

Chapter 1

Biofilm Formation

INTRODUCTION

The term microbiologically influenced corrosion (MIC) is used to designate corrosion due to the presence and activities of microorganisms, that is, those organisms that cannot be seen individually with the unaided human eye, including microalgae, bacteria, and fungi. Microorganisms can accelerate rates of partial reactions in corrosion processes or shift the mechanism for corrosion. Microorganisms do not produce unique types of corrosion; instead, they produce localized attack including pitting, dealloying, enhanced erosion corrosion, enhanced galvanic corrosion, stress corrosion cracking, and hydrogen embrittlement. Microbiologically influenced corrosion has been reported for all engineering metals and alloys with the exception of predominantly titanium and high chromium–nickel alloys, and has been documented for metals and nonmetals exposed to seawater, freshwater, distilled/demineralized water, crude and distillate hydrocarbon fuels, process chemicals, foodstuffs, soils, human plasma, saliva, and sewage. It occurs in environments where corrosion would not be predicted (e.g., low chloride waters) and the rates can be exceptionally high. According to a recent survey, damage due to corrosion in the United States is estimated at \$276 billion. Similar surveys in the United Kingdom, Japan, Australia, and Germany estimate the cost of corrosion to be 1 to 5 percent of the gross national product (www.corrosion-doctors.org). Microbiologically influenced corrosion is reported to account for 50 percent of the total cost of corrosion (Fleming, 1996). The industries most affected by MIC are power generation; oil production, transportation, and storage; and water distribution.

BIOLOGICALLY ACTIVE ENVIRONMENTS

Microorganisms require water, nutrients, and electron acceptors. Liquid water is needed for all forms of life and the availability of water influences the distribution and growth of microorganisms. Water availability can be expressed as equilibrium

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relative humidity or water activity (a_w) with values ranging from 0 to 1. Microbial growth has been documented over a range of a_w from 0.60 to 0.999, though none can grow at $a_w = 1$ (pure water) because there are no nutrients available to the organism. A representation of the relative requirements of the major elements required for typical microorganisms composition is as follows: $C_{169}(H_{280}O_{80})N_{30}P_2S$. Waters with suitable forms of carbon, nitrogen, phosphorus, and sulfur support microbial growth. Microorganisms can use a variety of electron acceptors for respiration, including oxygen, sulfate, nitrate, nitrite, carbon dioxide, Fe^{3+} , Mn^{4+} , and Cr^{6+} . The significance of electron acceptors will be discussed later in this chapter.

Microorganisms include bacteria, fungi, and microalgae. Algae are unicellular photosynthetic organisms found in a wide range of environments—from freshwater to concentrated brines (pH from 5.5 to 9.0) and temperatures from below 0 to 40 °C. In the presence of light, algae produce oxygen (photosynthesis). In the absence of light, algae consume oxygen (respiration) and reverse the process. Diatoms are microalgae that have silicon-containing frustules and are often the most conspicuous constituents within the biofilm (Figure 1-1*a, b*). Some diatoms can grow nonphotosynthetically. Many algae excrete organic acids and are primary producers of nutrients that are necessary to support other fouling species. Fungi are nonphotosynthetic, having a vegetative structure known as a mycelium that is the outgrowth of a single reproductive cell or spore (Figure 1-2*a, b*). Neither spores nor mycelia are capable of movement. Fungi often reach macroscopic dimensions due to mycelia growth. Fungi assimilate organic material and produce organic acids including oxalic, lactic, acetic, and citric. Yeasts are fungi that multiply by forming buds instead of mycelia. Fungi are the most desiccant-resistant microorganisms and can remain active down to $a_w = 0.60$, whereas few bacteria remain active at a_w values below 0.9.

Bacteria have received the most attention for their influence on corrosion. Bacteria can be subdivided into groups depending on shape (Figure 1-3*a–c*), requirements for oxygen, source of energy, and type of environment in which they survive.

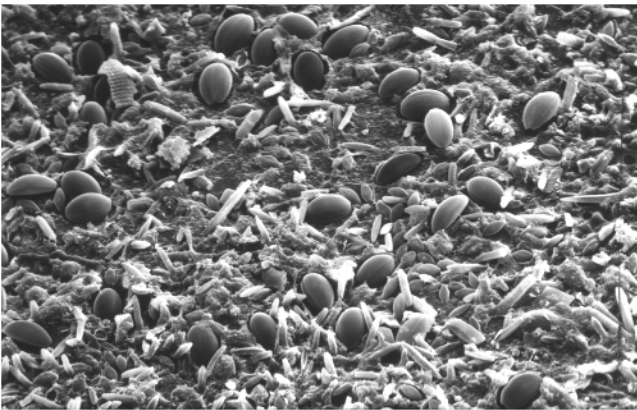


FIGURE 1-1a Pennate diatoms embedded in a biofilm. (Image by Richard Ray, Naval Research Laboratory, Stennis Space Center, MS.)

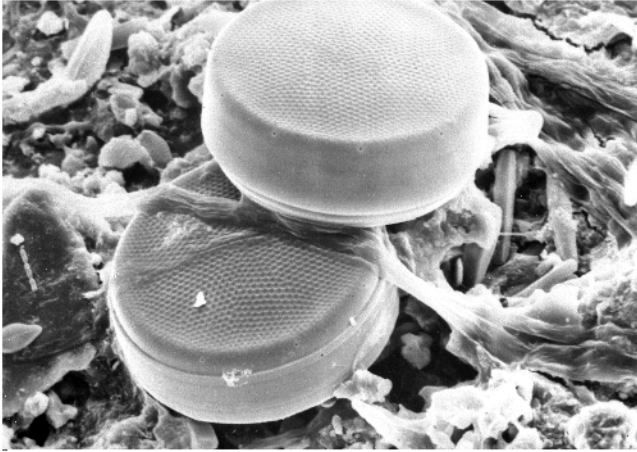


FIGURE 1-1b Centric diatom *Coccosinodiscus* sp. embedded in a biofilm. (Image by Richard Ray, Naval Research Laboratory, Stennis Space Center, MS.)

