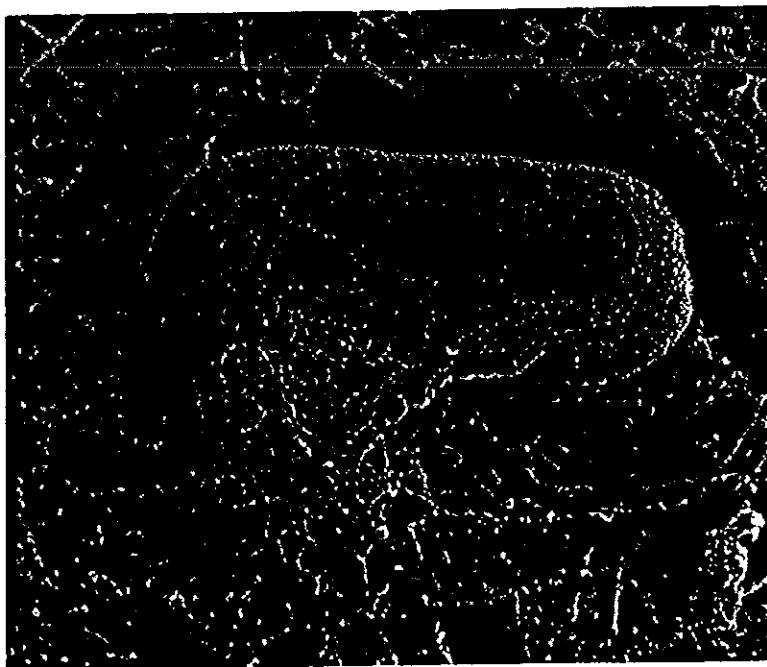




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GEOMICROBIOLOGY:
INTERACTIONS BETWEEN
MICROBES AND MINERALS

J.F. BANFIELD & K.H. NEALSON
Editors



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Chapter 4

SPATIAL RELATIONSHIPS BETWEEN BACTERIA AND MINERAL SURFACES

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INTRODUCTION

In aquatic environments, microbial cells attach to solids, including minerals and metals, and initiate biomineralization reactions. "Immobilized cells grow, reproduce and produce extracellular polymers which frequently extend from the cell forming a tangled matrix of fibers which provide structure to the assemblage termed a biofilm" (Characklis and Marshall 1990). 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 minerals and mineral replacement reactions that are not predicted by thermodynamic arguments based on the chemistry of the bulk medium.

While it has been established that the most devastating microbiologically influenced corrosion (MIC) takes place in the presence of microbial consortia in which many physiological types of bacteria, including sulfate-reducing bacteria (SRB), acid-producing bacteria, metal-oxidizing bacteria and metal-reducing bacteria (MRB), interact in complex ways within the structure of biofilms (Fig. 1) (Pope et al. 1984, Little et al. 1991), the realization that biomineralization takes place within biofilms has received inadequate attention. Spatial relationships of microorganisms to minerals has been studied to a limited extent as related to MIC. Microorganisms influence corrosion by both forming and dissolving minerals. Biomineralization that results in mineral deposition on a metal surface can shift the corrosion potential in either a positive or negative direction, depending on the nature of the mineral. Manganese oxide biodeposition on stainless steel surfaces forces a shift in the positive, more noble direction, moving the corrosion potential above the pitting potential and making some stainless steels more vulnerable to pitting and crevice corrosion. Bioprecipitated sulfides decrease hydrogen overvoltage at cathodic sites and stimulate the cathodic reaction so that sulfide formation on all metal surfaces moves the corrosion potential in a negative, more active direction, resulting in accelerated corrosion of some metals and alloys. Iron oxide formation can initiate a sequence of events that results in underdeposit corrosion of susceptible metals. Biomineral dissolution reactions remove passive layers or force mineral replacement reactions that lead to further dissolution. In the following sections biofilm formation and spatial relationships of microorganisms and minerals as related to corrosion will be described.

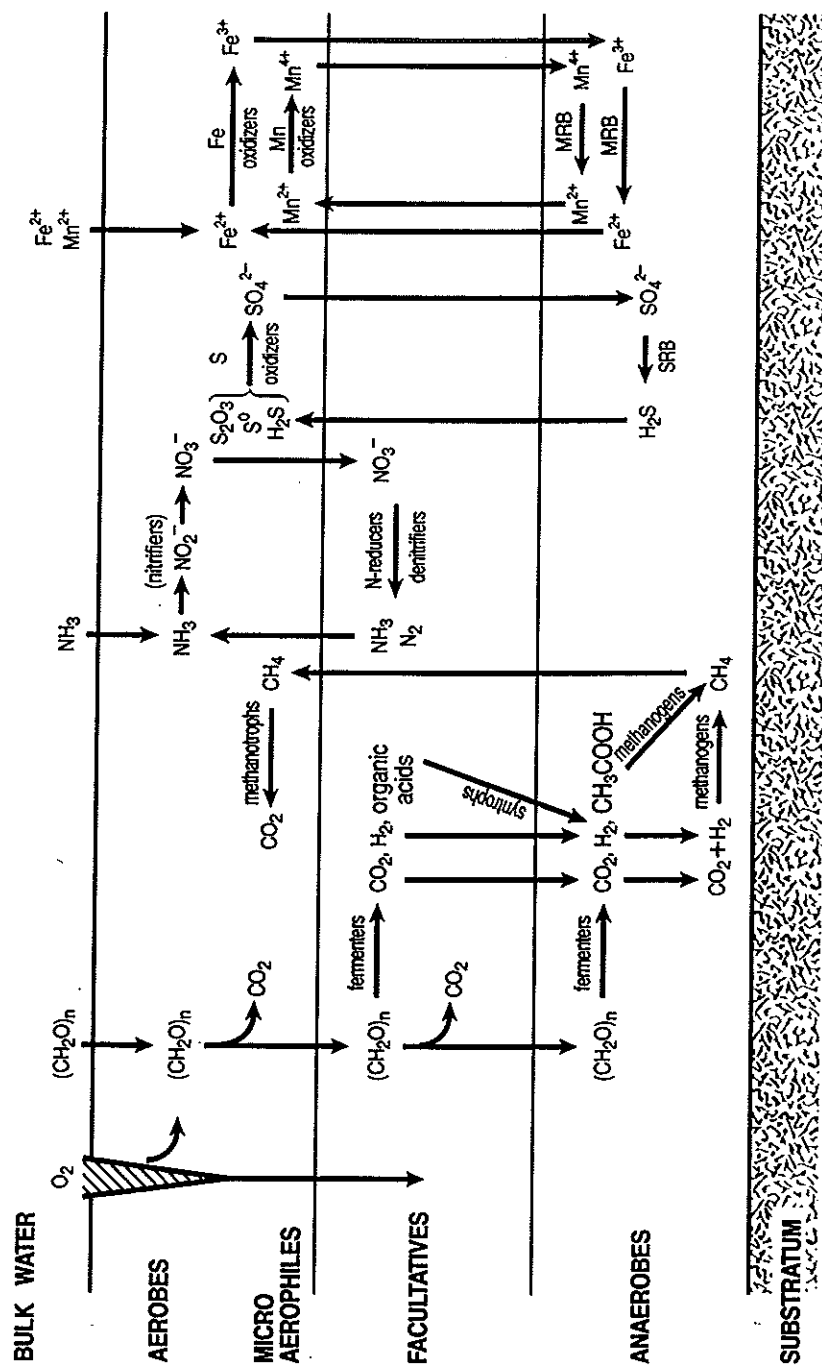


Figure 1. Strata within a typical biofilm and possible reactions within the strata.

OVERVIEW OF BIOFILM FORMATION

Initial events of biofilm formation

Biofilm formation consists of a sequence of steps and begins with adsorption of macromolecules (proteins, polysaccharides, and humic acids) and smaller molecules (fatty acids, lipids) at interfaces, including liquid/solid and gas/liquid interfaces. The following sections deal with specifics of biofilm formation on solid substrata. Adsorbed molecules form conditioning films which alter physico-chemical characteristics of the interface (Chamberlain 1992, Marshall et al. 1994). Conditioning films change surface hydrophobicity and influence surface electrical charge. Both parameters have proven effects on the extent and kinetics of microbial attachment. Surface charge may change because of the conditioning film and/or because of adsorption of ions from solution (Loeb and Neihof 1976). The amount of adsorbed organic material is a function of ionic strength, and can be enhanced on metal surfaces by polarization (Little 1985).

During initial stages of biofilm formation the major factor controlling rate of colonization is hydrodynamics (Duddridge et al. 1982). Microbial colonization begins with transport of microorganisms to the interface mediated by at least three mechanisms: (1) diffusive transport due to Brownian motion, (2) convective transport due to liquid flow, and (3) active movement of motile bacteria near the interface (van Loosdrecht et al. 1990). 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 number adhering to a surface to the cell number transported to this surface is termed "sticking efficiency." Sticking efficiency depends on many factors including surface properties, physiological state of organisms, and hydrodynamics near the surface (Escher and Characklis 1990).

Biofilm accumulation

Microbial cells transported with the stream of fluid above the surface interact with the conditioning films. Immediately after attachment, microorganisms initiate production of slimy adhesive substances, predominantly exopolysaccharides (EPS). Although the association of EPS with attached bacteria has been well documented (Fletcher and Floodgate 1973) there is no direct evidence suggesting that EPS participates in initial stages of adhesion. However, EPS definitely assists the formation of microcolonies and microbial films (Allison and Sutherland 1987). Recent genetic studies have clearly shown that adhesion to surfaces triggers the expression of several genes controlling polymer synthesis in *Pseudomonas* (Davies and Geesey 1995). According to Silverman et al. (1984) synthesis of adhesive substances is genetically controlled and influenced by environmental factors. For example, Little et al. (1997) documented copious EPS production by *Shewanella putrefaciens* grown on manganese oxide (birnessite), and absence of polymer when the same organism in the same medium was grown on iron oxides (hematite, goethite or ferrihydrite) (Fig. 2a,b). EPS bridges microbial cells with the substratum and permits negatively charged bacteria to adhere to both negatively and positively charged surfaces. EPS may also control interfacial chemistry at the mineral/biofilm interface.

Biofilm accumulation at surfaces is an autocatalytic process. Initial colonization increases surface irregularity and promotes further biofilm formation. Bouwer (1987) pointed out that 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 shelter from shear forces, and (3) increasing surface area for attachment. Biofilm accumulation is the net result of the following microbial processes: attachment,

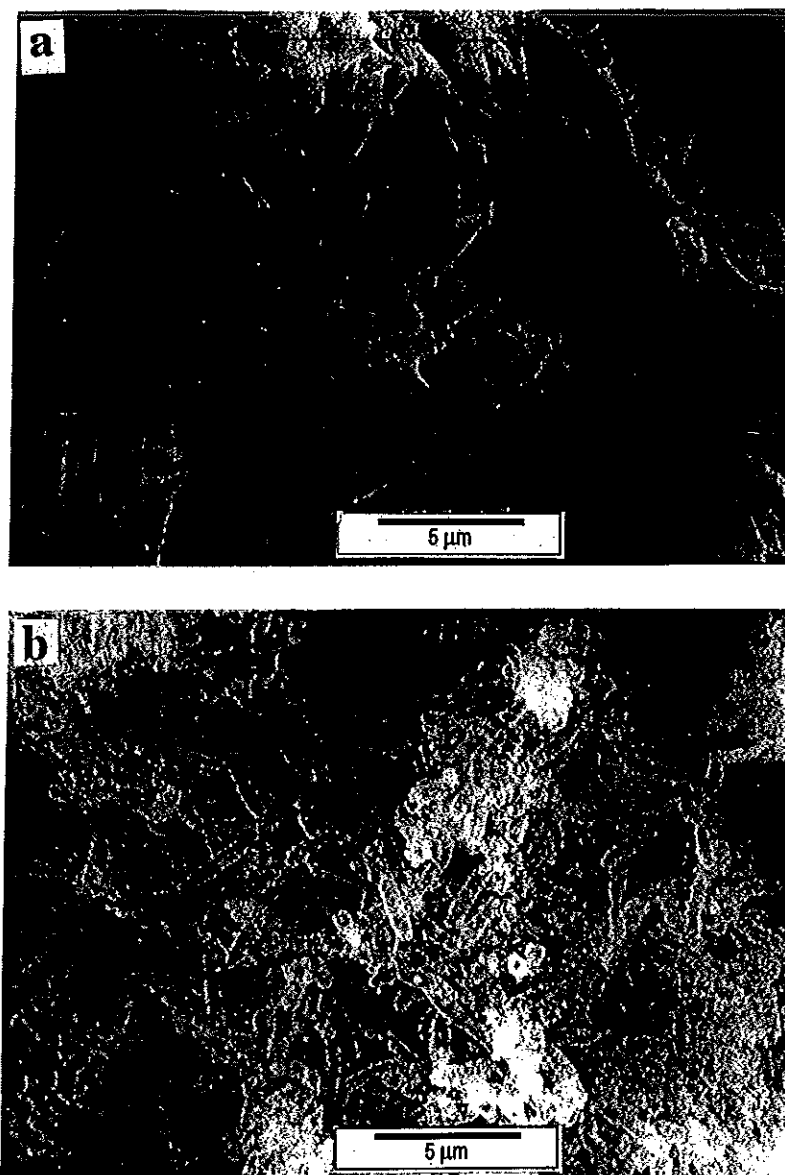


Figure 2. Mineral surfaces after exposure to *S. putrefaciens* (a) manganese oxide (birnessite) cells obscured by EPS, (b) iron oxide (goethite), no visible EPS.

growth, decay, and detachment (Fig. 3). More rigorous treatments introduce adsorption and desorption (Bryers and Characklis 1992, Escher 1986). However, 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. Growth is due to microbial replication and growth rate is traditionally described by Monod kinetics:

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

where $\mu_{\max}$ = maximum specific growth rate (t^{-1}), K_s = half saturation coefficient (mole L^{-3}), S = substrate concentration (mole L^{-3}). Each species in the biofilm has its own growth parameters. Growth rate in biofilms may depend on the spatial position of

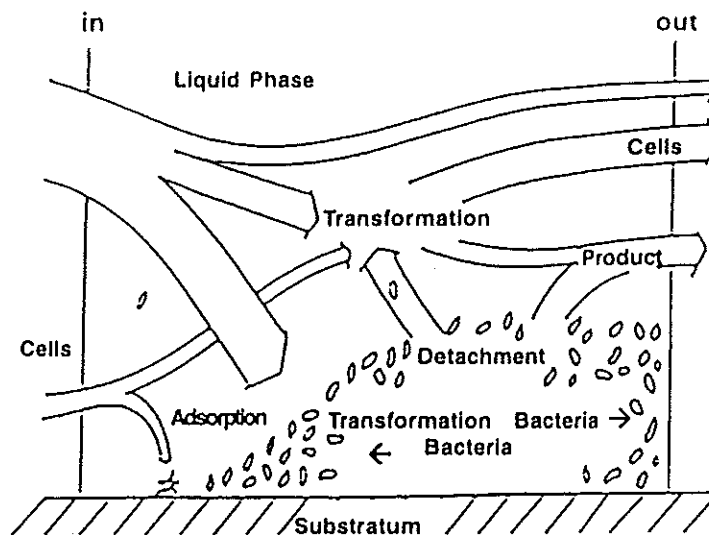


Figure 3. A composite of processes contributing to biofilm accumulation (WG Characklis, "Biofilm Processes," in *Biofilms*. ©1990 Reprinted by permission of John Wiley & Sons.)

microorganisms, unlike in suspended growth reactors. Assumption that all microorganisms of the same species have the same growth parameters in biofilms appears to be overly simplistic. Instead there is a spatial distribution of growth parameters.

