circulatory system. When an implanted device is surgically removed and replaced due to colonization by microbes, further opportunity for infection occurs. Such biofilms are often unresponsive to antibiotic therapy. When left unchecked, the biofouling can interfere with prosthesis function and ultimately lead to failure. Biofouling of artificial hearts can be life-threatening to patients (Gristina et al., 1988).

The sources of bacteria responsible for the vast majority of implant-associated infections are: (1) perioperative contamination, (2) exit site contamination for percutaneous devices, or (3) hematogenous spread from locations distal to the implant area (Mittelman, 1996). Each of these sources defines a different species diversity, severity of infection, and predisposing host condition. Although there have been numerous studies demonstrating the influence of material properties on bacterial colonization, no clear link to frequency of infection has been established. Circumstances related to device application appear to be the single most important criterion influencing infection.

It has been proposed that most vascular grafts, prosthetic heart valves, and orthopedic implants are colonized by bacteria via hematogenous dissemination. Percutaneous access devices are the major routes of entry of bacteria responsible for early-onset prosthetic valve endocarditis, which is the result of biofilm formation on the surface of heart valves (Keys, 1993). Oral microflora are the most common etilogical agents of bacterial endocarditis. Dental hygiene practices can introduce organisms such as *Actinobacillus* spp. into the bloodstream.

Indwelling urethral catheters are tubular, latex, or silicone instruments used to drain urine from the bladder. Prevalence surveys have revealed the large-scale use of indwelling urethral catheters in modern medicine in both hospital and community care. This device provides easy access for bacteria from a contaminated environment into a vulnerable body cavity. Urinary tract infection is a common occurrence in catheterized patients. Organisms colonizing the periurethral skin migrate along the epithelial surface of the urethra and the external surface of the catheter. Bacterial multiplication in the urine allows high numbers of cells to accumulate in the urine drainage bags. These bacteria migrate back up the drainage tube and catheter and into the bladder. The various routes of infection are summarized by Stickler (1996).

In the first week of catheterization, infection is usually by a single species of bacteria, such as *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, or *Proteus mirabilis*. As the duration of catheterization increases, the more complex the community of microorganisms becomes. Bacteria such as *Providencia stuartii*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Klebsiella pneumoniae* are common gram-negative, nosocomial species that persist in this type of device-related infection.

Both the external and luminal surfaces of the catheter develop bacterial biofilms. Fibrinogen, along with a number of other blood plasma proteins deposited by the body at the site of tissue injury caused by the catheter, may promote bacterial adhesion and biofilm development on these surfaces. Biofilms range from patchy, discontinuous films across the

device surfaces to layers several hundred cells in thickness that cover large areas of surface. The biofilms form encrustations of inorganic minerals on the catheterization device that block fluid flow (Stickler, 1996).

While our bodies have evolved a sophisticated defense system to counter infection, implantation procedures compromise some of the important barriers. By subjecting our bodies to invasive surgery or indwelling catheters, we lose the barrier provided by our skin. Peritoneal dialysis catheters do not allow the skin epithelial cells to form a tight seal at the point of perforation. Instead, necrotic epidermal cells and keratin line the sinus tract, creating a habitat conducive for microbial colonization. Peritonitis is the most common complication associated with peritoneal dialysis and a major cause of morbidity.

By using immune-repressing drugs, we compromise the ability of our immune system to seek and destroy foreign substances that enter the body. Device-related infections possess an intrinsic resistance to topically or systemically applied antimicrobials. Although not yet fully understood, microbial biofilms, which have accumulated on surfaces, resist antibiotic doses that irreversibly inactivate individual, unattached bacterial cells (Nickel and Costerton, 1992; Vrany et al., 1997).

The matrix biopolymers excreted by surface-associated bacteria modulate host immune responses that are usually effective in clearing individual, free-living bacteria. The matrix protects bacterial cells embedded deep in the biofilm from antibodies and other antigen-seeking and neutralizing molecules of our immune system. The matrix also enables the device-associated bacterial populations to remain attached to the surface and to each other, thereby resisting engulfment by macrophages and other cell-mediated processes. Until a weak link in the defense system of biofilm microorganisms is discovered and exploited to control their growth, the potential for prosthetic devices to replace natural human body parts will always come with a certain risk of infection.

8.7 ENGINEERING CONSIDERATIONS OF BIOFILM FORMATION ON INDUSTRIAL SURFACES

8.7.1 How Surface Biofouling Influences Mass Transport

Until recently, most mathematical models treated biofilms as a layer of uniform thickness and cell density. Previously, when biofilms were treated as uniformly porous structures, molecular diffusion was the only mass transport process considered. Although difficult to demonstrate, substrate uptake by bacterial cells attached to a surface is at least as fast as or faster than mixing can replace the substrate removed from the bulk aqueous phase at the liquid–surface interface (Gjaltema et al., 1994). This leads to the formation of substrate concentration gradients within a biofilm at the interface. Localized substrate depletion occurs within the biofilm as the thickness of the biofilm increases. Substrate depletion can lead to biofilm sloughing. In a continuously stirred tank reactor of the Roto-Torque design, sloughing occurs most prominently from the lower surfaces where the biofilm is thicker (Gjaltema et al., 1994).

8.7.1.1 How Biofilm Heterogeneity Affects Mass Transfer.

Biofilm communities exhibit specialized biofilm specific activities that are a manifestation of the combined effects of species diversity, surface attachment, and physicochemical gradients. Bacterial

growth in biofilms is strongly influenced by local environmental pressures; pH, oxygen, and redox local concentrations are key variables that may affect the colonization structure of a biofilm. The physical microstructure of a biofilm may influence the formation of pH and Eh gradients through its impact on diffusive and convective fluid flow characteristics. These transport phenomena influence the access of substrates for bacterial metabolism and, in turn, the removal of metabolic end-products. Cell:cell communication is dependent upon the exchange of minute levels of signal molecules that must travel through the microstructure of the biofilm. The ability of antimicrobial agents to penetrate to their intended targets depends upon diffusive and convective transport processes, which are directly influenced by the microstructure of the biofilm.