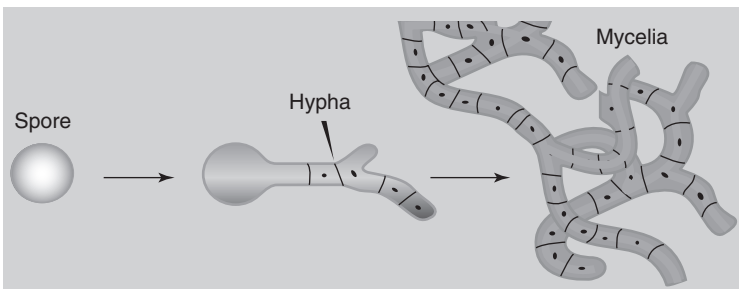


FIGURE 1-2a Schematic of fungal spore development into mycelia.

They may occur individually, but tend to form colonies, reproducing by binary fission or cell division. Bacteria range in size from 0.2 μm wide to 1 to 10 μm long. Some filaments may be several hundred millimeters long. Dwarf cells can form in oligotrophic (i.e., nutrient-deprived) waters. Bacteria can be grouped according to their requirements for oxygen and sources of energy. Obligate aerobes require oxygen for survival and growth. Microaerophilic bacteria require low oxygen concentration and facultative anaerobic bacteria can grow under aerobic or anaerobic conditions. Obligate anaerobic microorganisms cannot tolerate oxygen for growth and survival. Obligate anaerobic bacteria are, however, routinely isolated from oxygenated environments associated with particles, crevices, and, most importantly, other bacteria that effectively remove oxygen from the immediate vicinity of the anaerobe. In aerobic respiration, energy is derived when electrons are transferred to oxygen, the terminal electron acceptor. In anaerobic respiration, a variety of organic

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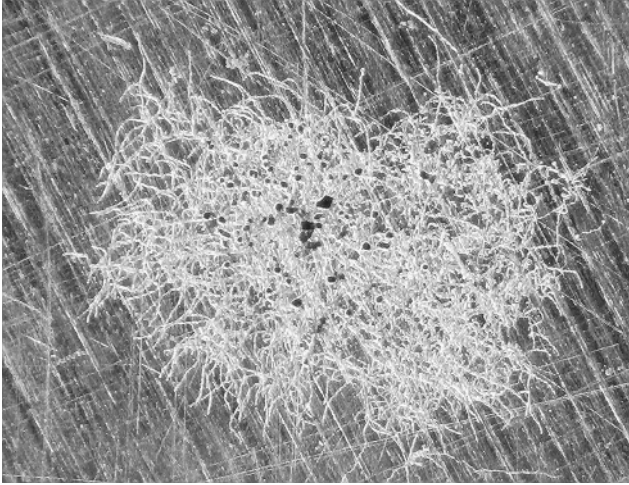


FIGURE 1-2b Fungal spores and mycelium on a metal surface. (Image by Richard Ray, Naval Research Laboratory, Stennis Space Center, MS.)

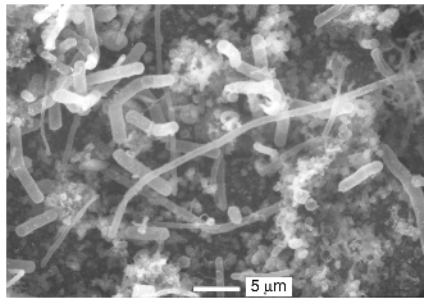


FIGURE 1-3a Filamentous and rod-shaped bacteria. (Image by Richard Ray, Naval Research Laboratory, Stennis Space Center, MS.)

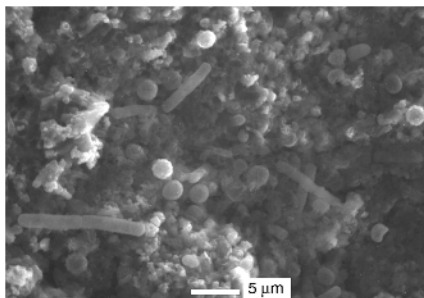


FIGURE 1-3b Spherical and rod-shaped bacteria. (Image by Richard Ray, Naval Research Laboratory, Stennis Space Center, MS.)

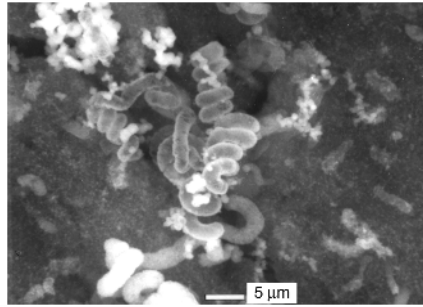


FIGURE 1-3c Spirillum-shaped bacteria. (Image by Richard Ray, Naval Research Laboratory, Stennis Space Center, MS.)

TABLE 1-1 Types of Respiration and Examples of Electron Acceptors

Electron acceptor	Product(s)	Organisms
<i>Aerobic respiration</i>		
O ₂	H ₂ O	Strictly aerobic or facultative anaerobic organisms
<i>Anaerobic respiration</i>		
NO ₃ ⁻	NO ₂ ⁻ , N ₂ O, N ₂	Denitrifying bacteria
S ²⁻	SO ₄ ²⁻	Obligate anaerobe bacteria, sulfate-reducing bacteria
S	S ²⁻	Facultative and obligate anaerobic bacteria
CO ₂	Acetate	Acetogenic bacteria
	Methane	Methanogenic bacteria
Fe ³⁺ , Mn ⁴⁺ , Cr ⁶⁺	Fe ²⁺ , Mn ²⁺ , Cr ³⁺	Metal-reducing bacteria

and inorganic compounds may be used as the terminal electron acceptor (Table 1-1). Bacteria are grouped based on the terminal electron acceptor in anaerobic respiration, for example, sulfate- and metal-reducing bacteria.

Bacteria can also be grouped according to their nutritional requirements. Heterotrophic bacteria derive energy from a wide range of organic molecules. As a group, heterotrophic bacteria can assimilate almost any available carbon molecule, from simple alcohols and sugars to complex polymers. Many heterotrophic microorganisms can grow on trace nutrients in natural waters or distilled water. Bacteria can adapt to a variety of nutrient sources. For example, *Pseudomonas fluorescens* can use over 100 different compounds, including sugars, lipids, alcohols, phenols, and organic acids, as sole sources of carbon and energy. Diversity among organic substrates is peripheral to the issue of energy-generating and energy-conserving processes. Autotrophic bacteria

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oxidize inorganic compounds, elements, or ions (e.g., NH_3 , NO_2^- , CH_4 , H_2 , SO_4^{2-} , Fe^{2+} , Mn^{2+}) as sources of energy. When both autotrophic and heterotrophic mechanisms operate simultaneously, the metabolism is mixotrophy. Phototrophic bacteria can use light as a source of energy.