Detachment includes two processes: erosion and sloughing. Sloughing is the process in which large pieces of biofilm are rapidly removed, frequently exposing the surface. 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. Frequently detachment is identified with erosion, especially in conduits. An increase in shear stress increases erosion rate and decreases biofilm accumulation rate. Empirical observations indicate erosion rate is related to biofilm thickness and density.

Biofilm architecture

When biofilm populations in aquatic ecosystems are imaged by light and electron microscopy, they appear to be composed of bacterial cells enclosed in an EPS matrix of uniform thickness and consistency (Costerton et al. 1987). Conceptual and numerical models have traditionally treated biofilms as a layer of matrix material within which bacterial cells are randomly distributed, and these models appear to be predictive of reaction rates in bioreactors (Wanner and Gujer 1986, Rittmann and Manem 1992). With time, however, it has become apparent that multi-species biofilms form highly complex structures containing voids, channels, cavities, pores and filaments, with cells arranged in clusters. Such complex structures have been reported in a wide variety of biofilms including methanogenic films from fixed-bed reactors (Robinson et al. 1984), aerobic films from wastewater plants (Eighmy et al. 1983, Mack et al., 1975), nitrifying biofilms (Kugaprasatham et al. 1992), and pure culture biofilms of *Vibrio parahaemolyticus* (Lawrence et al. 1991) and *Pseudomonas aeruginosa* (Stewart et al. 1993). High resolution confocal laser scanning microscopy (CLSM), coupled with the sophisticated image analysis (Wilson 1990, Caldwell et al. 1992) produced detailed images of the intrabiofilm environment. Lawrence et al. (1991) presented images of a complex biofilm architecture in which cells grow in matrix-enclosed microcolonies separated by water-filled voids. This

microcolony/void structure of biofilms was confirmed by Keevil and Walker (1992) who noted that the microcolonies formed stacks that extend as many as 500 micrometers away from the colonized surface. Wolfaardt et al. (1994) described this same architecture in biofilms formed by microbial populations in natural ecosystems.

Studies of the structure of biofilms using CLSM followed by studies of flow near microbially colonized surfaces using nuclear magnetic resonance imaging (NMRI), (Lewandowski et al. 1992, 1993a, 1994) and CLSM (de Beer et al. 1994a,b; Stoodley et al. 1994) delivered detailed information on the structure of biofilms and the nature of water flow in biofilm systems. The conceptual image of biofilms became much more complex (Massol-Deya et al. 1995, Gjaltema et al. 1994, Zhang and Bishop 1994 a,b) than the uniform layer with imbedded microorganisms that dominated the early studies. Lewandowski and Stoodley (1995) presented a concept of an intricate interplay between hydrodynamics and viscoelastic biopolymers leading to formation of "streamers" and characteristic biofilm matrix oscillation. Using NMRI, Lewandowski et al. (1993b) demonstrated that water flows through biofilms. This observation was corroborated and quantified by tracking fluorescent beads (0.2 μm diameter) through pores in a biofilm (Fig. 4) using CLSM (Stoodley et al. 1994, de Beer et al. 1994b). These and other experiments led the authors to conclude that biofilms form compliant surfaces which actively interact with the hydrodynamic boundary layer. It is now generally accepted that microorganisms in biofilms are aggregated in cell clusters or microcolonies separated by interstitial voids (Fig. 5) (Costerton et al. 1995). The new conceptual model assumes an inherent biofilm heterogeneity and constitutes the foundation for studying biofilm structure and the consequences of this structure to physical and chemical microenvironments. It has been speculated that biofilm structure represents an optimal arrangement for influx of nutrients but no direct evidence has been presented. There is no doubt, however, that interstitial voids participate in supplying nutrients to deeper layers of biofilms (Lewandowski et al. 1995).

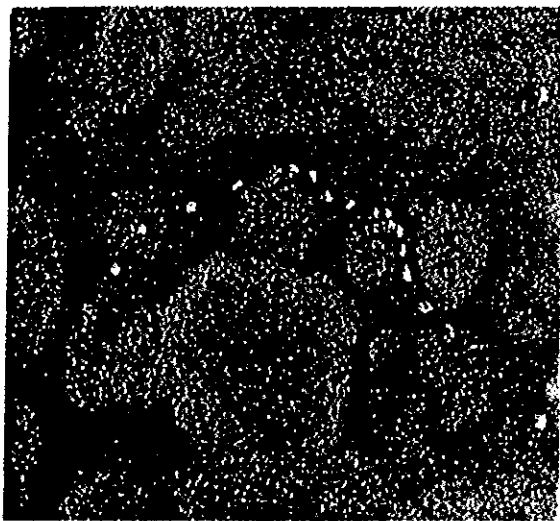


Figure 4. Superimposed time sequence image of an autofluorescing bead moving through a biofilm channel (Stoodley et al. 1994).

SPATIAL RELATIONSHIPS BETWEEN BIOFILMS AND MINERALS

Spatial relationships between bacteria and metal corrosion products have been investigated because of the economic impact to industry and military. MIC has been documented for metals exposed to seawater, fresh water, demineralized water,

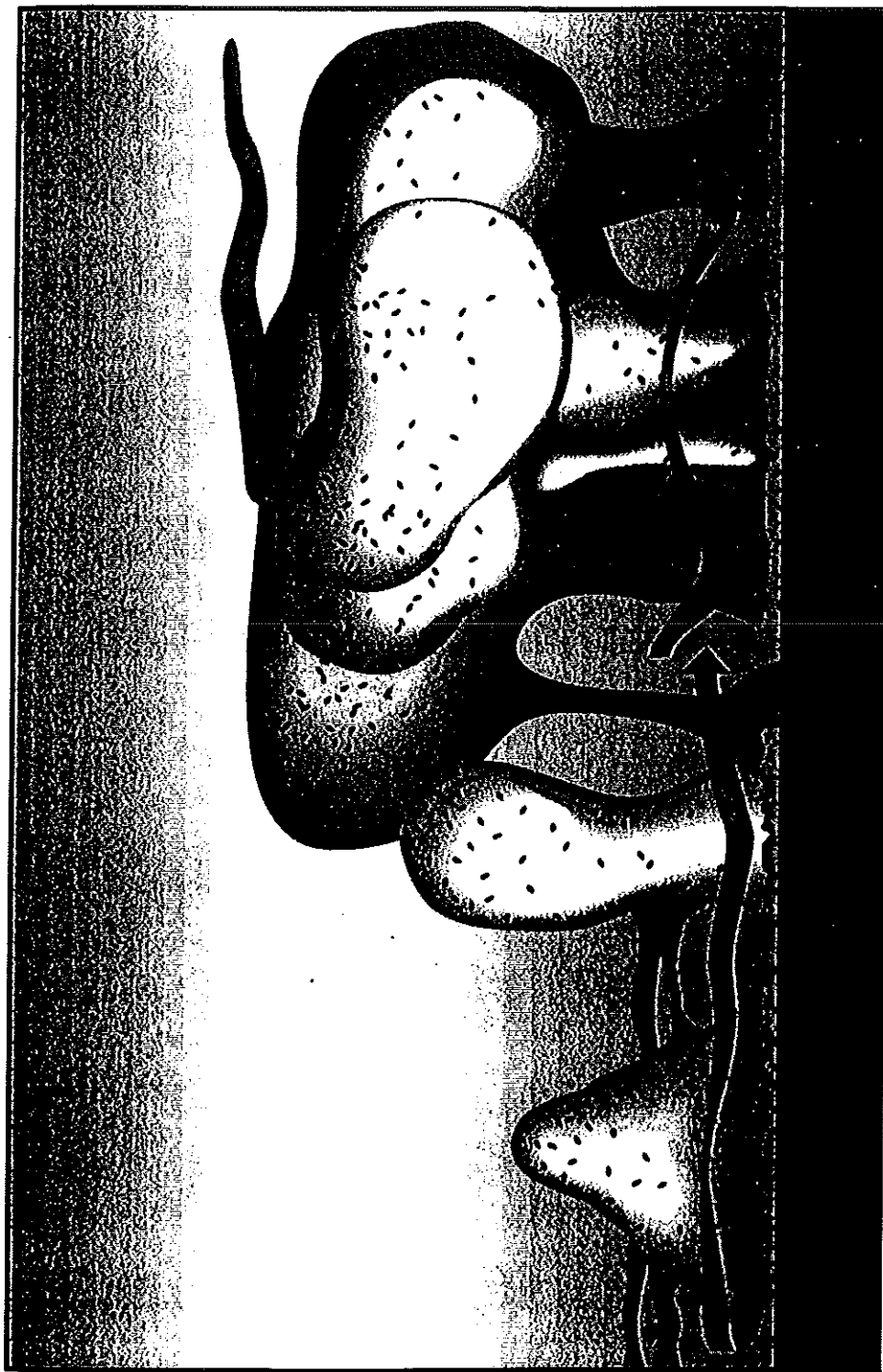


Figure 5. Model of a biofilm showing microbial microcolonies and interstitial voids filled with water. Arrows indicate convective flow (Lewandowski et al. 1995).

process chemicals, food stuffs, soils, aircraft fuels, human plasma, and sewage. The following is a brief introduction to corrosion terms (Uhlig and Revie 1985) that will be used throughout this chapter.

A metal surface is a composite of electrodes electrically short-circuited through the body of the metal. As long as the metal remains dry, local-action current and corrosion are not observed. But on exposure of the metal to water or aqueous solutions, local electrochemical cells are established and are accompanied by chemical conversion of the metal to corrosion products. The electrode at which chemical oxidation occurs is called the anode and the electrode at which reduction takes place is called the cathode. Corrosion of metals usually occurs at the anode. When a specimen is in contact with a corrosive liquid the specimen assumes a potential (relative to a reference electrode) termed the corrosion potential, E_{corr} . A specimen at E_{corr} has both anodic and cathodic currents present on its surface. However, these currents are exactly equal in magnitude so no net current can be measured. The specimen is at equilibrium with the environment even though it may be visibly corroding. E_{corr} can be defined as the potential at which the rate of oxidation is exactly equal to the rate of reduction.

An important concept that will be discussed in following sections is that of passivity. Passivity can be defined as the loss of chemical reactivity exhibited by certain metals and alloys under specific environmental conditions. That is, metals and alloys such as chromium, iron, nickel, titanium, and alloys containing these elements, become passive or essentially inert and act as if they were noble metals. Passivity is due to the formation of a surface film which acts as a barrier to further corrosion. Pitting potential is that potential required to allow penetration of the passive film. Eventually, as potential increases in the noble direction either the oxide is undermined by condensation of migrating vacancies, or cations of the oxide undergo dissolution at the electrolyte interface. In the absence of aggressive anions in the electrolyte, defects in the passive film can heal or repassivate (Szlarska-Smialowska 1986).

Experimental evidence indicates that microbial colonization changes the properties of metal surfaces and, in some cases, makes them more susceptible to corrosion. In an experiment conducted by Pendyala (1996), stainless steel coupons were exposed for 18 days to a biofilm consisting of three species: *Klebsiella pneumoniae*, *Pseudomonas fluorescens* and *Pseudomonas aeruginosa*. Elemental and chemical composition of the surface was analyzed by X-ray photoelectron spectroscopy (XPS) and other surface sensitive spectroscopies. Figure 6a and 6b show changes in the composition of the passive film resulting from microbial colonization. The most dramatic differences are seen in the first 50 Å. Comparison of elemental composition of the passive layer of materials as-received (same as the control) and after the 18-day exposure to microorganisms indicates that microbial action depleted the relative concentrations of chromium and nickel by ~10%.

Mineral deposition

Oxides. Biomineralization of iron and manganese oxides occurs widely in natural waters, and is a dominant control in geochemical cycling of these elements (Gounot 1994). Mineralization can be carried out by a variety of organisms including bacteria, yeast, and fungi (Nealson et al. 1988), but is particularly associated with genera of the so-called iron and manganese bacteria, *Siderocapsa*, *Gallionella*, *Leptothrix*, *Sphaerotilus*, *Crenothrix*, and *Clonothrix*.