8.7.1.1.1 Surface Area Considerations. The heterogeneity displayed by some biofilms affects mass transport into and within the biofilm. Biofilm heterogeneity can increase contact area between the bulk liquid and biofilm. The larger contact area between the bulk liquid phase and a biofilm of varying thickness promotes mass transport between these phases. Rough surfaces increase external mass transfer by increasing eddy diffusion. The formation of filamentous strands of biofilm extending from a base film into the bulk liquid is estimated to increase the biofilm surface area at the bulk liquid interface from 1.5 to 11 times that of the surface area of the underlying substratum, depending on the density and dimensions of the filaments (Gjaltema et al., 1994). The undulating movement of the filaments in a flow field has been estimated to increase mass transport in and out of the biofilm. A patchy, discontinuous biofilm, on the other hand, offers a smaller biofilm-bulk liquid interface than a homogeneous layer containing the same amount of biomass. The structural heterogeneity of many biofilms makes it extremely difficult to estimate the interfacial area of the biofilm-bulk liquid boundary because direct measurements either give an average over the sampled areas or provide unrelated local values (Gjaltema et al., 1994).

8.7.1.1.2 Volume Considerations. Biofilm heterogeneity extends beneath the biofilm surface-bulk liquid interface. Cell densities vary greatly within a multilayer biofilm. Although it has been known for some time that within trickling filter biofilms, bacteria develop as microcolonies separated by voids or water channels of low cell density (Mack et al., 1975; DiSalvo and Daniels, 1975), quantitative differences have not been established until recently. By cutting biofilms into 10–20 μm thick sections and determining total suspended solids, phospholipid concentrations, and dye absorption for each section, the densities and porosities have been determined using a theoretical model (Zhang and Bishop, 1994a). Biofilm densities were found to be 5–10 times higher at the bottom of the biofilm than at the top. Correspondingly, porosity decreased from 84–93% in the top layers to 58–67% in the bottom layers, but the mean pore radius of the biofilm decreased from the top to the bottom of the biofilm. Both tortuosities and effective diffusivities depend on the density and porosity of the biofilm. The ratio of effective diffusivity within the biofilm to that in the bulk reportedly varies from 68–81% in the top layer to 38–41% in the bottom layer of a biofilm (Zhang and Bishop, 1994b).

Recent evidence indicates that there is convective fluid flow not only at the biofilm-bulk liquid interface but also at the biofilm-substratum interface in biofilms that contain areas of low cell density near the substratum (Lewandowski et al., 1995). Using oxygen microelectrodes and confocal scanning laser microscopy (CLSM), de Beer et al. (1994) found that the

flux of oxygen between areas of low cell density and areas of high cell density, within an 80 μm-thick biofilm, was similar to the flux of oxygen between the bulk liquid and the surface of the biofilm. The flux within a biofilm has both a horizontal and vertical component, suggesting the need for a three-dimensional approach to modeling mass transport processes in biofilms. Thus, new models of mass transport within biofilms must take into account the varying effective diffusivity and flux at different planes within a biofilm.

8.7.1.1.3 Advective Mass Transport of Particles into and Within Biofilms. Biofilms promote the entrainment of particles suspended in the bulk fluid. Drury et al. (1993) showed that latex spheres the diameter of bacterial cells were entrained by bacterial biofilms. The eddies formed as a result of variable biofilm thickness and filament formation entrain particles that would tend to remain in suspension if transported across a biofilm-free surface. The heterogeneity of biofilms allows advective mass transport of particles through voids deep within the biofilm (Lewandowski et al., 1995). Neutrally buoyant, 0.3 μm diameter latex spheres have been tracked at velocities ranging from 10–20 μm/sec in a mixed species, laboratory-cultivated biofilm (Stoodley et al., 1994). Particle velocities within a biofilm vary depending on the biofilm-to-void ratio. Where voids make up a large portion of the volume of a biofilm, the advection term in the mass transport equation increases in importance relative to the diffusion terms (see Eq. (11.6) in Gujer and Wanner, 1990).

The structure of a bacterial biofilm is not the uniform gel-like matrix, entrapping cells, as implied by numerous mathematical models and experimental studies. Rather, a biofilm can be highly heterogeneous in its structural architecture, with very dense cell clusters separated by crevasses or water channels. These water channels are of various sizes and dimensions and render the biofilm gel matrix a labyrinth of tortuous cavities through which fluid, dissolved solutes, and suspended particles can migrate.

Consequently, biofilm properties can no longer be estimated as overall biofilm lumped parameters but rather must be estimated locally. Estimates by the half-cell diffusion chamber method were inadequate to provide an accurate measure of true mass transfer coefficients. Bryers and Drummond (1999) report experiments where pure culture *P. putida* biofilms were cultivated under controlled conditions to a desired overall biofilm thickness, then employed within classical half-cell diffusion chambers to estimate, from transient solute concentrations, the effective diffusion coefficient for several macromolecules of increasing molecular weight and molecular complexity.

The basic idea of a half-cell diffusion system is quite simple. Two compartments are separated by a known thickness of permeable material, the half-cells filled with a solvent, allowed to hydrostatically equilibrate, and at time equal zero, the solute is introduced into one cell and the concentrations of solute measured in both cells as a function in time. Concentrations of solute in both half-cells are then determined as a function of time; diffusion coefficients can then be calculated from Eq. (8.2),

$$ln\left[\frac{C_I(t) - C_{II}(t)}{C_I(t = t_1) - C_{II}(t = t_1)}\right] = \frac{A}{K}\left(\frac{1}{V_I} + \frac{1}{V_{II}}\right)(t - t_1) \qquad (8.2)$$

where V_I = volume of half-cell I (L^3); V_{II} = volume of half-cell II (L^3); $C_I(t)$ = concentration of solute at time t in half-cell I, ($M\ L^{-3}$); $C_{II}(t)$ = concentration of solute at time t in

half-cell *II*, ($M L^{-3}$); $C_{I,II}(t = t_1)$ = concentration of solute in half-cell *I* or *II*, respectively, at the onset of pseudo-steady state, time $t = t_1$, ($M L^{-3}$); t = time (t); A = surface area of transport connecting the two half cells, (L^2); and K is the overall composite mass transfer coefficient, ($L t^{-1}$). K can be defined for this work by Eq. (8.3),

$$K^{-1} = \left[\frac{L_M}{D_{\text{eff}-m}} + \frac{L_{\text{biofilm}}}{D_{\text{eff}-\text{biofilm}}}\right] \tag{8.3}$$

where L_M = thickness of permeable membrane separating the two half-cells and employed as a support for biofilm accumulation, (L); L_{biofilm} = thickness of the biofilm, (L); $D_{\text{eff}-m}$ = molecular diffusivity of solute through support membrane alone, ($L^2 t^{-1}$); and $D_{\text{eff}-\text{biofilm}}$ = molecular diffusivity of solute through biofilm, ($L^2 t^{-1}$).