The temperature range in which living organisms can grow is that in which liquid water can exist, approximately 0 to 100 °C. Microbial life is possible over a range of 10 pH units or more. Many microorganisms can withstand a 100-fold or greater variations in pressure. Pressure in the depths of the sea is only mildly inhibitory to the growth of many microorganisms. Heavy-metal concentrations as low as 10^{-8} M can inhibit the growth of some microorganisms, while others may be resistant to concentrations of 1,000,000-fold or greater. Microbial species show 1000-fold differences in susceptibility to ultraviolet, beta, and gamma irradiation. The following general statements about microorganisms are taken from Pope (1986):

- Individual microorganisms are small [from less than two-tenths to several hundred micrometers (μm) in length by up to 2 or 3 μm in width], a quality that allows them to penetrate crevices, and some other small spaces easily. Bacterial and fungal colonies can grow to macroscopic proportions.
- Bacteria may be motile and capable of migrating to more favorable conditions or away from less favorable conditions; for example, toward nutrients or away from toxic materials.
- Bacteria have specific receptors for certain chemicals, which allow them to seek out higher concentrations of nutrients.
- Bacteria and fungi can reproduce very quickly (generation times of 18 min have been reported).
- Individual cells can be widely and quickly dispersed by wind, water, animals, or aircraft.
- Microorganisms are resistant to many chemicals (antibiotics, disinfectants, etc.) by virtue of their ability to degrade them or by being impermeable to them [due to extracellular polymeric substances (discussed later), their cell walls, or their cell membrane characteristics]. Resistance may be acquired through mutation or acquisition of a plasmid by naturally occurring genetic exchange between cells.
- Microorganisms have developed several strategies for survival in natural environments: (1) spore formation, (2) biofilm formation, (3) dwarf cells, and (4) a viable, but nonculturable state. Many bacteria and fungi produce spores that are very resistant to temperature (some even resist boiling for over one hour), acids, alcohols, disinfectants, drying, freezing, and many other adverse conditions. Spores may remain viable for hundreds of years and germinate on finding favorable conditions. In the natural environment, there is a difference between survival and growth. Microorganisms can withstand long periods of starvation and desiccation. If conditions are alternately wet and dry, microbes may survive dry periods and will grow during wet periods.

BIOFILM FORMATION

It is convenient and informative to discuss the characteristics of individual groups of microorganisms; however, in natural environments microorganisms form synergistic communities that conduct combined processes which individual species cannot. The term biofilm embraces an enormous range of microbial associations generally found at phase boundaries (Wimpenny, 1996). In aquatic environments, microbial cells attach to solids, including metals. Immobilized cells grow, reproduce, and produce extracellular polymers forming a biofilm. Biofilm accumulation is the net result of attachment, growth, and detachment (Figure 1-4).

Biofilm formation consists of a sequence of steps and begins with adsorption of macromolecules (proteins, polysaccharides, and humic acids) and smaller molecules (fatty acids and lipids) at surfaces. Adsorbed molecules form conditioning films that alter physiochemical characteristics of the interface, including surface hydrophobicity and electrical charge. The amount of adsorbed organic material is a function of ionic strength and can be enhanced on metal surfaces by polarization.

Because of the complexity of microbial binding to surfaces, the terms attachment and detachment are frequently used without referring to specific physical processes. Attachment is due to microbial transport and subsequent binding to surfaces. The extent of bacterial adhesion and the adhesion pattern depend on bacterial characteristics, including cell-surface hydrophobicity and charge, cell size, presence of flagella and pili, and properties of the substratum such as chemical composition, surface roughness, crevices, inclusions, and coverage by oxide films or organic coatings, the composition and strength of the aqueous medium, and the hydraulic flow regime.

During initial stages of biofilm formation, the major factor controlling the rate of colonization is hydrodynamics. Microbial colonization begins with transport of

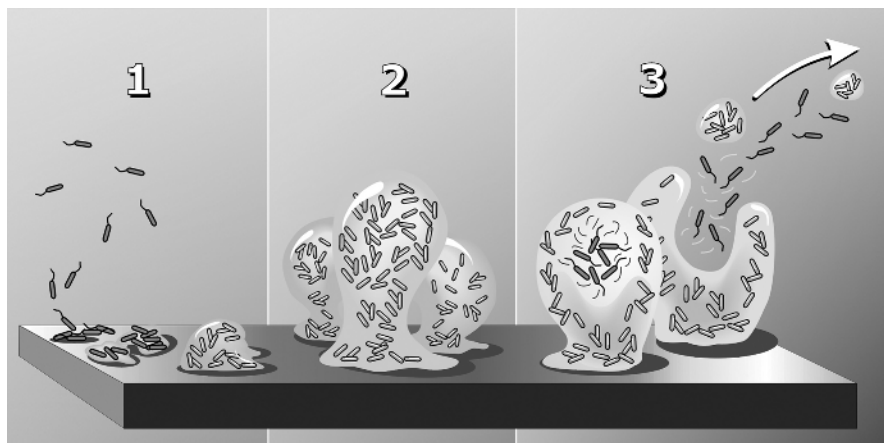


FIGURE 1-4 The biofilm life cycle in three steps: (1) attachment, (2) growth of colonies, and (3) detachment in clumps or “seeding dispersal.” (Stoodley and Dirckx, 2003a. Reprinted with permission from the Center for Biofilm Engineering at Montana State University, Bozeman.)

microorganisms to the interface mediated by at least three mechanisms: (1) diffusive transport due to Brownian motion, (2) convective transport due to the liquid flow, and (3) active movement of motile bacteria near the interface. The influence of convection transport exceeds the other two by several orders of magnitude. Once the microbial cell is in contact with a surface, it may or may not adhere. The ratio of cell numbers adhering to a surface to the cell numbers transported to this surface depends on surface properties, physiological state of organisms, and hydrodynamics near the surface. Biofilms grown at high shear stress develop elongated microcolonies (Figure 1-5) (Lewandowski and Stoodley, 1995).