Manganese. Manganese oxidation is coupled to cell growth and metabolism of heterotrophic substrates (Arnold et al. 1988, Jung and Schweisfurth 1976a,b). While the

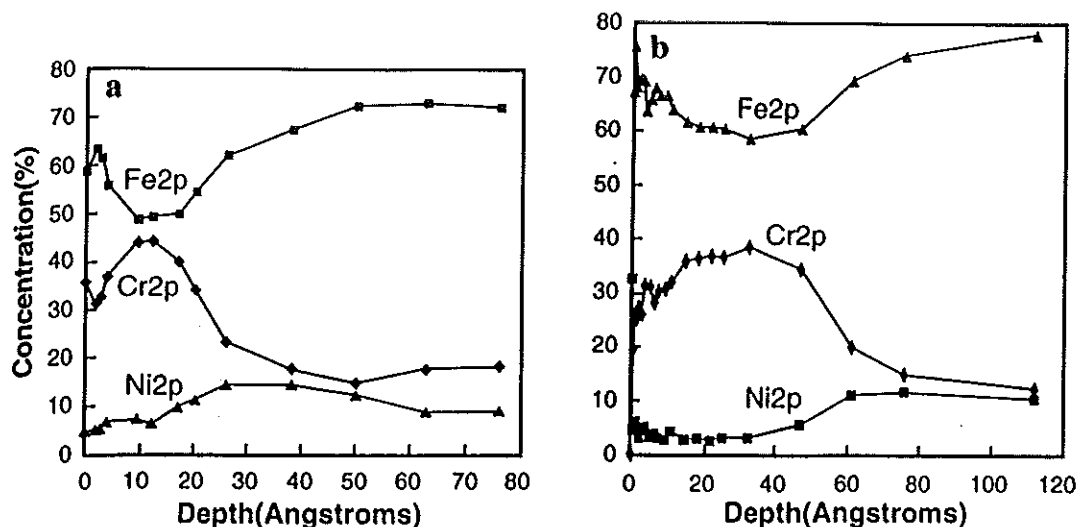


Figure 6. Depth profiles of normalized iron, chromium, and nickel concentrations in a stainless steel (a) as received and (b) after 18 days exposure to biofilm. (Courtesy of J. Pendyala and R. Avci, Dept. Physics, Montana State University.)

reduced form of manganese, generally identified as Mn^{2+} is soluble, all the various oxidized forms, Mn_2O_3 , $MnOOH$, Mn_3O_4 , MnO_2 , are insoluble. Microbially deposited manganese oxides have an amorphous structure as MnO_2 (vernadite) and sometimes form a black precipitate of MnO_2 (birnessite) found with *Leptothrix* and spores of *Bacillus* spp. (Gounot 1994). In the *Bacillus*, however, birnessite recrystallizes to octahedral Mn_3O_4 (hausmannite) (Nealson et. al. 1988). The relationship of manganese-depositing bacteria with MnO_2 can be demonstrated with X-ray microscopy. In Figure 7 a *Pseudomonas*-like



Figure 7. X-ray micrograph of manganese-depositing bacterium with Mn-oxides. Cells are dark ellipses, oxides are lighter material. (An unpublished micro-graph from the Center for X-ray Optics, Lawrence Berkeley Laboratories, Berkeley, CA, produced by B. Tonner, W. Meyer-Ilse & J. Brown from samples provided by K. Nealson, University of Wisconsin, Milwaukee, WI.)

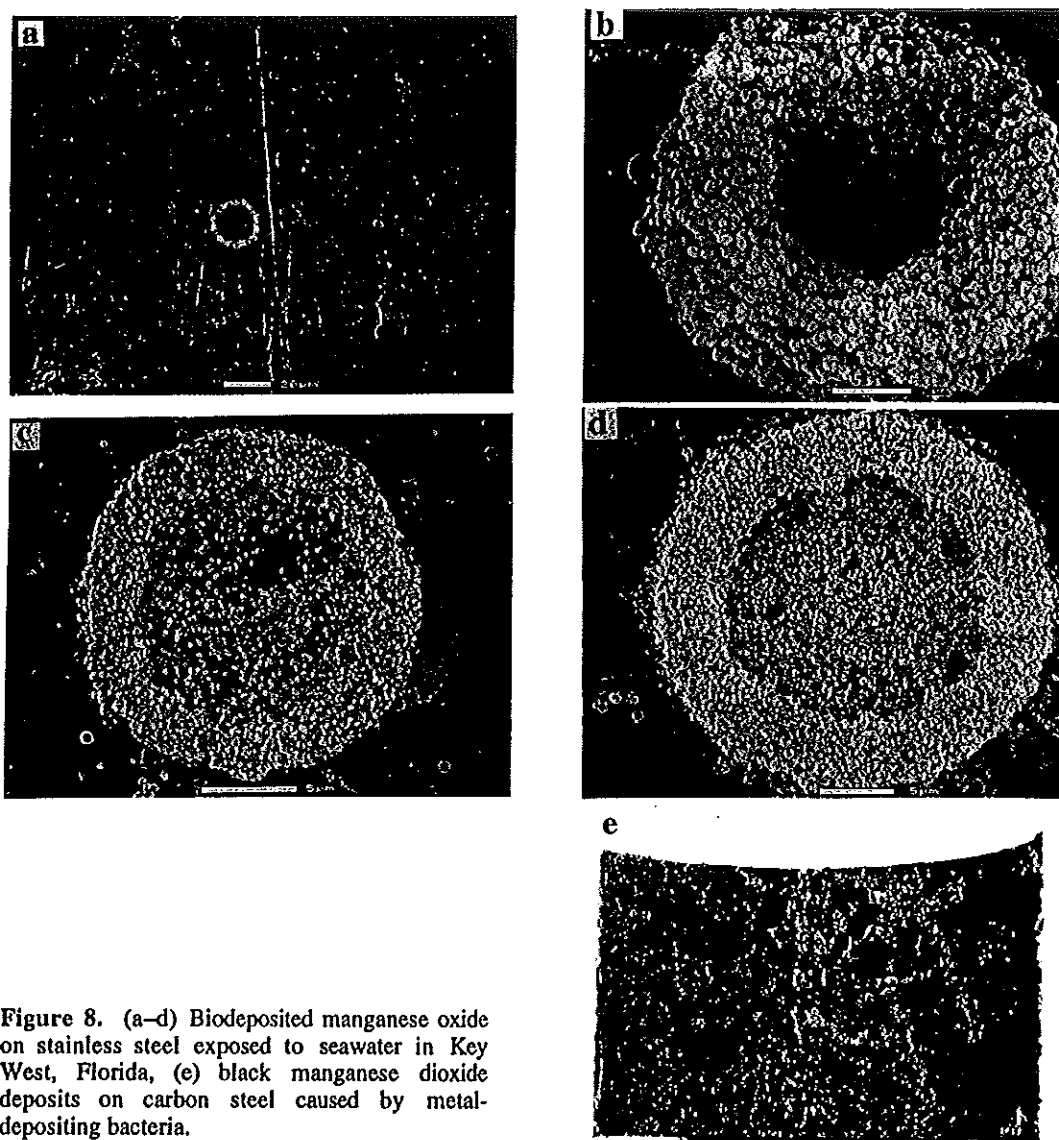


Figure 8. (a–d) Biodeposited manganese oxide on stainless steel exposed to seawater in Key West, Florida, (e) black manganese dioxide deposits on carbon steel caused by metal-depositing bacteria.

organism, originally isolated from freshwater (Jung and Schweisfurth 1976a,b), is imbedded in the manganese oxides it produced. As a result of microbial action, manganese oxide deposits are formed on submerged materials including metal, stone, glass, and plastic and can occur in natural waters with manganese levels as low as 10 to 20 ppb (Dickinson et al. 1996). Deposition rates of $1 \text{ mcoul cm}^{-2} \text{ day}^{-1}$ on stainless steel have been observed (Dickinson and Lewandowski 1996). Mature manganese deposits from both fresh water and marine sources can be identified as ring structures that become more and more numerous and dense with time (Fig. 8a–d). In other instances, surfaces become covered with manganese dioxide deposits (Fig. 8e).

It has been demonstrated that microbially deposited manganese oxide on a stainless steel (Dickinson and Lewandowski 1996) in fresh water (Fig. 9 a–c) caused an increase in E_{corr} and increased cathodic current density at potentials above -200 mV (vs saturated calomel reference electrode (SCE)). For mild steel corrosion under anodic control, the oxides can elevate corrosion current, but will cause little positive shift in E_{corr} . The increase in corrosion current may be significant, particularly for mild steel covered with

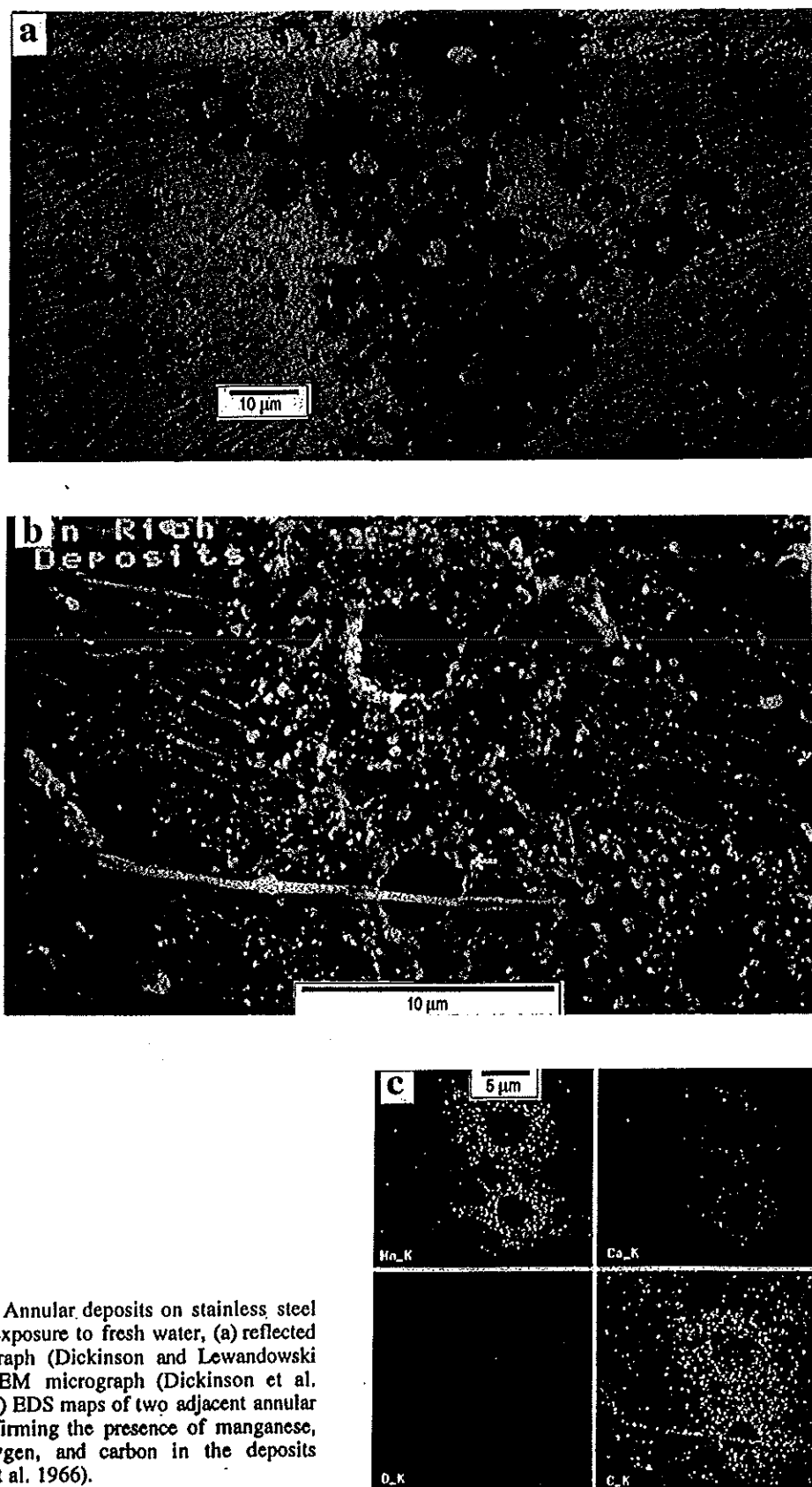


Figure 9. Annular deposits on stainless steel after 13-day exposure to fresh water, (a) reflected light micrograph (Dickinson and Lewandowski 1966), (b) SEM micrograph (Dickinson et al. 1966), and (c) EDS maps of two adjacent annular deposits confirming the presence of manganese, calcium, oxygen, and carbon in the deposits (Dickinson et al. 1966).

biomineralized oxides and tubercles that provide large mineral surface areas. Given sufficient conductivity in the tubercle, much of this material may serve as an oxide cathode to support corrosion at the oxygen-depleted anode within the tubercle. Continued biomineralization within the large tubercle may sustain a significant amount of the cathodic current. Both factors can increase the risk of stainless steel corrosion. Ennobled E_{corr} can exceed pitting potentials for low molybdenum alloys, enhancing risk of pit nucleation, while elevated cathodic current density impedes repassivation. Biomineralized manganic oxides are efficient cathodes and increase cathodic current density on stainless steel by several decades at potentials between roughly -200 and $+400$ mV_{SCE}. The extent to which the elevated current density can be maintained is controlled by the electrical capacity of the mineral reflecting both total accumulation and conductivity of the mineral-biopolymer assemblage (only material in electrical contact with the metal will be cathodically active). Oxide accumulation is controlled by the biomineralization rate and the corrosion current, in that high corrosion currents will discharge the oxide as rapidly as it is formed. This variation in accumulation causes the oxides to exert different modes of influence on the corrosion behavior of active metals compared with passive metals.

While biomineralized manganic oxides are expected to elevate corrosion current on mild steel, on passive metals they serve primarily to initiate localized attack. Passive metal corrosion currents of the order 10 nA cm^{-2} allow biomineralized material to accumulate. E_{corr} then shifts in the noble direction as increasing areal coverage anodically polarizes the metal. E_{corr} may exceed the pitting potential for low molybdenum alloys in dilute chloride media, increasing the risk of pit nucleation. Once nucleation occurs, cathodic current sustained by the MnO_2 cathode impedes repassivation by holding the corrosion potential above the protection potential. More available cathode material will support a greater number of pitting sites, increasing the probability that a metastable site will become fixed. Localized corrosion current that exceeds the biomineralization rate will discharge the oxide cathode so that eventually the corrosion rate becomes limited by the oxide biomineralization rate and by availability of other cathodic reactants (typically dissolved oxygen).

Iron. Iron-depositing bacteria produce orange-red tubercles of iron oxides and hydroxides by oxidizing ferrous ions from the bulk medium or the substratum (Fig. 10). Using environmental scanning electron microscopy (ESEM) it is possible to demonstrate iron-depositing bacteria within tubercles, twisted filaments with iron-rich deposits along their length (Fig. 11). Iron-depositing bacteria are microaerophilic and may require syner-



Figure 10. Iron oxide deposits on galvanized pipe (1.6x).