Diffusivity estimates were based on calculations using Eqs. (8.3) and (8.4) and data considered valid during the steady state portion of each diffusion trial (i.e., after a time period t_1 that varied with solute and biofilm thickness). Results of the effective diffusivity calculations based on Eqs. (8.2) and (8.3) are summarized in Table 8.2. Also reported in Table 8.2 is the ratio of the estimated solute diffusivity in biofilm relative the calculated value for the solute in pure water at the same temperature as estimated from Eq. (8.4),

$$D_{AB} = kT/6\mu_B \pi R \tag{8.4}$$

where k is the Boltzmann constant, T is temperature (°K), μ_B is the solvent viscosity, and R is the solute Stokes radius. Results in Table 8.2 indicate that regardless of solute molecular weight or size (radius of hydration), the diffusivities of solutes considered were all reduced in the biofilm by about the same degree relative to pure water, which is erroneous.

Water channels or perforations in the biofilm structure were observed that were between 10 and 30 μm wide at the outer edge of the biofilm and penetrated deep into the biofilm,

TABLE 8.2. Diffusion Coefficients for Fluorescently Labeled Molecules in a Bacterial Biofilm of 180 μm Using Diffusion Half-Cells

		Diffusion Coefficients ($cm^2/sec \times 10^{-7}$)		
Diffusing Molecule, *i*	Molecular Weight	In Pure Water[a]	In Biofilm	$D_{\text{biofilm}}/D_{i-\text{water}}$
Fluorescein	332.	55.0	54.0	0.98
Dextran	10,000.	2.2	1.96 (0.1)	0.89
Dextran	70,000.	1.7	1.44 (0.15)	0.85
BSA[b]	68,000.	6.9	5.9 (0.3)	0.86
IgG[b]	146,000.	3.8	NA	NA
HK[b]	102,000.	5.9	4.1 (0.23)	0.69
Catalase	225,000.	4.1	2.78 (0.15)	0.67
DNA	3.2×10^6	0.008	0.005 (0.001)	0.63

[a]Calculated from Einstein's Equation, see Table 8.3 for definition.
[b]Abbreviation definitions found in Table 8.3.
Note: Numerical values in parenthesis are standard deviations.

often times completely to the base substratum. The outer edge of the biofilm also exhibited an undulating topography, with the distance between the top and bottom of a "wave" averaging 10 μm. Presence of these perforations was documented by variation in the levels ("heights") of latex particles deposited onto a biofilm sample. Further, the continuous microscopic observation of a latex bead in a water channel as it settled due to gravity allowed estimates of the bead's terminal settling velocity. An average 1.3 μm/sec was observed over several experiments, which agrees well with the value of 1.6 μm/sec calculated from Stokes Law for a pure water system.

These qualitative observations suggest the half-cell diffusion trials just reported may not be able to accurately determine solute transport properties, which apparently vary locally within the biofilm. The presence of perforations in the biofilm coupled with the added surface area for transport afforded by the sides of these water channels, makes it likely that transport of the solutes in question from one half-cell to another was not impeded by the biofilm itself. It is more likely that the solutes were able to diffuse through the water channels at a rate similar to that in pure water. Eventually, solute may diffuse laterally into the biofilm matrix itself.

Thus, an alternative analytical technique was refined by Bryers and Drummond (1999) to determine the local diffusion coefficients on a microscale in order to avoid the errors created by the biofilm architectural irregularities. This technique is based on the fluorescence return after photobleaching (FRAP), which allows image analysis observation of the transport of fluorescently labeled macromolecules as they migrate into a microscale photobleached zone.

In concept FRAP is a simple technique (Axelrod et al., 1976). A small region of a surface or volume containing mobile fluorescent molecules (or fluorescently labeled molecules) is exposed to a brief intense pulse of light, thereby causing irreversible (assumed but often times not true) photochemical bleaching of the fluorophore in that region. Transport properties of the fluorescently labeled molecule are determined by measuring the rate of fluorescence recovery that occurs due to transport of unbleached molecules migrating into the bleached area from the unirradiated surroundings. Overall fluorescence is achieved using a separate source of light with a much attenuated intensity. Because the initial bleaching beam can be focused to bleach a region of very small dimensions, it is possible to acquire several FRAP samples over a sample plane very rapidly.

FRAP measurements provide an estimate of the "intradiffusion" coefficient for a component in a multicomponent system. Intradiffusion is used here rather than the more ambiguous terms of "self" or "tracer" diffusion (Albright and Mills, 1965; Westrin, 1991). Consider a homogeneous sample of a multicomponent system is at a uniform temperature. At time zero, all the molecules of a single component in a small subsection of the entire sample becomes an isotopically labeled form of that component. The labeled solute's concentration can now be uniquely measured as different from that of its unlabeled counterpart. Further, there are no chemical differences between the labeled and unlabeled components with regard to their transport properties. Equivalent and opposite concentration gradients are established for the component. The mutual diffusion between the two chemically equivalent components is defined as intradiffusion. Intradiffusion coefficients are obtained by treating the system as a two-component system and using Fick's Law. Intradiffusion coefficients are numerically equivalent to tracer diffusion coefficients but the terms have two significantly different meanings. In tracer diffusion the tracer molecule may or may not have a chemically equivalent unlabeled counterpart in the system and the tracer is applied

in very low concentrations. In intradiffusion, the resultant diffusion coefficient is independent of the ratios of the concentrations of the two components and depends only on the sum of their concentrations and the concentration of other components in the system. Self-diffusion is a special case of intradiffusion that deals with a binary system of only the two diffusing components, labeled and unlabeled.