Dense biofilms form as a result of high shear stress or starvation (Beyenal and Lewandowski, 2002). van Loosdrecht et al. (1995) discussed the effects of substrate loading, shear stress, and growth rate on biofilm structure. Immediately after attachment, microorganisms initiate production of slimy adhesive substances, termed extracellular polymeric substances (EPS), which assist in the formation of microcolonies and microbial films. Extracellular polymeric substances bridge microbial cells with the substratum and permit negatively charged bacteria to adhere to both negatively and positively charged surfaces. They may also control interfacial chemistry at the substratum–biofilm interface. Azeredo and Oliveira (2000a) examined the role of exopolymers in biofilm formation and composition. They reported that exopolymers are essential for cell-to-cell adhesion and for biofilm formation. Exopolymers are also responsible for cohesive forces within biofilms and biofilm stability.

Biofilm accumulation at surfaces is an autocatalytic process. Initial colonization increases surface irregularity and promotes further biofilm formation. Increased surface irregularity due to biofilm formation can influence particle transport and attachment rate by (1) increasing convective mass transport near the surface, (2) providing

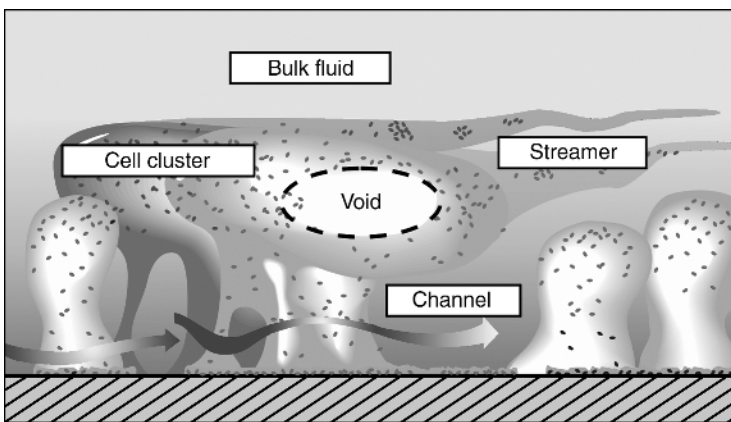


FIGURE 1-5 Conceptual illustration of the heterogeneity of biofilm structure, with labeled bacterial clusters, streamers, and water channels. (Lewandowski and Dirckx, 1996. Reprinted with permission from the Center for Biofilm Engineering at Montana State University, Bozeman.)

shelter from shear forces, and (3) increasing surface area for attachment. Growth is due to microbial replication and growth rate is traditionally described by Monod kinetics:

$$\mu = \frac{\mu_{\max} S}{K_s + S}$$

where μ_{\max} is the maximum specific growth rate (t^{-1}), K_s the half-saturation coefficient (mol L^{-3}), and S the substrate concentration (mol L^{-3}). Each species in the biofilm has its own optimum growth parameters.

Hydrodynamic shear stress, related to flow, influences transport, transfer, and reaction rates within the biofilm, as well as detachment. Detachment includes two processes: erosion and sloughing. Sloughing is the process in which large pieces of biofilm are rapidly removed, frequently exposing the surface. The reasons for biofilm sloughing are not well understood. Biofilm erosion is defined as continuous removal of single cells or small groups of cells from the biofilm surface and is related to shear stress at the biofilm–fluid interface. Frequent detachment is identified with erosion, especially in conduits. An increase in shear stress increases erosion rate and decreases biofilm accumulation rate. Empirical observations indicate that the erosion rate is related to biofilm thickness and density.

Influence of Conditioning Films

Many investigators have demonstrated that materials with diverse surface properties (e.g., wettability, surface tension, and surface charge) are rapidly conditioned by absorbing organics when exposed to natural waters. The impact of conditioning films on subsequent microbial attachment and growth has been the subject of extensive research and controversy. Poleunis et al. (2002) used surface analytical techniques to monitor the chemical composition and growth kinetics of the adsorbed layer on 316L stainless steel (UNS S31603) immediately after immersion in natural seawater. They reported successive adsorption of two types of compounds before any bacterial attachment—nitrogen-containing species (conjectured to be proteins) followed by carbohydrates. They monitored an increase in adsorbed material for 24 h. Even after a 24-h immersion, there was no continuous conditioning film on the surface. The authors indicated that even in the presence of adsorbed potential nutrients, the substratum influences are more important to bacterial adhesion than the conditioning film. Bradshaw et al. (1997) evaluated the influence of conditioning films on biofilm development using bacteria isolated from the oral cavity and concluded that conditioning films have a role in the degree and pattern of oral biofilm development. However, Ostuni et al. (2001) demonstrated that there is little or no correlation between adsorption of protein on surfaces and adhesion of bacteria. Busscher et al. (1997) concluded that a 1.5-h adsorbed salivary conditioning film appears to slow deposition of yeasts and some bacteria on silicone rubber. They reported that adhesion to silicone rubber is weaker with a salivary conditioning film

compared with the same surfaces without the conditioning film, that is, attached cells are easier to remove from conditioned surfaces.

Influence of the Substratum

The surface to which microorganisms attach, the substratum, plays a major role in biofilm processes during the early stages of biofilm accumulation and may influence the rate of cell accumulation and cell distribution. There is a vast literature investigating the influence of surface composition roughness, wettability, and polarization on the attachment of bacteria. It has been demonstrated that the composition of a metal substratum influences the formation rate and cell distribution of microfouling films in seawater during the first hours of exposure. Gerchakov et al. (1977) demonstrated that initial bacterial attachment is more rapid on glass and 304 stainless-steel (UNS S30400) surfaces compared to 60/40 copper-zinc brass (UNS C28000) and 90/10 copper-nickel (UNS C70600) surfaces (Figure 1-6). Hydrated oxide and hydroxide passivating films on