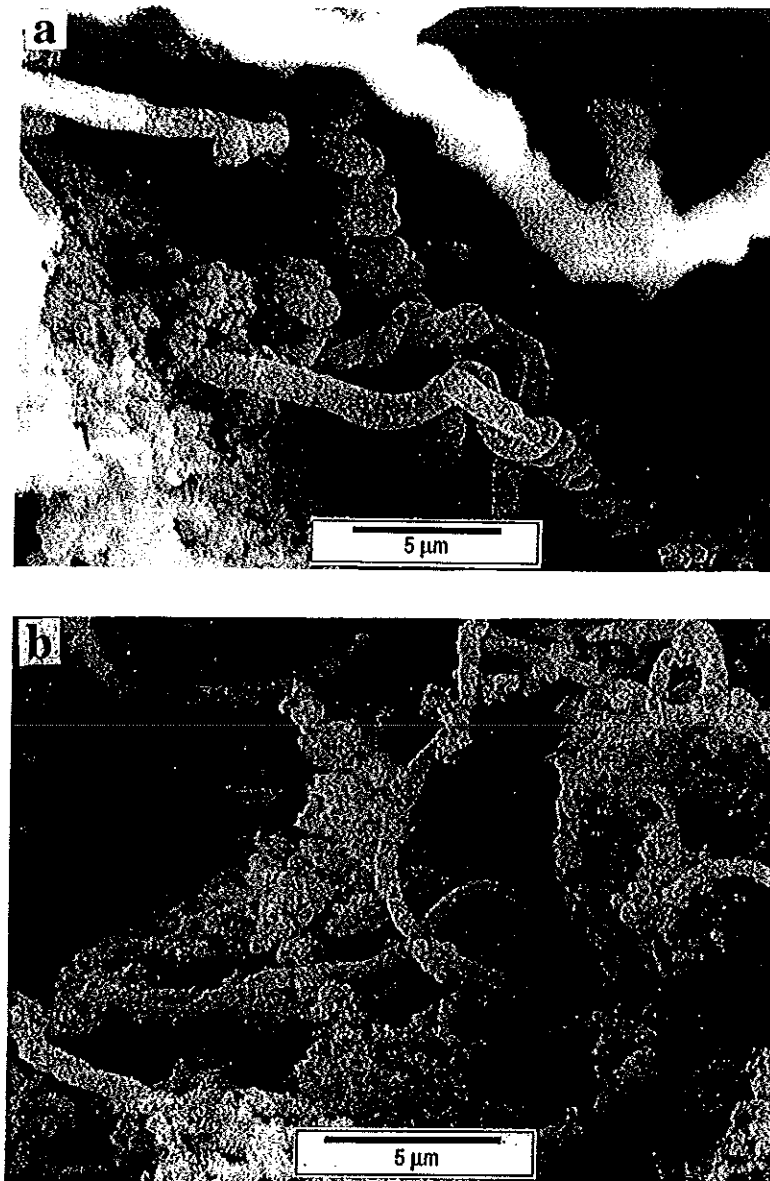


Figure 11. Iron-depositing bacteria within tubercles from Figure 10.

gistic associations with other bacteria to maintain microaerophilic conditions in their immediate environment. Bacilli and cocci were imaged on the surface of the tubercle in Figure 10. Deposits of cells and metal ions create oxygen concentration cells (Fig. 12) that effectively exclude oxygen from the area immediately under the deposit and initiate a series of events that are individually or collectively very corrosive. In an oxygenated environment, the area immediately under individual deposits becomes deprived of oxygen. That area becomes a relatively small anode compared to the large surrounding oxygenated cathode. Cathodic reduction of oxygen may result in an increase in pH of the solution in the vicinity of the metal. The metal will form metal cations at anodic sites. If the metal hydroxide is the thermodynamically stable phase in the solution, the metal ions will be hydrolyzed by water with the formation of H^+ ions. If cathodic and anodic sites are separated from one another, the pH at the anode will decrease and that at the cathode will increase. In addition, Cl^- ions from the electrolyte will migrate to the anode to neutralize any buildup of charge, forming

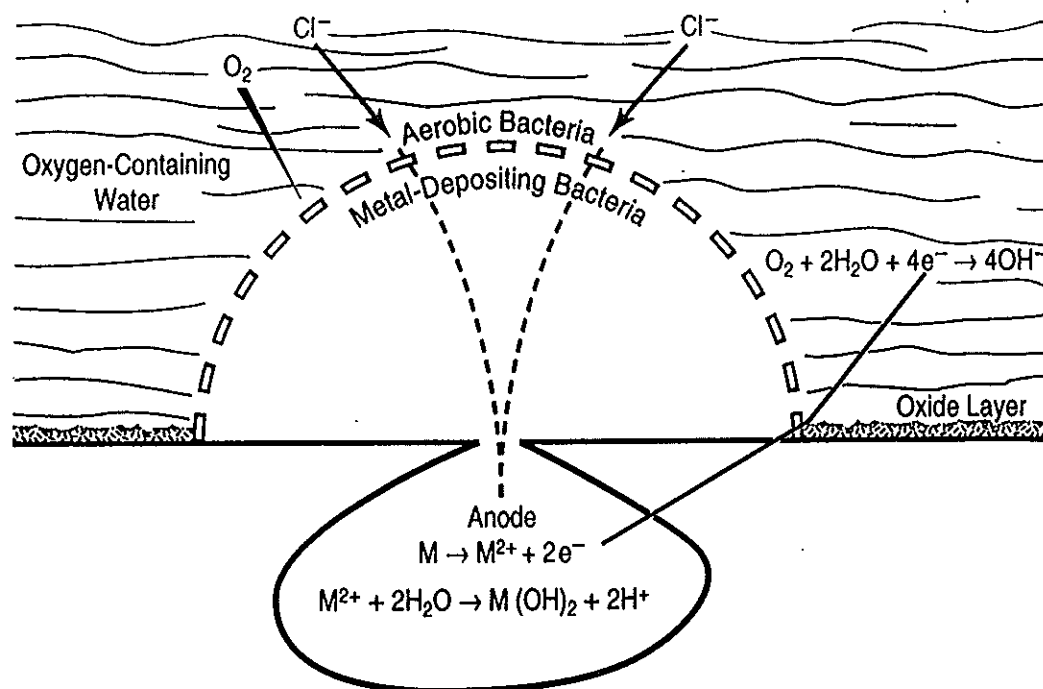


Figure 12. Possible reactions under tubercles created by metal-depositing bacteria.

heavy metal chlorides that are extremely corrosive. Under these circumstances, pitting involves the conventional features of differential aeration, a large cathode to anode surface area and the development of acidity and metallic chlorides. Pit initiation depends on mineral deposition by bacteria. Pit propagation is dependent not on activities of the organisms but on metallurgy (George 1996). This also means that attempts to kill the organisms within mineral deposits using biocides will not result in a cessation of pit propagation (Miller and Tiller 1970). The pH at the anode depends on specific hydrolysis reactions (Table 1). The largest pH decrease is found in alloys containing chromium. Stainless steels containing 6% or more molybdenum are not vulnerable to this type of attack.

Table 1. Specific hydrolysis reactions.

HYDROLYSIS REACTION	EQUILIBRIUM pH
$Fe^{2+} + 2H_2O \rightleftharpoons Fe(OH)_2 + 2H^{+}$	$pH = 6.64 - 1/2 \log a_{Fe^{2+}}$
$Cr^{3+} + 3H_2O \rightleftharpoons Cr(OH)_3 + 3H^{+}$	$pH = 1.53 - 1/3 \log a_{Cr^{3+}}$
$Ni^{2+} + 2H_2O \rightleftharpoons Ni(OH)_2 + 2H^{+}$	$pH = 6.5 - 1/2 \log a_{Ni^{2+}}$
$Mn^{2+} + 2H_2O \rightleftharpoons Mn(OH)_2 + 2H^{+}$	$pH = 7.66 - 1/2 \log a_{Mn^{2+}}$

Sulfides. Many sulfide minerals under near-surface natural environmental conditions can only be produced by microbiological action on specific precursor metals. SRB are a diverse group of anaerobic bacteria that can be isolated from a variety of environments (Pfennig et al. 1981, Postgate 1979) including seawater where the concentration of sulfate is typically 25 mM (Postgate 1979). Even though seawater is generally aerobic (typical

values above the thermocline are in the range 4 to 6 ppm), anaerobic microorganisms survive in anaerobic microniches until conditions are suitable for their growth (Costerton and Geesey 1986, Staffeldt and Kohler 1973). If the aerobic respiration rate within a biofilm is greater than the oxygen diffusion rate, the metal/biofilm interface can become anaerobic and provide a niche for sulfide production by SRB (Little et al. 1990).

In the following sections, SRB sulfide production will be reviewed for iron, copper, copper alloys, silver, zinc and lead. The metal interface under the biofilm and corrosion layers will be referred to as base metal to differentiate it from layers of minerals and metal ions that have been derivatized by corrosion reactions. Mineralogical data, thermodynamic stability diagrams (Pourbaix 1966, Wagman et al. 1982) and the simplicity principle for precipitation reactions (McNeil et al. 1991) will be used to rationalize corrosion product mineralogy in fresh and saline water and to demonstrate the action of SRB.

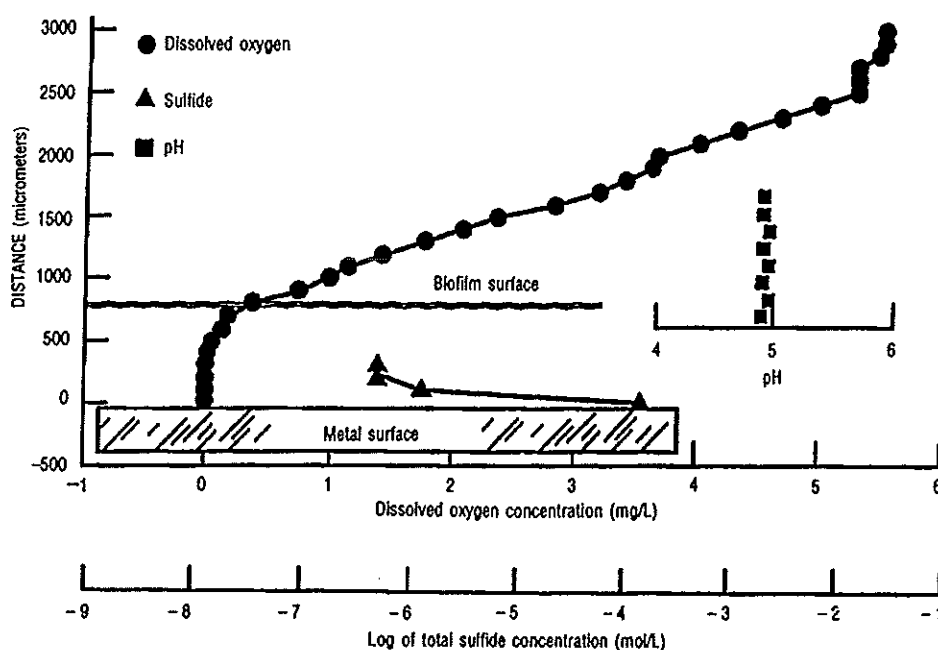


Figure 13. Concentration profiles of sulfide, oxygen, and pH in a biofilm on mild steel (Lee et al. 1993).

Iron. The corrosion rate of iron in the presence of hydrogen sulfide is accelerated by the formation of iron sulfide minerals (Wikjord et al. 1980) that stimulate the cathodic reaction through a decrease in hydrogen overvoltage at cathodic sites. Once electrical contact is established, mild steel behaves as an anode and electron transfer occurs through the iron sulfide. In the absence of oxygen, the metabolic activity of SRB causes accumulation of hydrogen sulfide near metal surfaces. This is particularly evident when metal surfaces are covered with biofilms. Figure 13 shows concentration profiles of sulfide, oxygen, and pH in a biofilm accumulated on the surface of a mild steel corrosion coupon. The concentration of sulfide is highest near the metal surface where iron sulfide forms quickly and covers the steel surface if both ferrous and sulfide ions are available. At low ferrous ion concentrations, adherent and temporarily protective films of iron sulfides are formed on the steel surface with a consequent reduction in corrosion rate. High rates of SRB-induced corrosion of mild steel are maintained only in high concentrations of ferrous ion.

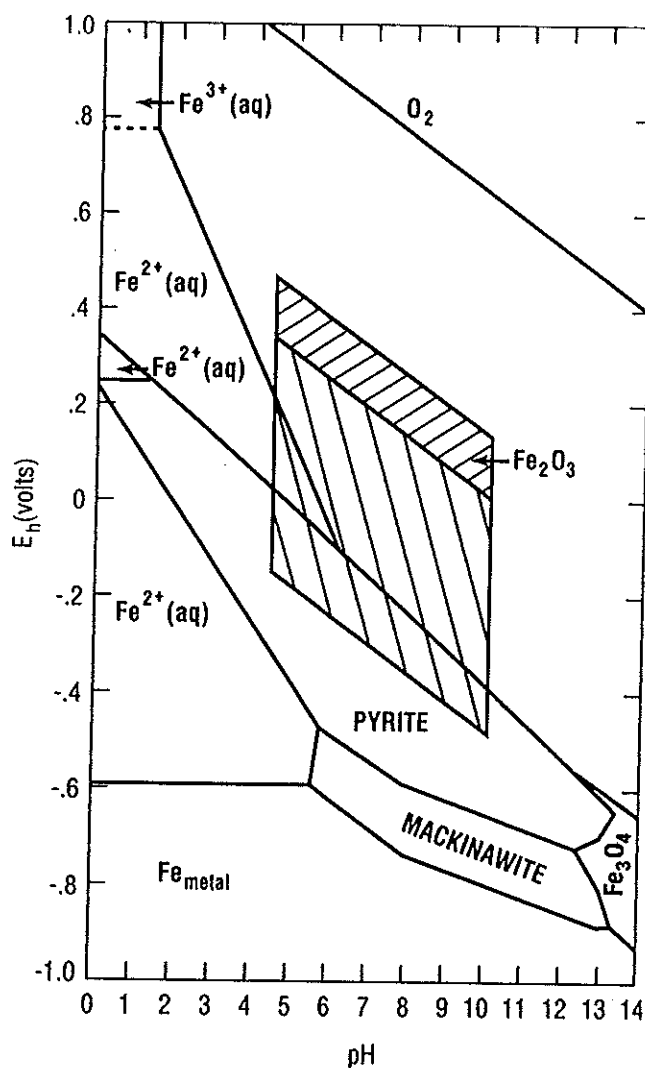


Figure 14. Iron stability diagram: water without chloride ions; total sulfide 10^{-2} M.