For pure molecular diffusion, fluorescence recovery is as given by Axelrod et al. (1976) as Eq. (8.5),

$$f(t) = \sum_{n=0}^{\infty} \frac{(-\kappa)^n}{n!} \frac{1}{[1 + n\{1 + 2t/\tau_d)\}]} \tag{8.5}$$

where κ is the sample bleach constant and τ_d, is the two-dimensional (2-D) characteristic diffusion time, defined as,

$$\tau_d = \omega^2/4D \tag{8.6}$$

where ω is the e^{-2} laser beam radius for both the bleaching and monitoring phases of the FRAP experiment and D is the sample lateral diffusion coefficient.

Estimates of the local diffusion coefficient are made by correlation between the theoretical fluorescence recovery curve and experimental observation, with the only adjustable parameter being the diffusivity. As the FRAP tests are repeated across a plane in the biofilm, areas of locally specific D_{eff} can be determined for all solutes. As depth is changed and another horizontal scan is carried out, it became evident, within the water channels, that $D_{eff} \sim D_w$. A summary of the various solute (D_{eff}/D_w) ratios taken within biofilm polymer-cell clusters is provided in Table 8.3.

8.7.2 How Biofilms Influence Momentum Transport

Biofouling of a surface in contact with a flowing fluid alters the velocity of that fluid at the fluid–surface interface. The presence of a biofilm may increase or decrease the fluid frictional resistance of the surface depending on the degree of biofouling.

Characklis and co-workers (Zelver, 1979; Picologlou et al., 1980) provided in 1980 what remains today to be the benchmark paper on the effects of biofilm accumulation on momentum transfer (i.e., friction factor) for fluid flow in a tube with an hydraulically smooth surface. With water velocity maintained constant in the tube, then pressure drop will increase as biofilm accumulates. Increasing pressure drop increases pumping costs. If the biofilm forms on a ship hull, power consumption for propulsion at constant velocity will increase. As biofilm accumulates on a tube or pipe surface in turbulent flow, the friction factor increases regardless of whether velocity or pressure drop are maintained constant since it is a dimensionless parameter that takes pressure drop, velocity, and pipe geometry into consideration.

Potential mechanisms, postulated by Zelver (1979) and Zelver et al. (1985), governing the significant losses in momentum due to biofilm accumulation were the following:

- Pipe diameter constriction
- Increase in pipe wall rigid roughness

- Biofilm creep or biofilm being pushed along the wall in the axial direction
- Biofilm compliancy
- Biofilm viscoelasticity
- Biofilm filament oscillation
- Increase in fluid viscosity due to soluble biofilm products
- Rigid pipe wall roughness imparted by the biofilm

All but biofilm compliancy, viscoelasticity, and oscillations of the biofilm irregular, filamentous surface structures were proved to be insignificant contributions to the overall dissipation of kinetic energy by a biofilm. In Bryers (1980) experiments indicate that biofilm filamentous nature to some extent is set by the fluid dynamic regime, which in turn dictates the degree to which biofilm influence momentum. At lower Reynolds number (Re), biofilm filaments are longer and for the same overall thickness create larger pressure drops while at higher Re, filaments are short and their impact on frictional resistance is relatively less. Viscoelasticity measurements by Kirkpatrick et al. (1979) indicated that biofilm exhibited both a viscous and an elastic modulus, meaning that fluid flow through a conduit with a compliant viscoelastic wall structure would potentially dissipate significant fluid kinetic energy. These facts were corroborated by Stoodley et al. (1998) and again in Stoodley et al. (1999); a study which for the first time also verified that under certain conditions, biofilm can exhibit a degree of creep (e.g., sliding of biofilm en masse down the tube wall) in material in the axial direction).

Nuclear magnetic resonance imaging studies show that in a rectangular cross-section flow channel the presence of a biofilm can affect the velocity distribution of flow

TABLE 8.3. Diffusion Coefficients for Fluorescently Labeled Molecules in Water and in a Bacterial Biofilm using the FRP Method

		Diffusion Coefficients (cm^2/sec) $\times 10^{+7}$			
Diffusing Molecule, i	Molecular Weight	In Pure Water[a]	Biofilm Location Water Channel	$D_{i-\text{biofilm}}$ Cell Cluster	$D_{i-\text{water}}$
Fluorescein	332.	55.0	54.0	50.	0.91
Dextran	10,000.	2.2	2.0	0.62 (0.01)	0.28
Dextran	70,000.	1.7	1.7	0.26 (0.02)	0.15
BSA	68,000.	6.9	6.8	3.73 (0.02)	0.54
IgG[b]	146,000.	3.8	NA	NA	NA
HK[b]	102,000.	5.9	5.0	2.89 (0.01)	0.49
Catalase	225,000.	4.1	4.0	1.27 (0.02)	0.31
DNA	3.2×10^6	0.008	0.007	0.0015 (0.0003)	0.19

Note: Biofilm thickness was 232 μm in overall thickness; results in this table were averaged from FRP scans taken at an overall depth of 120 μm.

[a]Calculated from Einstein's Equation: $D_{i-\text{water}} = k_b T/6\pi\mu r_s$, where k_b = Boltzmann's constant; T = temperature, °K; μ = viscosity of solvent:solute mixture at temperature, T; and r_s = Stokes molecular radius.

[b]Results for IgG suspect due to binding of the immunoglobulin to biofilm matrix and cell wall. I Ratio reported are for values taken in biofilm cell clusters relative to pure water values.

BSA = bovine serum albumen; IgG = G class of human immunoglobulins, antibodies; HK = hexokinase. NA = not applicable.