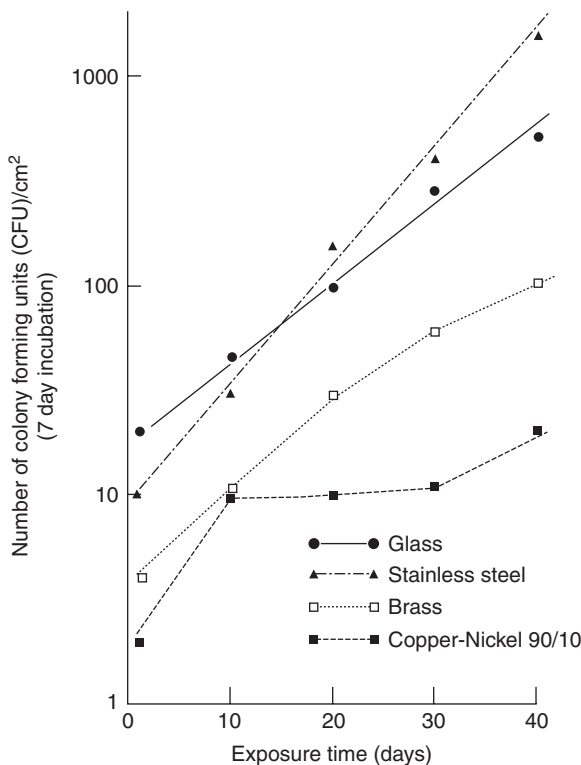


FIGURE 1-6 Numbers of marine heterotrophic bacteria cultured from various substrates in relation to exposure time on surfaces including glass, 304 stainless steel (UNS S30400), 60/40 copper-zinc brass (UNS C28000), and 90/10 copper-nickel (UNS C70600). (Gerchakov et al., 1977. Reprinted with permission of the author.)

metal surfaces provide bacteria with sites for firm attachment (Kennedy et al., 1976). Similarly, spalling or sloughing of corrosion products forces the detachment of the biofilm associated with corrosion products (Characklis et al., 1983).

Conflicting reports have been published regarding the influence of surface topography on microbial attachment. The term roughness is defined as the pattern or texture of surface irregularities that are inherent in the production process, excluding waviness and errors of formation. In some cases, higher surface roughness increased the extent of bacterial accumulation and adhesion took place at surface irregularities, whereas other authors found reduced adhesion to rougher surfaces. Nickels et al. (1981) demonstrated that the microbiota colonizing silica grains of the same size and water pore space but different microtopography show differences in biomass and community structure after 8 weeks of exposure to seawater. Absence of surface cracks and crevices resulted in a marked diminution of total biomass. Medilanski et al. (2002) used four bacterial species comprising three phyla with a variety of physiochemical characteristics to evaluate the influence of surface topography on colonization of UNS S30400. Five types of surface finishes corresponding to roughness values (R_a) between 0.03 and 0.89 μm were produced. Adhesion of all four bacteria was minimal at $R_a = 0.16 \mu\text{m}$, whereas smoother and rougher surfaces gave rise to more adhesion (Figure 1-7).

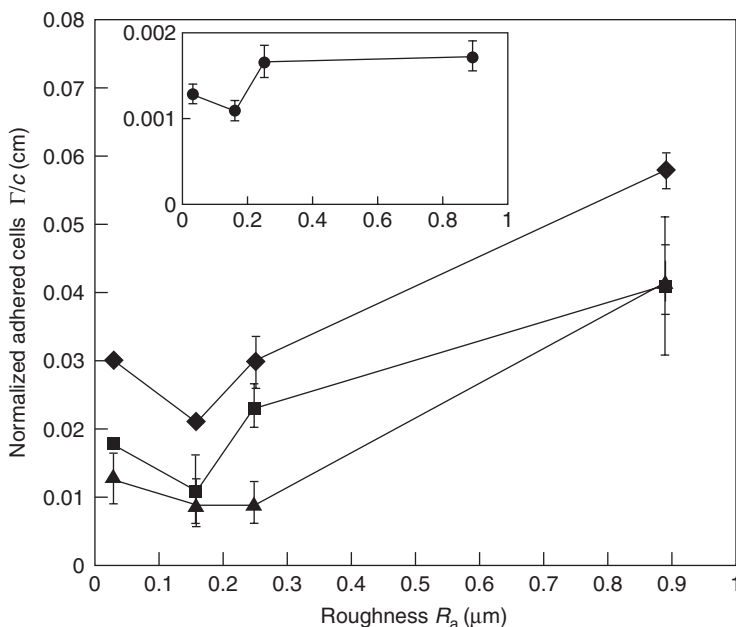


FIGURE 1-7 Normalized levels of adhesion of *Pseudomonas putida* mt2 (■), *Pseudomonas aeruginosa* PAO1 (◆), *Rhodococcus* sp. C125 (▲), and *Desulfovibrio desulfuricans* (●) on UNS S30400 stainless-steel surfaces of different roughness. (Reprinted from Medilanski et al., 2002, with permission from Taylor and Francis Ltd. <http://www.tandf.uk/journals>.)

The $R_a = 0.16 \mu\text{m}$ surface exhibited parallel scratches $0.7 \mu\text{m}$ in width, in which a high proportion of bacteria was aligned. This particular surface roughness corresponded to the approximate width of the cells, but was smaller than their length. Bacteria fit these scratches in longitudinal orientation only. The generally higher adhesion to the roughest surfaces may be due to the increased surface area. Reduced adhesion was attributed to unfavorable interactions between the surface and bacteria oriented other than parallel to the scratches. Interaction energy calculations and considerations of microgeometry confirmed this mechanism. Rougher surfaces exhibiting wider scratches allowed a higher fraction of bacteria to adhere in other orientations, whereas the orientation of cells adhered to smoother surfaces was completely random. Flint (1997) suggested that opposing observations regarding the influence of surface roughness on bacteria adhesion are probably related to the degree of surface roughness, the bacterial species tested, the physiochemical parameters of the surface, the bulk fluid phase under study, and the adhesion method used to detect bacteria. Korber et al. (1997) suggested that increased surface area provided at the microorganism–material interface might facilitate more film attachment by providing contact points. Little et al. (1988) demonstrated that porous welds provide increased sites for colonization compared to smooth pipe surfaces. Sreekumari et al. (2001) evaluated bacterial attachment to 304L stainless-steel (UNS S30403) weldments and the significance of substratum microstructure. They found that welded metal samples show more attachment while base metals show the least. The area of attachment was inversely proportional to the average grain size. Bacterial colonization started on grain boundaries. The weld area had more grains and more grain boundaries (Figure 1-8). Furthermore, the authors established a direct relationship between increased attachment and the onset of MIC. There is some evidence that nanometer roughness enhanced the adhesion of the conditioning layer to the substratum (Gold, 1999).