Figure 14 is a stability diagram for an iron-water-reduced sulfur system with lines for 10^{-6} M ferrous iron and 10^{-2} M sulfide. For clarity, pyrite (FeS_2) and mackinawite (FeS_{1-x}) are the only sulfides indicated. Parallelograms superimposed on the diagram are bounded by the highest and lowest pH values commonly found in natural fresh and saline surface waters. The upper portion of the hatched area applies to waters less than 10 m from the surface; the lower portion (oppositely hatched) represents waters at depths greater than 10 m. Conditions in the upper hatched parallelogram represent those readily achieved in stagnant waters. The lower portion indicates conditions not found in near-surface environments. Mackinawite is a tetragonal mineral that may be unstable altogether but is clearly unstable above 150°C . It cannot be produced by conventional techniques. Greigite is a thiospinel with formula Fe_3S_4 . Smythite is a hexagonal compound with formula $\text{Fe}_{3-x}\text{S}_4$, $0 < x < 0.25$. Cubic FeS can be produced artificially. By applying H_2S pressures in the range of one atmosphere, Berner (1969) produced "tetragonal FeS," which has the same symmetry as mackinawite but contains somewhat less sulfur and has slight but systematic differences in lattice parameter. Presumably, further increases in H_2S pressure could produce material equivalent to natural mackinawite. Thermodynamic analyses indicate that, under redox and sulfide activity conditions in surface waters, only pyrite is stable; furthermore, pyrite forms relatively easily in nonbiological corrosion, so

the preferential formation of less stable sulfides is difficult to attribute to slow pyrite formation kinetics. The region of stability of mackinawite is wholly outside the region defined by surface water conditions, excluding waters influenced by peat bogs, coal mines, volcanic activity and industrial effluents.

During corrosion of iron and steel in the presence of SRB, a thin (approximately 1 μm), adherent layer of "tarnish" is first formed. This was originally termed "kansite," but has since been identified as mackinawite. As it thickens, the layer becomes less adherent. If ferrous ion concentration in the electrolyte is low, the mackinawite alters to greigite. This alteration is not observed in nonbiological systems. If ferrous ion concentration is high, mackinawite is accompanied by green rust 2, a complex ferrosferic oxyhydroxide. The presence of green rust 2 may be due to a solubility effect and accounts for reduced corrosion rates when the electrolyte is rich in ferrous ions and has a very low renewal rate.

In summary, mackinawite (tetragonal FeS_{1-x}) is easily produced from iron and iron oxides by consortia of microorganisms that include SRB. The presence of mackinawite in corrosion products formed in shallow water environments with the exclusions previously delineated is proof that the corrosion was SRB-induced. Recent work indicates that on continued exposure to SRB mackinawite alters to greigite (Fe_3S_4), smythite (Fe_9S_{11}) and finally to pyrrhotite (FeS_{1+x}) (McNeil and Little 1990). SRB in thin biofilms on pottery surfaces (Duncan and Ganiaris 1987, Heimann 1989) and silver (McNeil and Mohr 1993) can produce pyrite films from iron-rich waters. Pyrite is not a typical iron corrosion product, but SRB can produce pyrite from mackinawite in contact with elemental sulfur (Berner 1969). Abiotic aqueous synthesis of these minerals, with the possible exception of pyrite, requires H_2S pressures higher than those found in shallow waters.

Copper. Cuprite (Cu_2O), the first product of copper corrosion, forms epitaxially as a direct reaction product of copper with dissolved O_2 or with water molecules (North and Pryor 1970). Cuprite has a high electrical conductivity and permits transport of copper ions through the oxide layer so they can dissolve in the water and reprecipitate. If the water chemistry approximates that of seawater, copper ions reprecipitate as botallackite ($\text{Cu}_2(\text{OH})_3\text{Cl}$) (Pollard et al. 1989) which can alter in minutes or hours to either paratacamite or atacamite (other crystal structures of $\text{Cu}_2(\text{OH})_3\text{Cl}$) depending on local water chemistry. Paratacamite, the more common form, gives the appearance of having precipitated from solution, but may be a pseudomorph after botallackite (Kato and Pickering 1984).

The impact of sulfides on the corrosion of copper alloys has received considerable attention, including published reports documenting localized corrosion of copper alloys by SRB in estuarine environments (Little et al. 1988, 1989) and a report of the failure of copper alloys in polluted seawater containing waterborne sulfides that stimulate pitting and stress corrosion cracking (Rowlands 1965). Copper alloys suffer accelerated corrosion attack in seawater containing 0.01 ppm sulfide after 1-day exposure (Gudas and Hack 1979). A porous layer of cuprous sulfide with the general stoichiometry Cu_{2-x}S , $0 < x < 1$, forms in the presence of sulfide ions (Syrett 1980). Copper ions migrate through the layer, react with more sulfide, and produce a thick, black scale (Fig. 15).

Figure 16 is a stability diagram for copper and its minerals drawn for 10^{-6} M total dissolved copper and 10^{-2} M total sulfide. Parallelograms superimposed on the diagram are similar to those described for Figure 14 and are appropriate for the analysis of corrosion mineralogy under non-hydrothermal conditions. McNeil et al. (1991) used Figure 16 to interpret results from laboratory experiments. They exposed mixed cultures known to

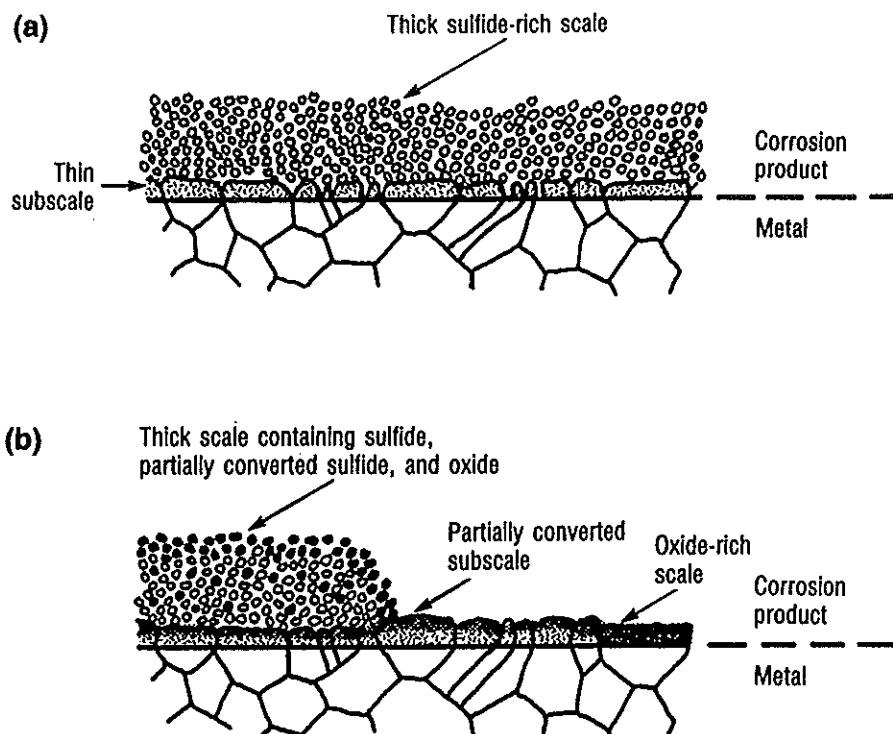


Figure 15. Schematic of (a) thick sulfide-rich scale on copper alloy and (b) disruption of sulfide film. (Reprinted from B.C. Syrett, "The Mechanism of Accelerated Corrosion of Copper-Nickel Alloys in Sulfide-Polluted Seawater," *Corrosion Science*, Vol. 21, 1981, with permission from Elsevier Science Ltd, Kidlington, UK.)

contain SRB to copper and copper/nickel alloys in a variety of natural and synthetic waters containing sulfates for 150 days. The pH values of the waters, measured after two weeks, were between 5.5 and 6.8. All copper-containing metals exposed to SRB in isolated cultures and in the natural augmented waters were covered with black sulfur-rich deposits (Fig. 17). The thickness and tenacity of the surface deposits varied among the metals and cultures. Corrosion products on commercially pure copper were consistently nonadherent. Corrosion products on copper alloys were more adherent and in some cases difficult to scrape from the surface. In all cases, bacteria were closely associated with sulfur-rich deposits (Fig. 18 a,b). Many bacteria were encrusted with deposits of copper sulfides (Fig. 19). Most scanning electron microscopy (SEM) micrographs of SRB on copper surfaces indicate a monolayer of cells overlaying a sulfide layer. Transmission electron microscopy has been used to demonstrate that bacteria are intimately associated with sulfide minerals and that on copper-containing surfaces the bacteria were found between alternate layers of corrosion products and attached to base metal (Blunn 1986).

Bio-mineralogy of copper sulfides has been studied for over a century (Daubree 1862, de Gouvernain 1875, Baas-Becking and Moore 1961, Mor and Beccaria 1975, Syrett 1977, 1980, 1981, McNeil and Little 1992). The complexity of the resulting observations reflects the complexity of the copper-sulfur system, especially in the presence of alloying elements or iron in the environment (Ribbe 1976, Kostov and Minceva-Stefanova 1981). Chalcocite (Cu_2S), digenite (Cu_9S_5), djurleite ($\text{Cu}_{1.93}\text{S}$ – $\text{Cu}_{1.97}\text{S}$), anilite (Cu_7S_4), spionkopite ($\text{Cu}_{39}\text{S}_{28}$), geerite (Cu_8S_5), and covellite (CuS) have been reported. In long-term corrosion where waters contain significant iron, chalcopyrite is a common product

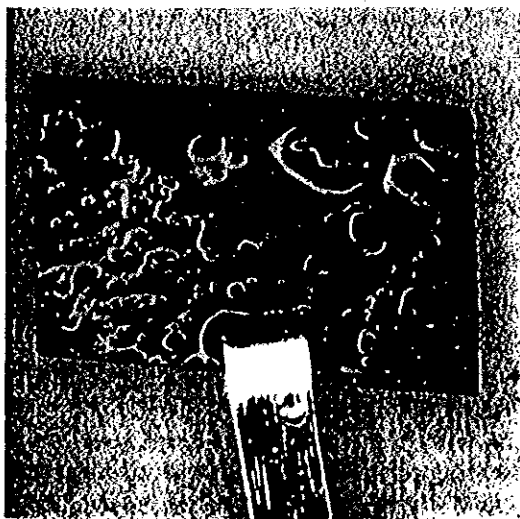
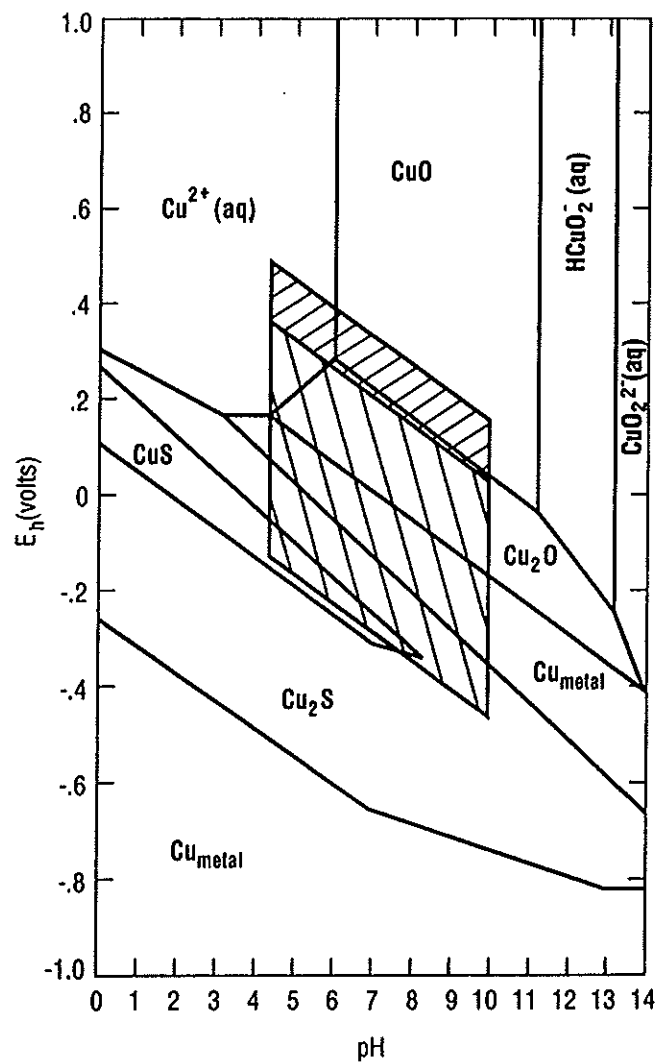


Figure 16 (above, right). Copper stability diagram: water without chloride ions; total sulfide 10^{-2} M.

Figure 17 (left). Black sulfide film on copper foil after 4-month exposure to SRB.

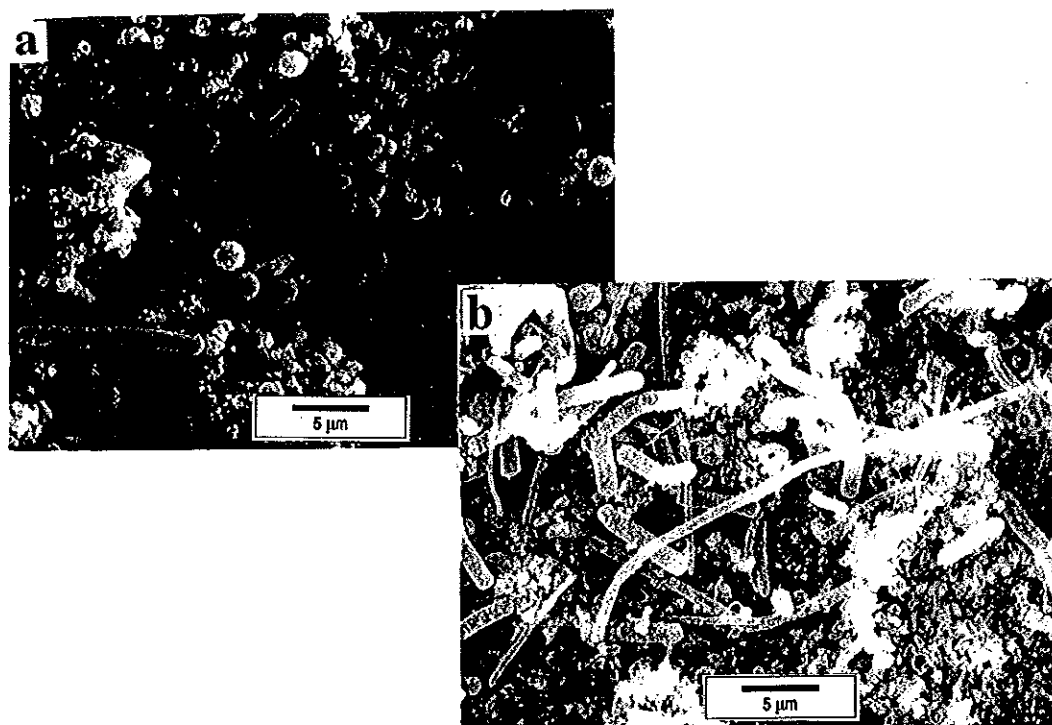


Figure 18. (a,b) ESEM micrographs of bacteria within corrosion products on copper-containing foils.