(Lewandowski et al., 1995). Whereas a jet is formed at average flow velocities of 4.0 cm/s or greater in the reactor without biofilm, no jet is formed in reactors containing biofilms at these same average fluid velocities (Fig. 8.9). It has been proposed that the presence of a biofilm dramatically increases the wetted perimeter since the biofilm has a well-developed surface. This biofilm-induced increase in wetted perimeter decreases the Reynolds number according to the equation

$$\mathrm{Re} = V4A/\nu P$$

where υ is the kinematic viscosity, A is the cross-sectional area, and P is the wetted perimeter (Lewandowski et al., 1995). The average flow velocity profile is not precisely parabolic near the walls. At flow rates of 4.0 cm/s the flow velocity reaches zero at the reactor surface, not at the biofilm surface.

Fluid frictional resistance or shear increases with increased contact area between biofilm and a flowing fluid. This may promote either cell attachment or biofilm biomass detachment or both. Biofilm desquamation, sloughing, and erosion are three terms used to describe the detachment or loss of surface-associated biomass. Sloughing and desquamation typically refer to a catastrophic event involving the detachment of many cells and associated EPS, and other entrained particles. Sloughing may result in the removal of biomass clear down to the base of the biofilm, exposing the bare substratum. Erosion often refers to the independent detachment of single cells from the surface of the biofilm adjacent to the bulk liquid phase. These processes are very difficult to predict using mathematical models.

Resistance to sloughing is dependent on the limiting nutrient in the case of biofilms of the bacterium, *P. putida*, in a turbulent flow system (Applegate and Bryers, 1991). Oxygen-limited biofilms achieve (1) higher steady state biofilm organic carbon levels, (2) higher extracellular carbon/cellular carbon ratios, and (3) higher biofilm-bound calcium than carbon-limited biofilms. Oxygen-limited biofilms exhibit shear removal rates that are 20–40% of those observed for carbon-limited biofilms.

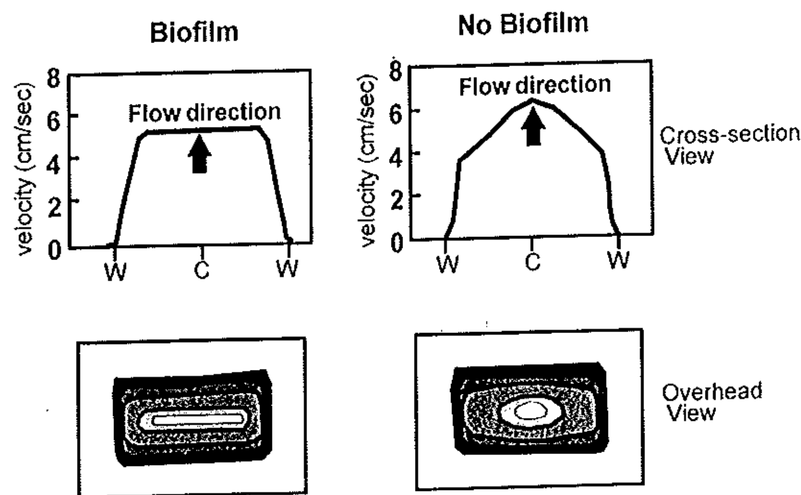

Figure 8.9 Cross-section and overhead views of fluid velocity profiles through a rectangular flow channel in the presence and absence of a biofilm. C is center of flow channel. W is the wall of the flow channel. (Adapted from Lewandowski, 1998.)

8.7.3 How Biofilms Influence Energy Transport

Heat is only one form of energy and, according to the First Law of Thermodynamics, energy and not heat is conserved. Nevertheless, in biofilm systems, the other forms of energy are generally negligible. Heat transport occurs frequently in nature and in process plants (e.g., the cooling of water as it exits a hot spring in Yellowstone Park or the heating of river water as it passes through a power plant condenser). Two mechanisms for heat transport are relevant to biofilm systems: (1) conduction, which is the transport of heat from a high temperature to a low temperature within a phase (intraphase) such as a solid or fluid by the motion of molecules or electrons and (2) advection, which is heat transport that results from bulk fluid motion usually relevant to interphase transport. Despite the tremendous effects of biofilm on heat transfer efficiency and intense research efforts investigating these effects in the late 1970s and early 1980s, ironically very little new research has since been initiated on the fundamental effects of biofilm accumulation on heat transfer.

Conductive heat transfer resistance results from the insulating layer formed by the biofilm and generally increases as the biofilm accumulates. Conductive heat transfer is the difference between overall and advective heat transfer resistance and can be calculated *a priori* if the biofilm thickness and thermal conductivity of the deposit is known. The conductive heat transfer resistance is dependent on thermal conductivity and thickness of the deposit. *Advective* heat transfer resistance results from fluid motion or turbulence and generally decreases as biofilm accumulates, since the roughness of the biofilm increases turbulence in the interfacial region. Advective heat transfer resistance can be calculated from the friction factor and the properties of water. Thus, the advective heat transfer resistance depends on the roughness characteristics of the biofilm and the shear stress at the heat transfer surface.

The overall heat transfer resistance determines the influence of biofilm on heat transfer efficiency. However, advective and conductive heat transfer resistance may be important because differences in deposit properties (apparent roughness and thermal conductivity) can result in significant differences in the relative contributions of conductive and advective processes to overall heat transfer resistance. Measurements related to biofilm accumulation and general fouling processes include monitoring overall heat transfer resistance and characterizing the biofilm or fouling deposit. A variety of different monitoring devices have been developed to obtain accurate fouling data under various experimental conditions. A detailed description of some of the devices can be found elsewhere (Knudsen, 1981). One type of instrument is the thick-walled heat exchanger (Characklis et al., 1981; Turakhia and Characklis, 1984; Zelver et al., 1985). Constant heat is supplied to the thick-walled heat exchanger and temperature at two points in the thick wall are measured, allowing one to calculate the heat flux in the system.