Verran and Boyd (2001) suggested that the surface roughness is also a factor in cell retention on surfaces. If the surface irregularities are much larger than the microorganisms, passive retention is minimal. Surface features on a nanometer scale impact cleanability of surfaces and surface defects on the microbiological scale may confer protection from shear forces in the surrounding environment (Figure 1-9).

Wiencek and Fletcher (1997) used self-assembled monolayers with a range of wettabilities to demonstrate that the greatest number of cells attached to hydrophobic surfaces. Armon et al. (2001) evaluated the impact of polarization on the adsorption of *Flavobacterium breve* (Figure 1-10) and *P. fluorescens* (Figure 1-11) to platinum, titanium (ASTM grade 2, UNS R50400), S31603 stainless-steel, copper (UNS C15000), aluminum alloy (UNS A95052), and carbon steel (UNS G10200). Maximal adsorption occurred in the potential range of -0.5 to 0.5 V (standard calomel electrode, SCE) for all metals. A shift of applied potential toward either the positive or negative direction caused a gradual decrease in bacterial adsorption.

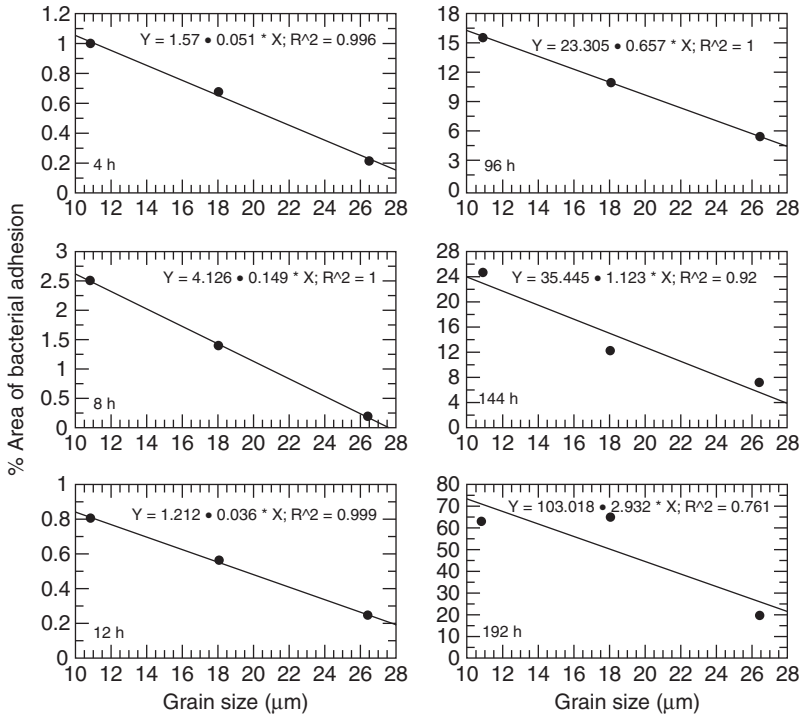


FIGURE 1-8 Results of regression analysis between the average grain size and the percentage areal cover of *Pseudomonas* sp. (Reprinted from Sreekumari et al., 2001, with permission from Taylor and Francis Ltd. <http://www.tandf.uk/journals>.)

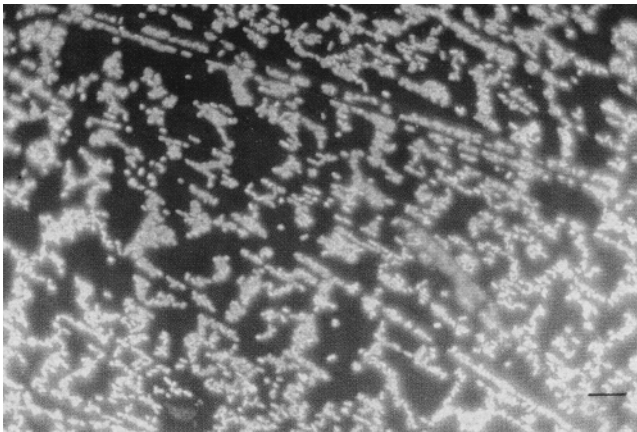


FIGURE 1-9 *Pseudomonas aeruginosa* (stained with acridine orange) retained in surface defects (scratches) on stainless steel, illustrating the role of surface defects (scratches) in cell retention. Bar = 10 μm. (Adapted from Verran and Boyd, 2001, with permission from Taylor and Francis Ltd. <http://www.tandf.uk/journals>.)

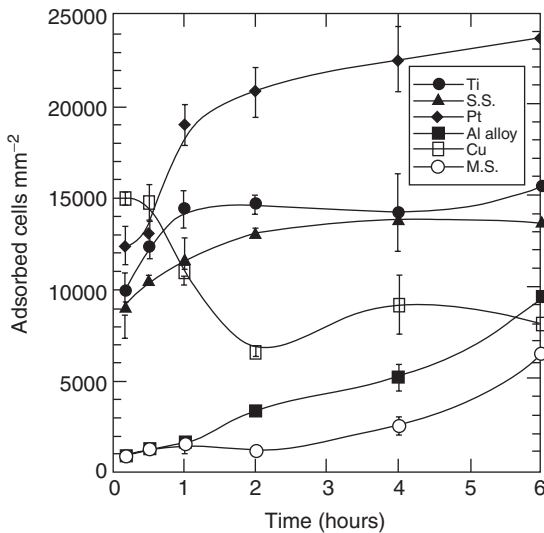


FIGURE 1-10 The effect of applied potential on the adsorption of *P. fluorescens* P17 on six different metal surfaces: platinum (Pt); UNS R50400 (ASTM grade 2) titanium (Ti); UNS 31603 stainless steel (SS); UNS C15000 copper (Cu); UNS A95052 aluminum alloy (Al); UNS G10200 carbon steel (MS). (Reprinted from Armon et al., 2001, with permission from Taylor and Francis Ltd. <http://www.tandf.uk/journals>.)