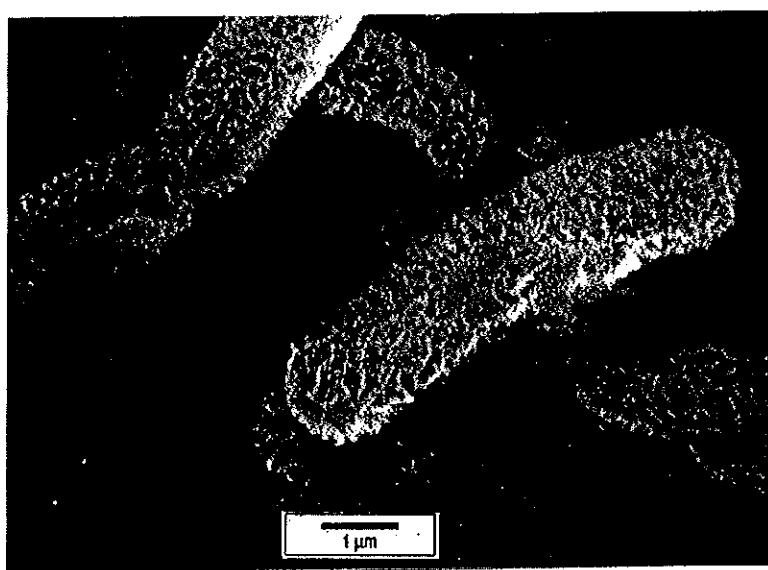


Figure 19. Encrustations of copper sulfide on bacterial cells.

(Daubree 1862, de Gouvernain 1875, McNeil and Mohr 1993). While chalcopyrite films can be formed abiotically in high sulfur concentrations (Cuthbert 1962), chalcopyrite and most other copper sulfides are not generally found as products of abiotic corrosion.

Detailed kinetics of individual reactions are not fully understood, and the consequences for corrosion depend on many factors, including mineral morphology and variations of

redox and pH with time (McNeil and Mohr 1993). Discussions of alteration kinetics are contained in a number of papers (Baas-Becking and Moore 1961, Roseboom 1966, Craig and Scott 1976, Putnis 1977, Evans 1979). The general phenomenology can be understood by the following approach. Microbial consortia, that include SRB produce anoxic, sulfide-rich environments in which the conversion of copper to copper sulfides is thermodynamically favored at a concentration of 10^{-2} M total sulfur (Fig. 16). Reactions appear to proceed as suggested by Ostwald's rule: the first sulfur-poor compounds are converted to sulfur-rich compounds. One would expect a layering effect with covellite on the outside and chalcocite next to unreacted copper metal. This has not been studied, and indeed would be very difficult to study on pure copper because of the porous and mechanically unstable corrosion products. In short-term experiments with excess of copper over available sulfur, chalcocite with little or no covellite is formed (McNeil and Little 1991). Covellite is produced if excess sulfide is available, either deliberately provided (Baas-Becking and Moore 1961) or naturally available (Daubree 1862, Mor and Beccaria 1975).

The presence of dissolved iron leads to other complications. Not only has chalcopyrite been observed, but also digenite (Baas-Becking and Moore 1961, Mor and Beccaria 1975, North and MacLeod 1986, McNeil et al. 1991), djurleite (Macdonald et al. 1979, McNeil et al. 1991), and the hexagonal high-temperature polytype of chalcocite (McNeil et al. 1991). These observations can be interpreted in terms of the simplicity principle (Goldschmidt 1953): impurities tend to stabilize high-entropy, high-temperature polytypes. Digenite stability is promoted by iron (Craig and Scott 1976). It appears that nickel stabilizes djurleite on copper-nickel and the stabilization of djurleite has major practical consequences. McNeil et al. (1991) observed that corrosion layers showing strong digenite lines were never observed on pure copper, but frequently on copper-nickel alloys. Furthermore, corrosion products containing digenite showed substantial adherence and mechanical stability, while the corrosion products on pure copper, composed of chalcocite with only traces of other minerals, were powdery and nonadherent.

It has been argued that if the copper sulfide layer were djurelite, the sulfide layer would be protective (Nilsson et al. 1980). Even if such a sulfide film were technically passivating, the mechanical stability of the film is so poor that sulfide films are useless for corrosion protection. In the presence of turbulence, the loosely adherent sulfide film is removed, exposing a fresh copper surface to react with sulfide ions. For these reasons, turbulence-induced corrosion and sulfide attack of copper alloys cannot easily be decoupled. In the presence of oxygen, the possible corrosion reactions in a copper sulfide system are extremely complex because of the large number of stable copper sulfides (Ribbe 1976), their differing electrical conductivities, and catalytic effects. Transformations between sulfides or of sulfides to oxides result in changes in volume that weaken the attachment scale and oxide subscale, leading to spalling. Bared areas repassivate forming cuprous oxide.

The analysis of nonsulfide-induced corrosion of copper at near-room temperature in aqueous environments is extremely complex because of the numerous mineral species which can be formed. In seawater environments, chloride compounds dominate, but even in relatively low-chloride waters the strong tendency of chloride ions to migrate to anodic sites leads to formation of chloride-containing minerals. In general, corrosion of copper-nickel alloys in saline environments is less rapid than that of pure copper under the same circumstances. Earlier conjectures that this was due to changes in the electronic structure of the passive film (North and Pryor 1970) or of the base metal (Swartzendrubber et al. 1973) appear not to account adequately for the experimental data. A comprehensive review of more recent experimental work (Hack et al. 1986) indicates that, under hydrodynamic conditions that prevent accumulation of porous outer layers of hydroxylchloride or

hydroxycarbonate corrosion products, the critical effect of the alloying elements is to alter the cathodic oxygen reduction.

If conditions at the surface of a copper alloy permit precipitation of nantokite (CuCl) under the cuprite layer, the alloy becomes vulnerable to bronze disease (Scott 1990) or pitting corrosion (Lucey 1967) depending on mass transport conditions. A biofilm containing acid-producing bacteria could create the requisite conditions by increasing acidity in anodic areas and reducing copper ion transport from the surface, producing a higher local concentration (McNeil and Mohr 1992). While nantokite-based corrosion can also occur by nonbiological paths, the nonisometric morphology frequently observed on precipitated cuprite crystals suggests that microbiological poisoning of growth planes is a factor. The presence of alloying elements does not protect against the formation of nantokite and the consequent bronze disease corrosion (Mond and Cuboni 1893).

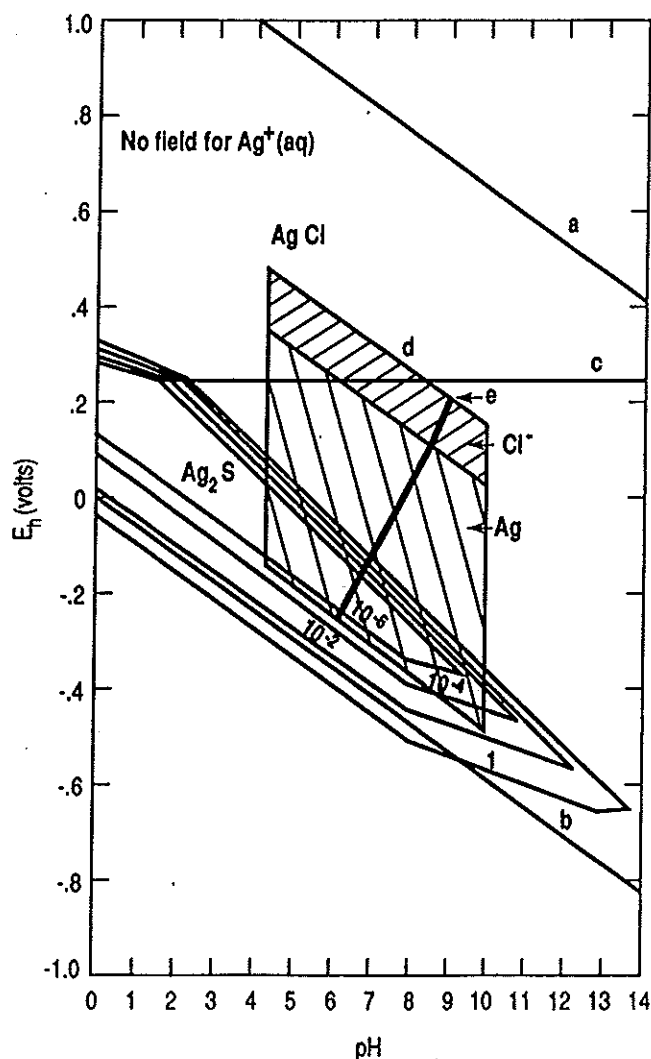


Figure 20. Silver-water-chloride-sulfur stability diagram for silver in seawater at varying reduced sulfur concentrations.

Silver. Figure 20 is a silver-water-chloride-sulfur system stability diagram. The upper diagonal line (a) is the oxygen line, above which water is thermodynamically

unstable with respect to oxygen generation. The lower diagonal line (b) is the hydrogen line, below which water is thermodynamically unstable with regard to hydrogen evolution. Waters do exist outside these boundaries under special conditions. Horizontal line (c) separates the regions in which chloride corrosion of silver can and cannot take place. The upper hatched area below line (d) approximates the region of effective redox/acidity conditions for near surface waters. Strongly acidic and basic regions are characteristic of groundwaters, but not seawaters. The effective redox potential of oxygenated water is less than would be calculated thermodynamically from oxygen concentrations because of kinetic effects. The effective redox potential also depends on water pollutants, temperature and temperature/pressure history. The lower, oppositely hatched parallelogram indicates redox/acidity conditions existing in natural waters but not characteristic of near surface waters. Bulk water conditions outside the parallelograms are found in peat bogs, coal mines and at depths not considered in this chapter. The heavy diagonal line (e) through the hatched area indicates redox conditions for a series of waters sampled from a brackish, stagnant pond in an industrialized area in Scandinavia where surface waters had a high oxygen content and deep waters contained $40 \text{ mg l}^{-1} \text{ H}_2\text{S}$ and significant amounts of decaying organic material (Garrels and Christ 1965). Pond conditions were used to define the upper and lower Eh limits near neutrality. The upper portion of the hatched area applies for waters less than 10 m from the surface; the lower portion represents waters at depths greater than 10 m. To achieve conditions outside those defined by the two parallelograms at a metal surface requires the presence of a biofilm of maintaining conditions radically different from those in the bulk environment.

Silver and its alloys are subject to corrosion by reduced sulfur species including H_2S , generally of microbiological origin. In air (e.g. in a museum) H_2S can be the consequence of biodegradation of sulfur-containing polymeric materials, producing monoclinic acanthite (Ag_2S) (Banister 1952, Bauer 1988). Figure 20 indicates the possible thermodynamically stable phases for silver equilibrated with varying total sulfur compositions in 0.46 M NaCl (typical for seawater). It is assumed that reduced sulfur species (S^{2-} , HS^- , H_2S) are in equilibrium. A straightforward type of silver corrosion is conversion of silver to cerargyrite (AgCl), as indicated above line (c). Below line (c) metallic silver is stable, except for the wedge-shaped areas pointing down and to the right indicating regions of stability for monoclinic acanthite (Ag_2S). The region between the diagonal lines bounding the upper hatched region approximates the effective oxidizing behavior of near-surface, fully aerated seawater (Garrels and Christ 1965). Most shallow sea chemistries fall into this region. Conditions in shallow land burials where the major source of groundwater is rain or surface water percolating through soils are near this region. Cerargyrite is stable in seawater and chloride-rich shallow-land burial conditions.

There are three polymorphs of Ag_2S . Monoclinic acanthite is stable up to 176°C (Kracek 1946). Body-centered cubic argentite (Kracek 1946) is stable from 176°C to a temperature between 586°C and 622°C , above which the stable form is a face-centered cubic polymorph (Djurle 1958, Barton 1980). The high temperature polymorph has never been observed in corrosion. Reactions between Ag_2S polymorphs are very fast. Pure body-centered cubic argentite (Ag_2S) is not found in nature at standard temperature and pressure, and artificial argentite made with pure silver cannot be quenched to room temperature (Roy et al. 1959).

Laboratory data on sulfide and derivation of silver can be summarized as follows: (1) corrosion of silver by reduced sulfides, whether H_2S (Sinclair 1982, Volpe and Peterson 1989) or organic sulfides (Sinclair 1982) produces acanthite, (2) CS_2 does not produce corrosion, (3) the corrosivity of organic sulfides appears to be controlled by transport mechanisms and thus by vapor pressures, and (4) the rate of sulfidation is

strongly affected by NH_3 and iron dissolved in the silver (Biestek and Drys 1987). Abiotic aqueous corrosion of silver in the presence of reduced sulfur species produces acanthite in bulk (Birss and Wright 1981, Campbell et al. 1982). Argentite is observed when objects made of impure silver (e.g. coins) are corroded in sediments over archaeological periods (Gettens 1963, North and MacLeod 1986). If Cl^- is present acanthite or argentite combined with cerargyrite is formed.

These observations support the hypothesis that formation of argentite is limited to precipitation of a silver-copper sulfide by reduced sulfide species. This theory is consistent with the observation that argentite corrosion products are sometimes accompanied by jalpaite ($\text{Ag}_{1.5}\text{Cu}_{0.5}\text{S}$) (North and MacLeod 1986). Argentite made of pure silver is unstable at room temperature, yet there are two reasons why argentite should precipitate during MIC of archaeological objects. Argentite is usually associated with jewelry and coinage containing several percent copper. Argentite, unlike acanthite, can accommodate nearly 30% copper in its lattice (Shcherbina 1978). The phenomenon is parallel to the production of akageneite rather than goethite in the corrosion of meteorites which is attributed to the ability of the akageneite lattice to accommodate significant Cl^- , whereas the goethite lattice can accommodate little or none (Buchwald 1977). Precipitation of a mineral from an impure environment favors a loose crystal structure capable of accommodating impurity atoms (Goldschmidt 1953).

Argentite formation occurs when an object made of silver-copper alloy is in a water-saturated deposit containing SRB in a biofilm capable of maintaining reducing conditions, and bacteria (perhaps ammonia producers) capable of solubilizing silver and copper atoms. A layer of sand or soil restricts the ability of the metal ions to escape, so that concentrations of copper and silver ions within the biofilm rise to levels which cause precipitation. The precipitation of argentite is favored for the reasons given above. Jalpaite forms in regions where the copper concentration is high. Argentite and jalpaite could, in principal, be stabilized by corrosion of pure silver in a copper-rich environment (e.g. silver coins with copper coins), but for practical purposes, the presence of either argentite or jalpaite implies that the silver artifact originally contained significant copper.