As biofilm accumulates, the overall heat transfer resistance for a tube changes due to increased biofilm conductive resistance and decreased advective heat transfer resistance resulting from increasing biofilm roughness. Characklis et al. (1981) have determined the relative changes in conductive and advective heat transfer resistance in a tube during biofilm accumulation. At the beginning of the experiment, conductive resistance due to the biofilm is zero and only advective resistance exists. As biofilm accumulates, conductive resistance increases proportional to biofilm thickness. Advective resistance decreases as a result of increased roughness reflected by an increase in friction factor. The Colburn analogy was used to calculate the advective resistance from friction factor measurements and therefore represents an approximation to the actual advective resistance. The biofilm thermal conductivity was measured in separate experiments.

8.8 MONITORING BIOFOULING

Monitoring is the key to biofouling control. The key to successful monitoring is (1) knowing exactly what it is your monitor is measuring, (2) deciding what it is you really need to monitor, and (3) relating that which you are monitoring to a performance criteria for a particular process of interest. By passing a process fluid over a substratum, organisms in the process fluid deposit on the substratum and form a biofilm. Selection of the appropriate engineering or biological parameters to monitor depends on the nature of the process that is influenced by the biofouling phenomenon. The most common biological parameters that have been used to monitor biofouling include surface-associated biomass, desquamation, and metabolic activities that impact the substratum or the bulk, aqueous phase (White et al., 1996). Common engineering parameters used to monitor biofouling include heat transfer resistance and fluid frictional resistance (velocity, pressure drop, or increase in torque). Details on methods of monitoring biofouling in various engineered systems may be found in several recent publications (Bryers, 1993; Flemming and Griebe, in press).

8.8.1 Microscopy

Early biofouling monitors consisted of transparent glass slides suspended in the aqueous phase (Henrici, 1933; Zobell and Allen, 1935). Once a biofilm accumulates on the slide, the slide is retrieved and examined by transmitted light microscopy. Following the development of epifluorescence microscopy and scanning electron microscopy, opaque surfaces were substituted for glass slides to examine the influence of different substratum properties on biofouling. These microscopic techniques also allowed better resolution of microbial cells in the presence of other material that was adsorbed to the surface. The recent availability of optical equipment to perform differential interference contrast (DIC) and CSLM has created new opportunities to evaluate the development of biofouling layers nondestructively in real time.

Much has been made of CSLM in recent years as a means of monitoring structural properties of biofilms. CSLM displays objects within a plane of focus (0.3 μm) while eliminating out-of-focus objects in other planes using a pinhole barrier. Images collected in different planes can be merged using computer-controlled image analysis to produce a 3-D reconstruction of a multilayer biofilm, showing depth-dependent relationship of light-absorbing structures (Lawrence et al., 1991; Caldwell et al., 1992). CSLM has been used in conjunction with different types of flow cell reactors, enabling biofilm development to be evaluated in 4 dimensions (x,y,z,t). Surman et al. (1996) have compared the types of biofilm information that can be obtained by the different microscopic techniques.

8.8.2 Sampling Devices

A variety of biofilm samplers have been developed, including the widely used Robbins Device, which can be fabricated of the same material as the fouled industrial surface and mounts flush with the surface of a conduit (McCoy et al., 1981). Coupons remain the most common sampling device, primarily because of their simplicity and versatility. Coupons of any composition and size can be suspended in either a static or dynamic aqueous medium and later sampled by a variety of methods.

8.8.3 Biofouling Reactors

8.8.3.1 Annular Reactors. There are nearly as many variations in the design of reactors to establish biofouling layers on surfaces as there are questions to answer regarding biofouling. The continuous flow stirred tank reactor (CFSTR), described by Characklis (1990), continues to be a popular system to use for development of biofouling layers on coupon surfaces. The Roto Torque, is a CFSTR that offers the opportunity to independently control shear and residence time of the liquid phase (Characklis, 1990). The Roto Torque contains multiple sampling slides that are flush-mounted on the reactor wall. Although wall shear can be controlled by the operator, there is considerable variation in the shear values obtained at different locations in the reactor (Gjaltema et al., 1994). The sampling slides contain a biomass/unit area that is not representative of the entire reactor surface area due to these local differences in shear. Since it is not possible to obtain representative biofilm samples from the Roto Torque, this reactor is not suitable for use in quantitative physiological or kinetic biofilm studies where it is essential that the total amount of active biomass in the system can be measured. The reactor can still be used for morphological and "black box" studies, however.

Substrate gradients and inhomogeneous shear fields are also difficult to avoid in other types of biofilm reactors, including flat plate channel-flow reactors and tubular reactors. Furthermore, because these sampling methods required "off-line" analysis, they offered only a "snapshot" in time of a very dynamic biological phenomenon, thus limiting our ability to detect desquamation or obtain accurate estimates of biofilm production.

8.8.3.2 Radial Flow Reactor. A radial-flow cell or Fowler Cell Adhesion Module, described by Fowler and McKay (1980), provides a continuous shear gradient across a substratum. Mittelman et al. (1990) have utilized a Fowler Cell Adhesion Module to reproducibly demonstrate the changes in cellular concentration and composition of a culture of *P. atlantica* on stainless steel as a function of applied shear force. The apparatus has also been used to observe microscopically through replaceable windows a developing biofilm as a bulk aqueous phase flows over the surface (Mittelman et al., 1992). Flow-through reactors have been developed that accommodate different flush-mounted substrata and surface coatings, offering the opportunity to assess substratum effects on biofilm accumulation under controlled laminar flow (Arrage et al., 1995).

A radial-flow chamber was used by Dickinson and Cooper (1995) to quantitatively describe attachment and detachment of cells of *S. aureus* as a function of shear stress on three different polyurethane surfaces conditioned with various blood fractions. An automated videomicroscope and image analysis system was used to achieve rapid counts of attached bacteria across the surfaces of the treated disc in the radial flow chamber over time. Shear-dependent rate constants were estimated for attachment and detachment by fitting the solutions of phenomenological models to the experimental data. The authors demonstrated the usefulness of the approach to resolve an influence of the various surface-conditioning molecules on cell attachment and detachment.

Dickinson et al. (1995) used the approach described previously to determine the probability of attachment and detachment of cells capable of producing a cell-surface clumping factor, coagulase, or both compared to mutants lacking these cell surface molecules on plasma-, fibrinogen-, or albumin-coated polyurethane surfaces. The result suggested that clumping factor played a pivotal role in enhancing adhesion to surfaces with adsorbed fibrinogen.