Influence of the Electrolyte

Electrolyte concentration, pH, and inorganic ions influence settlement. Fletcher (1988) found that an increase in the concentration of several cations in the electrolyte (sodium, calcium, lanthanum, and ferric iron) affects the attachment of *P. fluorescens* to glass surfaces, presumably by reducing the repulsive forces between the negatively charged bacterial cells and the glass surfaces. Energy derived from organic carbon drives heterotrophic microbial growth within biofilms. Starvation decreases adhesion of some species and does not affect others (Wienczek and Fletcher, 1997). Generally, increasing the total organic carbon (TOC) increases the substrate or carbon source available to the biofilm. Cowan et al. (1991) evaluated the influence of nutrient concentration on the colonization of glass substrata. They concluded that the ability of bacteria to colonize surfaces is to a large extent related to their ability to colonize the liquid phase and deposition of bacteria onto surfaces is positively correlated with the density of suspended cells. At high organic loadings, the substrate flux in the biofilm will reach a constant value as a result of one of the following events: (1) the growth rate of the microbial population in the biofilm reaches a maximum, (2) the thickness of the biofilm exceeds the penetration depth of the substrate into the biofilm, or (3) the electron acceptor or another nutrient becomes nutrient-limiting. Others have suggested that attachment to surfaces is a survival

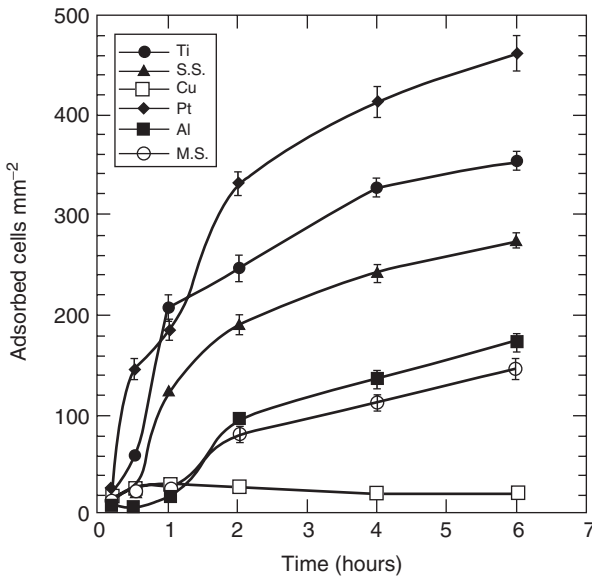


FIGURE 1-11 The effect of applied potential on the adsorption of *F. breve* on six different metal surfaces: platinum (Pt); UNS 31603 stainless steel (SS); UNS R50400 (ASTM grade 2) titanium (Ti); UNS C15000 copper (Cu); UNS G10200 carbon steel (MS); UNS A95052 aluminum alloy (Al). (Reprinted from Armon et al., 2001, with permission from Taylor and Francis Ltd. <http://www.tandf.uk/journals>.)

strategy and in oligotrophic waters cells attach to surface-associated organic matter. Organic carbon is present in all natural and processed waters, but its concentration can vary widely. For example, in distilled water TOC is 1 to 2 g m^{-3} ; 1 g m^{-3} in the seawater off the coast of Hawaii, 10 g m^{-3} in the Gulf of Mexico coastal water, 150 g m^{-3} in water produced in oil fields, and 200 g m^{-3} in untreated sewage. Biofilm formation has been documented over the widest possible range of substrate concentrations (Table 1-2) (Wimpenny, 1996). Biofilms form in highly oligotrophic waters including ultrapure water. At the other end of the spectrum, biofilms form on surfaces exposed to extremely high nutrient concentrations. Direct evidence indicates that at low nutrients, biofilms form as separate stacks or groups of cells around and through which water can move, and, in high nutrients, biofilms can appear to be dense and almost confluent. Azeredo and Oliveira (2000b) demonstrated that cells entrapped within thin biofilms are metabolically active within a relatively homogeneous matrix. Cells in the inner layer where nutrients are not limited produce a more heterogeneous, denser biofilm structure. In contrast, the inner layer of the thicker biofilm is metabolically inactive. Nutrient limitation in the deeper layer of thick biofilms is responsible for cell lysis and production of proteolytic enzymes.

Carbon is not always the growth-limiting nutrient for microorganisms. Phosphorus and nitrogen may be limiting in some aquatic systems. An electrolyte with a carbon–nitrogen ratio greater than 7:10 is considered nitrogen-limited for

TABLE 1-2 A Brief Survey of Biofilm Types in Relation to Nutrient Availability

Nutrient level	System
Extremely low	High purity water: granular activated carbon, reverse osmosis membrane, ion-exchange resin, degasifier unit, water storage tanks, microporous membrane filters. Water distribution and storage: water distribution pipes, domestic copper piping, storage tanks, oligotrophic stream, and river and lake epilithon
Low	Eutrophic water bodies Swimming-pool filters Domestic drains Car wash bottles Plant surfaces Phylloplane communities
Medium	Effluent treatment: trickling filter, anaerobic digester granules, rotating disc aerators, fluidized-bed reactors Production: membrane bioreactor, vinegar production Plant surfaces: rhizosphere
High	Food associated: food products (meat, etc.), food processing surfaces Animal surfaces: Oral surfaces—cheek, tongue, palate, epithelium, tooth surfaces Epithelia—gut, rumen, vagina, etc. Infection—lung, heart valves, etc. Prosthesis and catheters: pacemaker, metal plates, joints, heart, heart valve stents, indwelling catheters, etc. Contact lenses

Source: Reprinted from Wimpenny, 1996, with permission from Taylor and Francis Ltd. <http://www.tandf.uk/journals>.

microbial growth. Cells growing in such a medium tend to reproduce slowly and produce copious amounts of EPS (Wilkinson, 1958). Biofilm thickness has been shown to increase with increasing carbon–nitrogen ratios (Bott and Gunatillaka, 1983). McEldowney and Fletcher (1988) demonstrated that the carbon source can influence the adhesive qualities of cells.

Bulk water temperature influences the rate of most chemical and biochemical reaction processes as well as transport processes within the biofilm. Biofilm formation is generally considered to be more of a problem in the summer months because higher temperatures increase the rate of biological processes.