Other metals. SRB-induced corrosion of zinc produces a zinc sulfide reported to be sphalerite (ZnS) (Baas-Becking and Moore 1961). SRB on lead carbonates produces galena (PbS) (McNeil and Little 1990). Galena has been found more recently as a lead corrosion product in SRB-induced corrosion of lead-tin alloys (McNeil and Mohr 1993).

Carbonates. Apparent calcium carbonate (calcite) precipitation by bacteria, algae, and yeasts has been reported by several researchers (Pentecost and Bauld 1988, Bouquet et al. 1973, Novitsky 1981, Shinano 1973). It is generally observed that calcite crystal deposition is favored by addition of Ca^{2+} , CO_3^{2-} and/or an increase in pH to 8.0 and higher. Calcification has been observed in marine, brackish, and freshwaters in the presence of CaCO_3 supersaturation. Bouquet et al. (1973), described calcite production by 210 soil bacterial isolates on a solid medium with added calcium.

Proposed mechanisms of calcite precipitation by microorganisms include calcium concentration from the medium by microbial binding, metabolic alteration of the medium that results in changes in bicarbonate concentration and pH, and microbial bodies acting as crystal nucleation sites. Pentecost and Bauld (1988) proposed that calcite deposition by cyanobacteria is initiated at sheath polymeric sites, on heteronuclei bound to sheath surfaces, or upon associated bacterial surfaces. However, most researchers have concluded that calcite precipitation is basically induced by chemical alteration of the medium whether by microbial activities or by abiotic reactions such as evaporation or outgassing of

aqueous carbon dioxide.

Figure 21 is a stability diagram for copper that represents the condition when no sulfur is present and the electrolyte has the same chloride concentration as seawater and total carbonate content (CO_3^{2-} , HCO_3^- , H_2CO_3) is assumed to be in equilibrium with air. Stability regions for red cuprite (Cu_2O), malachite ($\text{Cu}_2(\text{OH})_2\text{CO}_3$), and paratacamite ($\text{Cu}_2(\text{OH})_3\text{Cl}$) are shown. Thermodynamic data (excluding malachite) are from Wagman et al. (1982); malachite data are from Woods and Garrels (1966). Azurite ($\text{Cu}_3(\text{OH})_2(\text{CO}_3)_2$) and georgite ($\text{Cu}_5(\text{CO}_3)_3(\text{OH})_4 \cdot 6\text{H}_2\text{O}$) are not included. There are no thermodynamic data for georgite, and azurite has no stability field in water having carbonate content in equilibrium with air. If the water contains little Cl^- but is in equilibrium with the atmosphere with regard to carbonate species, malachite will precipitate on copper surfaces. The hydroxylchlorides, atacamite and paratacamite, form in inhomogeneous layered structures. Malachite can form in layered structures, but sometimes forms botryoidal (Gettens 1969) or hairlike structures. The reasons for these differing morphologies are not known and all are found in association with bacteria.

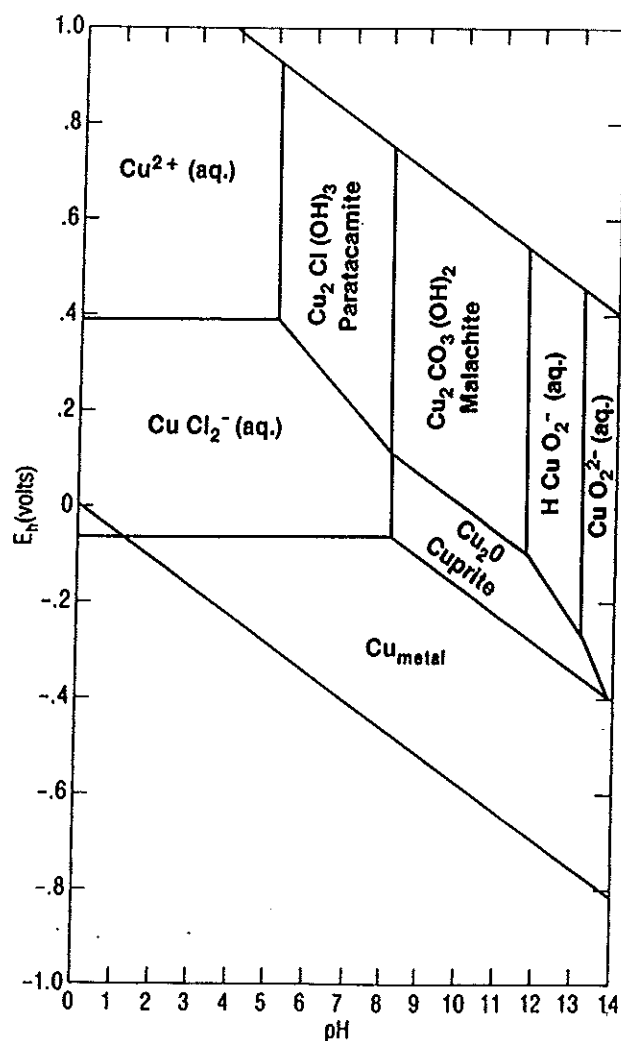


Figure 21. Copper stability diagram: seawater ($a_{\text{Cl}^-} = 0.319$), carbonates in equilibrium with air, no sulfides.

Mineral dissolution

Biofilm formation of copper alloys often results in selective dealloying. Zinc tends to be selectively removed from copper-zinc alloys, producing a spongy copper material (Walker 1977) though the incorporation of zinc in corrosion products to produce rosenite ($(\text{Cu,Zn})_2\text{CO}_3(\text{OH})_2$) (Gettens 1963). Dealloying of the copper from tin bronzes has been reviewed by Geilmann (1956). Partitioning of alloying elements between remaining metal ions in the corrosion product and the electrolyte has received little attention (Zolotarev et al. 1987).

Dissimilatory iron and/or manganese reduction occurs in several microorganisms, including anaerobic and facultative aerobic bacteria. Inhibitor and competition experiments suggest that Mn(IV) and Fe(III) are efficient electron acceptors similar to nitrate in redox ability and are capable of out-competing electron acceptors of lower potential, such as sulfate or carbon dioxide (Myers and Nealson 1988). Many of the recently described MRB are capable of using a variety of electron acceptors, including nitrate and oxygen (Myers and Nealson 1988). Myers and Nealson (1988) suggested that iron and manganese-reducing microorganisms must be in direct contact with oxides to reduce them. This conclusion is based on the observation that Fe(III) and Mn(IV) are not reduced if microorganisms capable of the reduction are separated from the oxides by a semi-permeable membrane that allows exchange of soluble molecules but prevents contact between the organism and the oxide. These experiments demonstrated that metal reduction by *S. putrefaciens* required cell/surface contact and the rate of reduction was directly related to surface area. ESEM and CLSM images of bacteria during both the iron and manganese reduction processes showed close contact of the cells with the oxides during the early stages of reduction. In later stages manganese oxides were coated with a layer of extracellular material that obscured the cells (Little et al. 1997).

Little et al. (1997) used synthetic iron oxides (goethite, $\alpha\text{-FeOOH}$; hematite, Fe_2O_3 ; and ferrihydrite, $\text{Fe}(\text{OH})_3$) as model compounds to simulate the mineralogy of passivating films on carbon steel. There is general agreement that oxide films formed on iron in air at temperatures below 200°C are composed of magnetite and hematite. Szklarska-Smialowska (1986) described the formation of hematite over a magnetite film. Ferric oxyhydroxides, including goethite and lepidocrocite ($\gamma\text{-FeO}\cdot\text{OH}$), have also been identified in protective layers on carbon steel. Under anaerobic conditions goethite, hematite, and ferrihydrite were reduced by *S. putrefaciens* (Table 2). Rates of reduction, measured by atomic absorption spectroscopy of Fe(II) in solution as a function of time, for the three minerals indicate that after a 24-h exposure to *S. putrefaciens*, initial reduction rates for goethite and ferrihydrite were approximately the same and were 5 times faster than the reduction rate for hematite. After 22 days the integrated reduction rates for goethite and ferrihydrite were much faster than those measured at 24 h. The hematite reduction rate actually slowed over the exposure period so that after 22 days the overall integrated rate was 50 times slower than reduction rates for goethite and ferrihydrite (Arnold et al. 1988, Roden and Zachara 1996).

Table 2. Relative rates of iron reduction based on Fe(II) in solution

	24 Hours mg/L/d	% Max	22 Days mg/L/d	% Max
Goethite	3.50	100	18.20	100
Ferrihydrite	2.60	74	14.10	77
Hematite	0.58	22	0.39	2

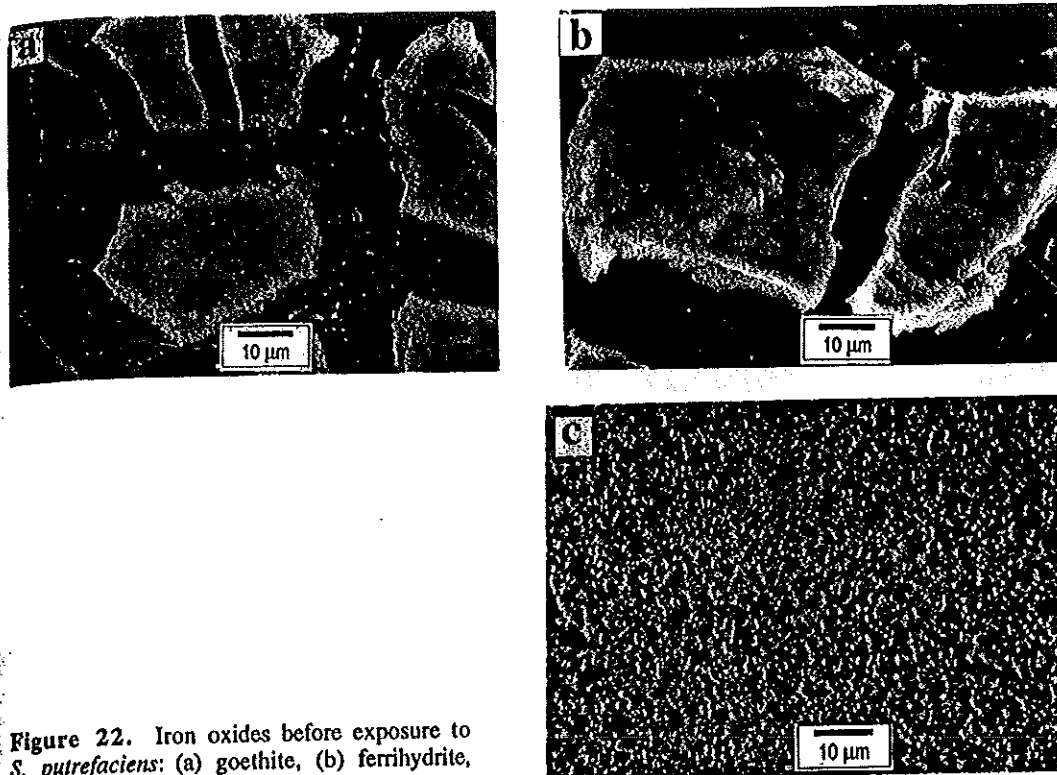


Figure 22. Iron oxides before exposure to *S. putrefaciens*: (a) goethite, (b) ferrihydrite, and (c) hematite.

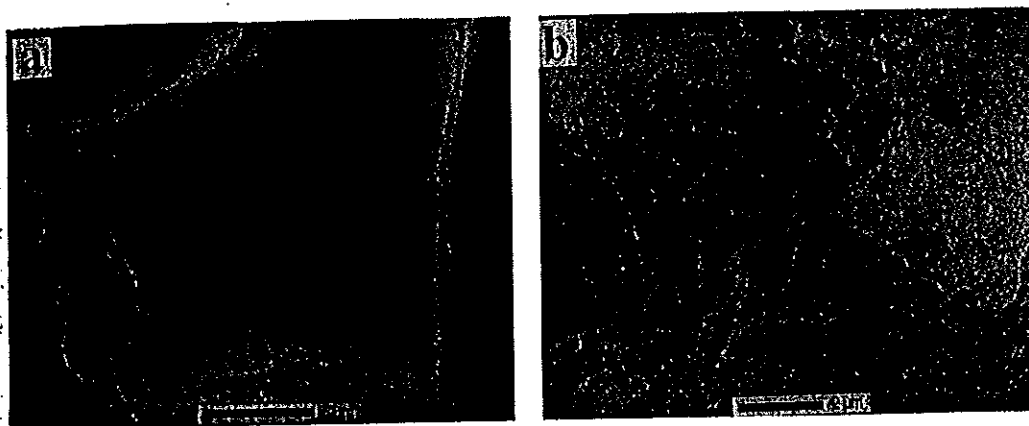


Figure 23. Bacteria on mineral surfaces after 48-h exposure to *S. putrefaciens*: (a) goethite and (b) ferrihydrite.

Differences in reduction rates may be due to the surface areas of individual oxides. Mineralogy and crystal structure must also play a role in microbial attachment and rate of biomineralization. Before exposure to *S. putrefaciens*, goethite and ferrihydrite appeared to form smooth platelet-like particles, while the hematite consisted of fine crystals (Fig. 22a-c). All iron oxides produced EDS spectra that were exclusively iron. After 48 h, isolated bacteria could be located on the surfaces of both the goethite and ferrihydrite (Fig. 23a,b), but not on the hematite. Minerals viewed with ESEM were wet and not coated with a metal coating as required for standard SEM. During initial stages of mineral dissolution, bacteria, composed of water and low atomic number elements, were not electron dense and were difficult to image. With reduction of the ferric iron, the bacterial cells became electron

dense and easier to recognize. When treated with a fluorescent stain that indicated viability, approximately 50% of all observed cells appeared to be actively metabolizing when viewed by CLSM. Occasionally dividing cells were observed. Surface microbial populations increased markedly after 72 h.