8.8.4 Nondestructive Monitoring of Biofilm Biomass

On-line biofilm monitoring techniques have been developed in recent years that offer real promise as practical monitoring tools to industry. Biofilm formation, succession, and stability can now be monitored using in-line, nondestructive techniques in flow-through cells. Biofouling has been quantified on the basis of biofilm microbial biomass determined by on-line monitoring of nicotinamide adenine dinucleotide (NADH), tryptophan, and chlorophyll fluorescence (Angell et al., 1993). The relative tryptophan fluorescence per cell has been used as a nondestructive measure of sublethal toxicity in biofilms exposed to antimicrobial compounds introduced to the bulk aqueous phase or impregnated into the substratum (Arrage et al., 1995). The intensity of the amide protein bands in the infrared has been used as a measure of microbial biofilm biomass (Geesey and Bremer, 1990; Suci et al., 1997). Using attenuated total reflectance Fourier transform infrared (ATR/FT-IR) spectroscopy, Nivens et al. (1993a) were able to detect as few as 5×10^5 cells of *Caulobacter crescentus* per cm^2 surface on a germanium internal reflection element (IRE) in a flow cell based on absorbance in the amide II region of the spectrum.

The quartz crystal microbalance (QCM) has been used to monitor initial fouling and subsequent biofilm development (Nivens et al., 1991). The QCM employs an AT-cut (refers to thickness-shear mode of cut relative to crystallographic axis) quartz crystal, and oscillator circuit, and a frequency counter to obtain a mass measurement. When bacteria in an aqueous suspension adsorb to a submerged quartz crystal containing a thin metal film, the frequency of oscillation decreases in proportion to the mass of adsorbed material. The QCM thus provides a direct measure of biomass. As few as 10^4 cells of *C. crescentus* were detected per square centimeter of a gold-coated quartz crystal (Nivens et al., 1991). A linear relationship was observed between frequency shift and adsorbed biomass over the range of 10^4–10^6 cells/cm^{-1}. One of the main limitations of this monitoring technique is the sensitivity of oscillation frequency to temperature. Furthermore, different crystals respond differently to temperature, making comparison of results among different crystals difficult to interpret.

On-line monitoring of bacterial behavior in biofilms based on bioluminescence has been achieved using a fiber optic probe and photomultiplier detector. Mittelman et al. (1993) monitored accumulation of the bioluminescent marine bacterium *Vibrio harveyi* on antifouling coatings in a flow-through cell containing quartz windows. This approach can be applied to other bioluminescent organisms, including those that have been genetically engineered to express the *lux* or *gfp* gene.

A biofouling monitor just recently introduced uses fiber optic sensors to capture reflected light, which is influenced by the accumulation of adsorbed material at the tip of the fiber (Tamachkiarowa and Flemming, 1996). The sensor can be flush-mounted on the surface undergoing fouling. The intensity of the back-scattered light is proportional to the intensity of the incident light and the projected area of the fouling layer on the sensor tip. However, the sensor is rather insensitive to the nature of the fouling layer in that it does not discriminate between biological and nonbiological material. Selectivity could be achieved through the use of specific wavelengths of energy that are absorbed by specific biological molecules.

8.8.5 Monitoring Biofilm Processes

ATR/FT-IR has also been used to monitor metabolic processes in biofilm populations nondestructively in real time. Nivens et al. (1993b) observed shifts in physiological status, based on an increase in the C=O stretch from the production of polyhydroxy alkanoic

acids, in *C. subvibriodes* cells attached to a germanium substratum following ammonium ion depletion. Bremer and Geesey (1991) used the same analytical approach to detect the production of polysaccharide in a bacterial biofilm population growing on a copper film. With this approach, polysaccharide production was related to the deterioration of the underlying copper film.

8.8.5.1 Molecular Biology Tools. Gene expression has also been monitored in microbial biofilms during surface fouling. Use of heterologous gene expression to characterize biofilm ecology is detailed extensively in Chapter 4. In studies using reporter genes, a *Pseudomonas* strain was constructed with the *lux* gene cassette under control of the promoter for algD, a gene involved in the commitment to synthesize alginic acid (Wallace et al., 1994). The effects of salt concentration and nitrogen balance on alginic acid synthesis by biofilm populations was determined on the basis of bioluminescence emitted from the biofilm (Rice et al., 1995). The brightness of the bioluminescence response in the engineered bacteria is influenced by factors other than promoter activity, however. Factors such as ionic strength, pH, trace metal concentration, carbon source, and plasmid copy number affect the light-emitting reaction (Heitzner et al., 1994).

A *lacZ* reporter gene cassette has been used to monitor the level of expression of *algC*, a "housekeeping" gene that is involved in the production of the extracellular polysaccharide alginic acid in *P. aeruginosa* (Davies et al., 1993). Using the fluorogenic substrate methylumbelliferyl β-D-galactopyranoside (MUG), sufficient quantities of the substrate were converted to fluorescent product by the reporter gene product β-galactosidase, that up-expression could be detected in individual bacterial cells within the biofilm. Three important observations were made in biofilms containing cells carrying these reporter genes (Davies and Geesey, 1995). First, not all members of an isogenic population behave the same in terms of gene expression during residence on a surface. Second, association with a surface promotes expression of genes not normally expressed when cells exist individually in suspension. Third, gene expression in cells on a surface can be dynamic and transient in nature. While sufficient quantities of fluorescent product remained associated with cells to microscopically resolve gene expression in individual cells in the studies cited above, the product often diffuses out of other types of cells as rapidly as it is produced, thus limiting its use in monitoring gene expression in individual cells. This limitation may be overcome by the use of reporter genes whose autofluorescent products remain inside the cells (e.g., green fluorescent protein) or enzyme substrates that not only fluoresce but also precipitate at the site of conversion (e.g., ELF™ substrates; Larison et al., 1995).