SUMMARY

Biofilms form on all engineering materials exposed in biologically active environments. They form compliant surfaces that actively interact with the hydrodynamic boundary layer (Figure 1-12), and can form in extreme environments such as

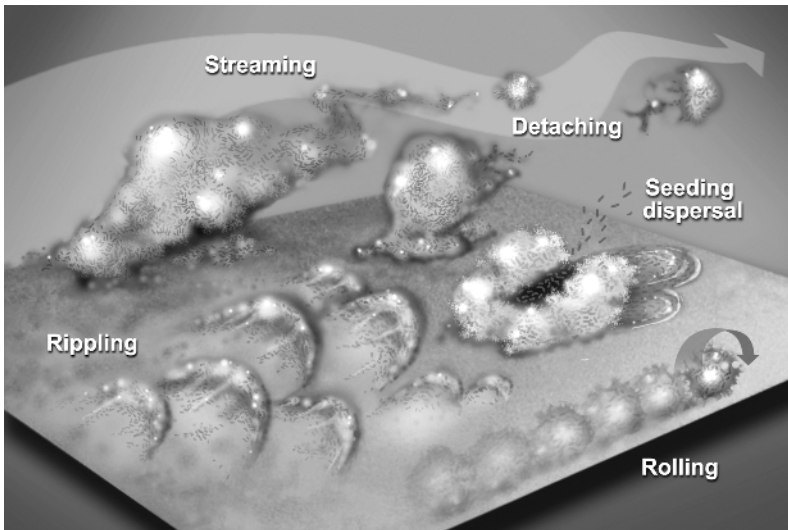


FIGURE 1-12 Biofilm bacteria can move in numerous ways: collectively by rippling or rolling across a surface, or by detaching in clumps or streaming, and individually, through a “swarming and seeding” dispersal. (Stoodley and Dirckx, 2003b. Reprinted with permission from the Center for Biofilm Engineering at Montana State University, Bozeman.)

ultrapure waters (McFeters et al., 1993; Kulakov et al., 2002) or highly radioactive conditions. Lewandowski (1998) hypothesized that biofilm development optimizes survival of the biofilm constituents and maximizes transport of nutrients into the biofilms. Biofilms also provide protective environments for bacteria and in most cases allow different types of bacteria to flourish within different strata of the biofilm (Figure 1-13) (Harrison et al., 2005). For example, obligate anaerobic bacteria are routinely isolated from oxygenated environments in association with other bacteria that effectively remove oxygen from the immediate vicinity of the anaerobe.

Bacteria within the biofilm act symbiotically to produce conditions more favorable for the growth of each species. Bacteria near the fluid phase are provided with complex nutrients and oxygen. These bacteria use oxygen, break down carbon sources, and produce simple polymers and fatty acids. Bacteria within the biofilm, removed from the bulk phase, use waste products generated by other bacteria as nutrients that are metabolized to fatty acids, carbon dioxide, and hydrogen. Cole (1982) reviewed the interaction between bacteria and algae in aquatic systems. Haack and McFeters (1982) demonstrated a flux of dissolved algal products into heterotrophic bacteria. The cycling of carbon and oxygen in algal bacterial aggregates and biofilms has also been observed in the marine environment (Azam and Ammerman, 1984). Successive stages of degradation depend on the chemistry of the liquid phase and the bacterial species. For example, acetogenic bacteria can convert

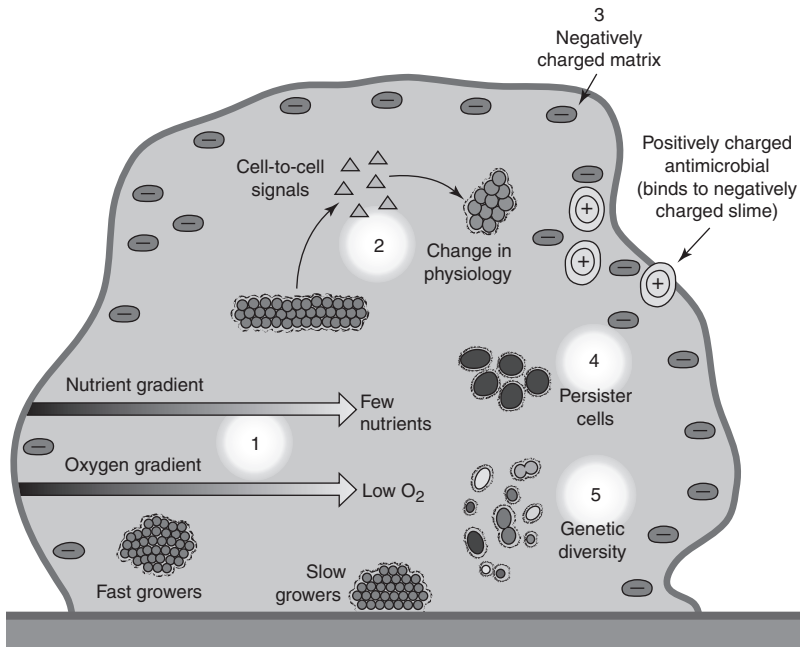


FIGURE 1-13 Biofilms derive their extraordinary tolerance to antimicrobial compounds from several factors. Bacteria near the center of a microcolony grow very slowly because they are exposed to lower concentrations of oxygen and nutrients (**1**). They are thus spared the effects of antibiotic drugs, which are much more effective against fast-growing cells. Intercellular signals (**2**) can alter the physiology of the biofilms, causing members to produce molecular pumps that expel antibiotics from the cells and allow the community to grow even in the presence of a drug. The biofilms matrix is negatively charged (**3**) and so binds to positively charged antimicrobials, preventing them from reaching the cells within the colony. Specialized populations of persister cells (**4**) do not grow in the presence of an antibiotic, but neither do they die. When the drug is removed, the persisters can give rise to a normal bacterial colony. This mechanism is believed to be responsible for recurrent infections in hospital settings. Finally, population diversity (**5**), genetic as well as physiological, acts as an “insurance policy,” improving the chance for some cells to survive any challenge. (Harrison et al., 2005. Reprinted with permission of the authors.)

nonfermentable compounds into acetic acid and hydrogen. Other microorganisms can consume acetate and hydrogen.

It is extremely difficult to predict the impact of biofilms on degradation processes. Cells within biofilms may be viable but nonculturable (Oliver, 1993; del Mar Lleó et al., 2000), making it impossible to detect their presence with traditional culture techniques. Microorganisms within biofilms are capable of maintaining environments at biofilm–surface interfaces that are radically different from the bulk in terms of pH, dissolved oxygen, and other organic and inorganic species. In some cases, these interfacial conditions could not be maintained in the bulk medium at room temperature near atmospheric pressure. As a consequence, microorganisms

within biofilms produce reactions that are not predicted by thermodynamic arguments based on the chemistry of the bulk medium.

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