The limitations associated with the reporter systems described above can be circumvented by the use of the green fluorescent protein (GFP) reporter system. This system utilizes the *gfp* gene whose product exhibits inherent fluorescence that is less sensitive to conditions in the cell than the *lux* reporter system, and does not require a diffusable substrate like the *lacZ* reporter system. Fluorescence of GFP at 509 nm can be easily detected after excitation at 395 nm. A suicide plasmid containing a promoterless *gfp* gene that recombines with the bacterial chromosome has been constructed to establish *gfp* fusions in a wide variety of gram-negative bacteria (Kalogeraki and Winans, 1997). Alternatively, Tn5- and Tn10-based transposons carrying either a promoterless *gfp* gene or a *gfp* gene expressed from a broad-host-range promoter has been created to tag the chromosome of diverse bacterial species (Matthysse et al., 1996; Stretton et al., 1998). A new *gfp* reporter system has recently been described that results in a fluorescent GFP product that degrades

rapidly following synthesis, allowing transient gene expression to be followed nondestructively in real time (Andersen et al., 1998).

GFP fluorescence but not synthesis involves an oxidation reaction requiring molecular oxygen (Heim et al., 1994). This would suggest that GFP may only be useful in anaerobic biofilms. However, recent studies indicate that dissolved oxygen concentrations of 0.73 mg l^{-1} or less is sufficient to promote detectable fluorescence in individual cells of facultatively anaerobic dissimilatory iron reducing bacteria cultured under anaerobic conditions (unpublished results). Furthermore, cultures of the dissimilatory sulfate reducing bacteria, *Desulfovibrio desulfuricans*, carrying a constitutively expressed *gfp* gene, display GFP fluorescence in individual cells under conditions of cell replication (unpublished results). Thus, these genetic tools offer unique opportunities to study the surface behavior of individual cells or populations of a wide variety of surface-fouling bacteria.

Introduction of fluorescent probes that report cell identity, viability, and activity to a flow cell reactor containing mixed population communities offers the opportunity to resolve through CSLM associations and interactions between individual cells and aggregations of cells (Korber et al., 1997; Moller et al., 1997). CSLM has been used to follow expression of chitinase genes in marine bacteria carrying the *gfp* gene under the control of promoters of chitin-degrading genes in cells that have formed a biofilm on squid pen surfaces (Stretton et al., 1998).

8.8.5.2 Microsensors. Microsensors offer yet another way to evaluate microbial processes in biofilms. Microsensors for N_2O, NH_4^+, NO_2^-, NO_3^-, and O_2 have been used to describe microscale chemical gradients from microbial activities in sediments, fluidized bed bioreactors, and biofilms. Microsensors have also been developed to determine diffusion coefficients and directly measure mass transport into and within biofouling layers on surfaces (Lewandowski, 1993; Rasmussen and Lewandowski, 1998).

Lee and de Beer (1995) have used pH and O_2 microelectrodes to probe respiratory activity in a biofilm on corroding steel. In this instance, the position of the electrodes above the substratum was controlled by a computer and stepper motor. Data was collected at different depths within the biofilm, providing profiles of pH and O_2. Through this approach, it was possible to demonstrate the consumption of O_2 by the biofilm above a tubercle and establish the pH at anodic and cathodic sites.

A highly selective liquid nitrite microsensor has been used to evaluate denitrifying, nitrifying biofilms from wastewater treatment plants (de Beer et al., 1997). Using these microsensors, local areas of elevated nitrite have been located in narrow zones of less than 1 mm. Such sensors have the potential to elucidate which factors control the nitrite concentrations in biofilms.

8.8.6 Concatenation of Reactor Design, Molecular Biology, Microscopy, and Microsensors to Obtain Multidimensional Information on Biofouling of Surfaces

One of the next steps to take in monitoring biofouling is to combine the techniques described previously to characterize surface biofouling processes. This will enable us to obtain a better understanding of the relationships between structure and function in multispecies surface fouling layers. Combining microscopy and the scanning vibrating electrode, Angell et al. (1994) established a relationship between the locations and activities of bacterial microcolonies and the formation of anodic electrochemical activity on a

corroding metal coupon. Such small-scale processes must be studied with multiple, non-destructive, on-line techniques that offer high spatial resolution.

Bacterial aggregates from a nitrifying fluidized bed have been investigated with microsensors and rRNA-based oligonucleotide probes (Schramm et al., 1998). Microprofiles of O_2, NH_4^+, NO_2^-, and NO_3^- revealed the occurrence of nitrification in the outer 125 μm of the aggregates. Fluorescent probes identified members of the Nitrosospira group as the primary ammonia oxidizers in this region of the aggregate. Another important step that needs to be taken is the modification of techniques just described to make them useful in the field. While the on-line biofilm monitoring techniques described previously have, so far, been limited to laboratory bench experimentation, theoretically, they should be adaptable to industrial scale biofilm monitoring.

8.9 SUMMARY AND CONCLUSIONS

Biocontamination of engineered materials and systems is difficult to quantify, predict, and assess. Surface biofouling is not governed by easily modeled, predictable processes. Robust three-dimensional models are likely to be required to predict the behavior of complex, spatially dependent populations of surface colonizing species of living organisms under the influence of hydrodynamics and surface chemical phenomena. Indications that such modeling is feasible and often necessary has recently been demonstrated (Picioreanu et al., 1998a; 1998b).

In spite of the heroic financial investments by industry and government to better understand biofilm development and biofouling processes, our ability to manipulate these processes in reproducible, predictable ways has, except in rare instances, been painfully inadequate. Hopefully, the next generation of biofilm researchers will possess the creativity and motivation to develop and effectively utilize the advanced technologies needed to achieve better control over biofilm processes. The development of sensitive devices to monitor biofilm parameters that we consider important is essential before we can demonstrate, at the most rudimentary level, "reproducible" biofilms. Without this capability, progress in understanding fundamental biofilm processes that we seek to control will continue to be slow, while the damage biofilms inflict on engineered materials and processes will continue to occur and cost more to abate. Without a higher level of understanding of biofilm processes, control of biofouling will likely continue to be defined by what amount of damage we can afford to accept in a particular system rather than by a priori knowledge of controllable behavior of the biofilm population in that system.

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