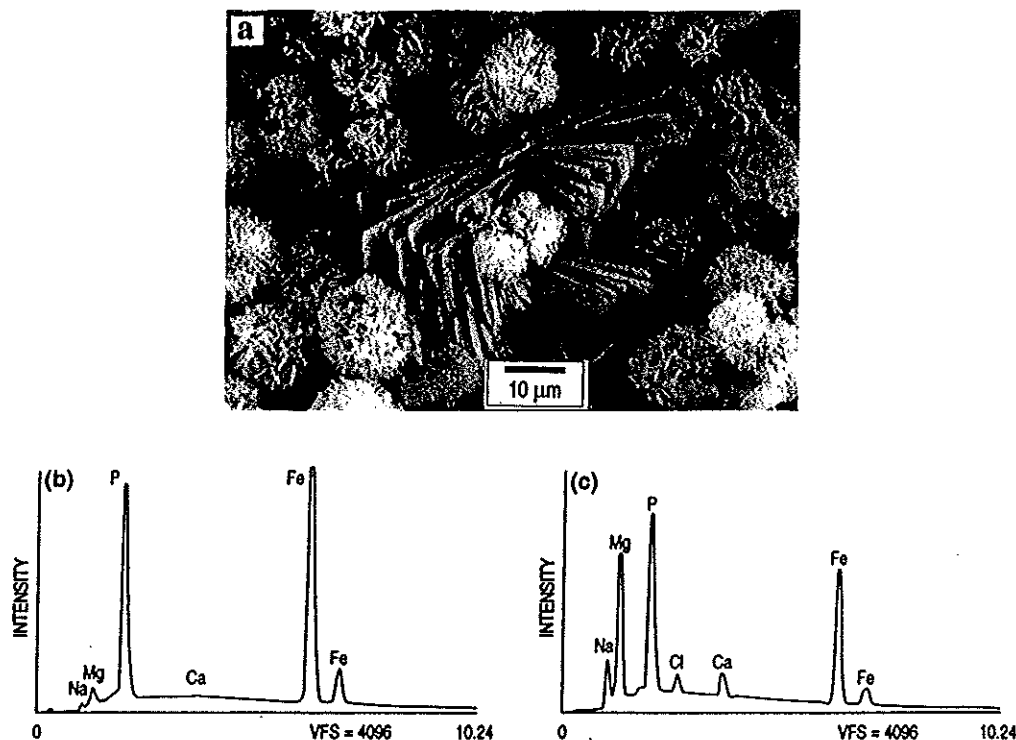


Figure 24. Goethite after 190 hr exposure to *S. putrefaciens*: (a) ESEM image of particle, (b) EDS spectrum of crystalline plate forms, and (c) EDS spectrum of globular forms.

After 190 h, ESEM images demonstrated that goethite and ferrihydrite surfaces had been altered during microbial reduction. Mineral particle size decreased and crystalline structure was transformed as the number of bacterial cells increased. Residual goethite particles consisted of both highly crystalline plates and more globular forms (Fig. 24a). EDS spectra of the two newly formed structures showed that the crystalline plate consisted of nearly stoichiometric amounts of iron and phosphorous, with a small enrichment of magnesium, suggesting that this mineral might be iron phosphate (vivianite) (Fig. 24b). Globular forms contained magnesium in addition to iron, phosphorous and magnesium (Fig. 24c). In previous experiments iron oxides were converted to siderite (FeCO_3) and other carbonates when experiments were conducted in closed containers from which carbon dioxide could not escape. ESEM images of residual goethite particles documented large accumulations of cells (Fig. 25a,b). The CLSM preparation of goethite particles contains a variety of sizes at different elevations within the preparation. The optical section in Figure 25c shows some particles that are completely surrounded with bright fluorescent cells. Other goethite particles have been optically cross-sectioned so that it is obvious that bacterial cells were associated with the exterior surfaces and had also created channels into the particles. Figure 26 shows the relationship between bacterial cell concentrations and Fe(II) in solution over time for goethite.

After 190 h CLSM images of optical sections indicated that individual particles of ferrihydrite could no longer be differentiated (Fig. 27). Instead, bacterial cells were

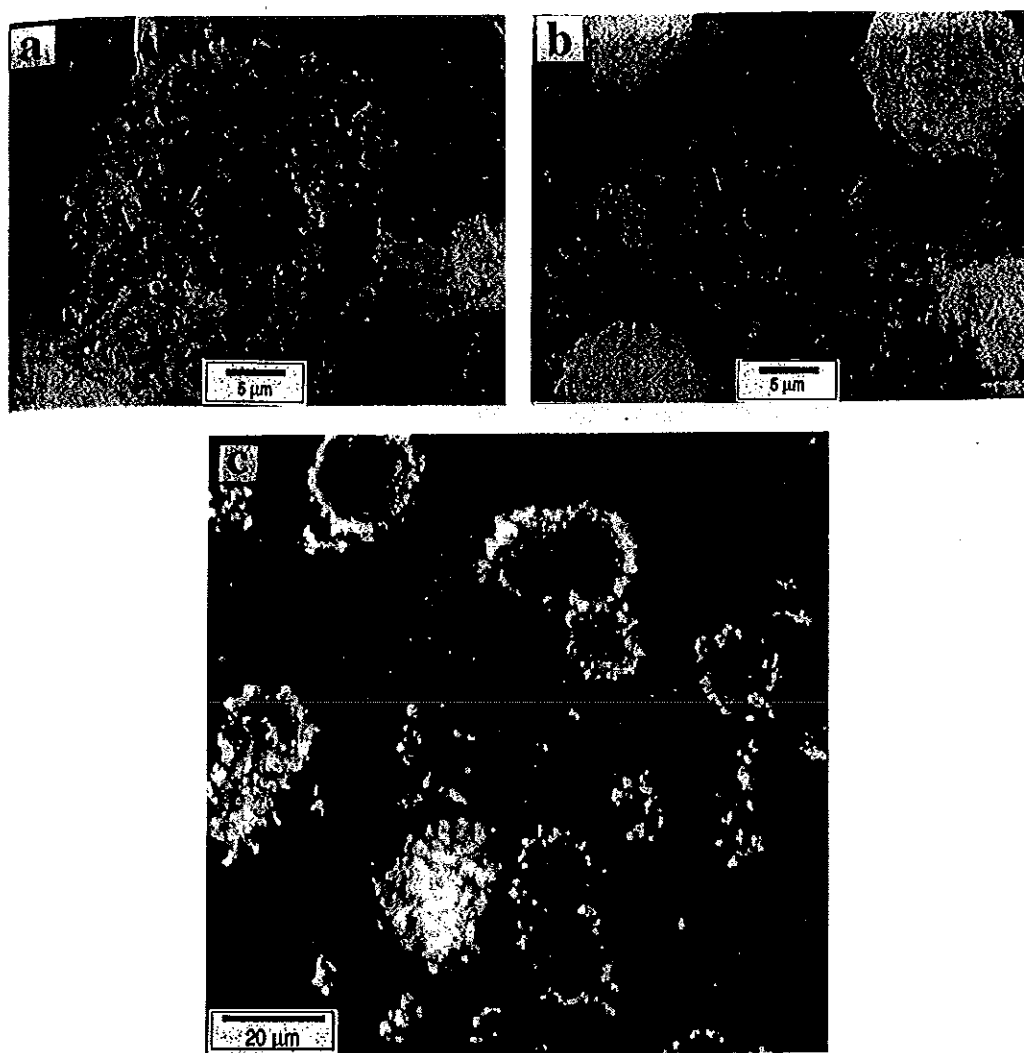


Figure 25. (a-b) Bacterial cells on goethite surface after 190-h exposure to *S. putrefaciens*. (c) CLSM image of optical cross-section of goethite particle.

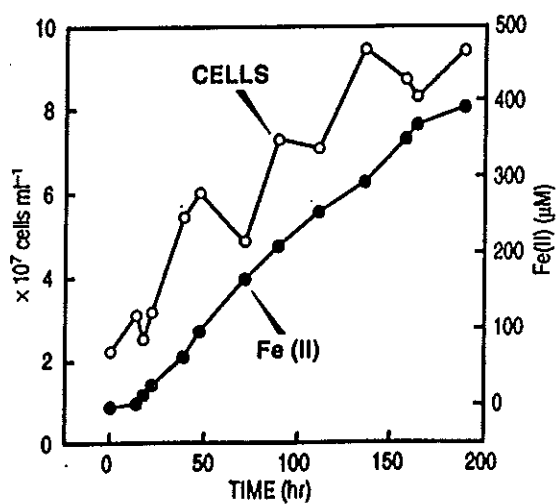


Figure 26. Bacterial reduction of goethite.

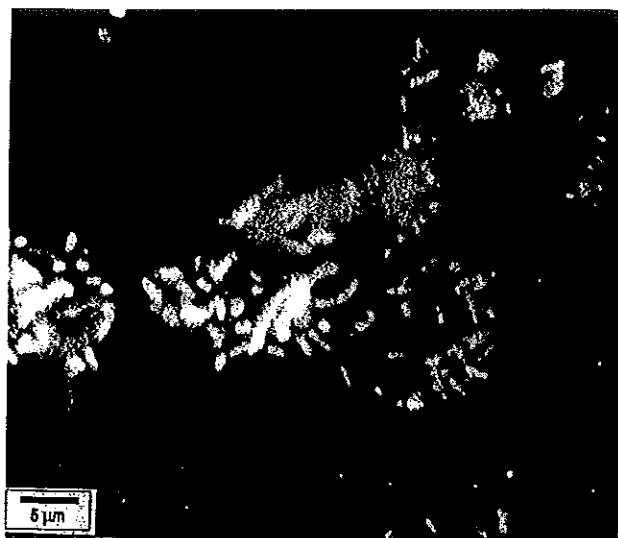


Figure 27. CLSM image of optical cross-section of ferrihydrite after 190-h exposure to *S. putrefaciens*.

distributed throughout all optical depths. Although iron reduction of hematite occurred (Table 2), no major changes in the elemental composition or crystal structure of the surface (Fig. 28a) were noted. EDS spectra of hematite indicated pure iron even after 190-h exposure. Scattered bacterial cells could be demonstrated in association with hematite surfaces (Fig. 28b).

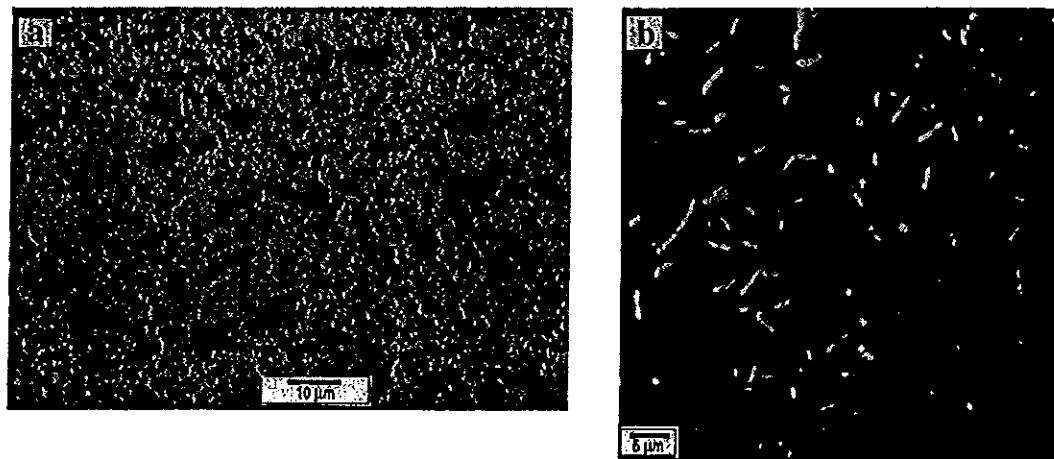


Figure 28. Hematite surface after 190-h exposure to *S. putrefaciens*: (a) ESEM image and (b) CLSM optical cross-section.

Current noise measurements of carbon steel exposed to pure cultures of *S. putrefaciens* in media with and without the combination of sodium chloride and thiosulfate were used to monitor corrosion (Fig. 29a,b). In both cases current noise showed active surface changes. Fluctuations in noise records of a corroding metal are usually interpreted as being due to the sudden rupture of a protective oxide film followed by immediate repassivation. *S. putrefaciens* can reduce thiosulfate to produce sulfide. Obuekwe et al. (1981) evaluated corrosion of mild steel under conditions of simultaneous formation of ferrous and sulfide

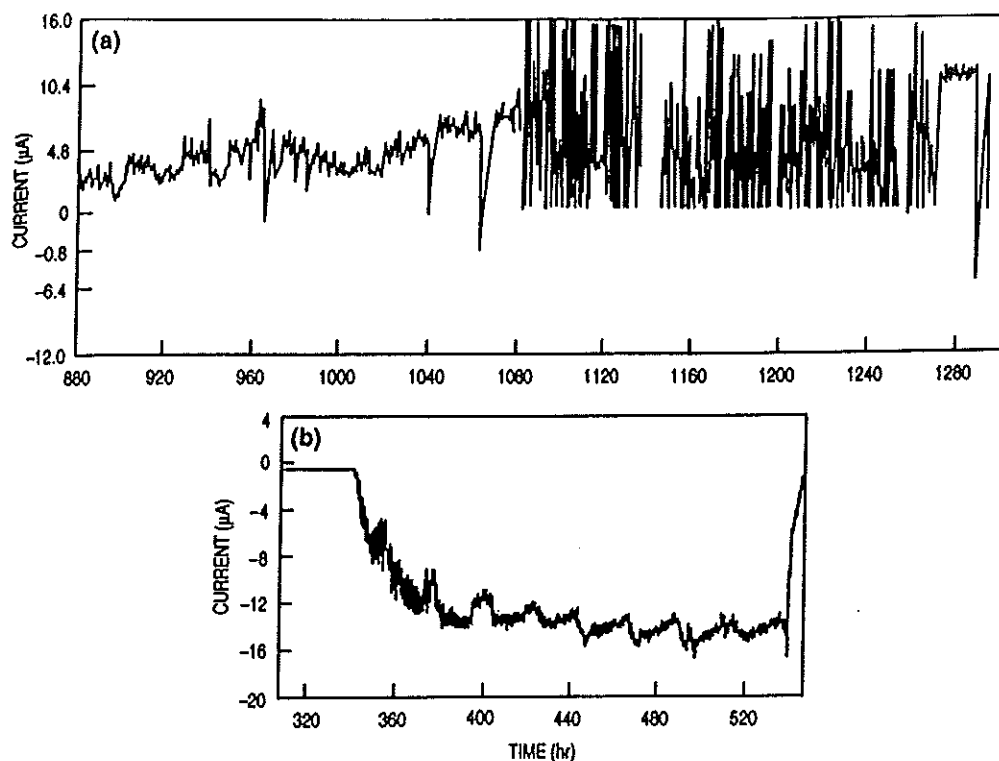


Figure 29. Electrochemical noise plots for carbon steel exposed to *S. putrefaciens* (a) in medium including thiosulfate and sodium chloride and (b) in medium without thiosulfate and sodium chloride.

ions. They reported extensive pitting when both processes were active. When only sulfide was produced, initial corrosion rates increased but later declined due to formation of a protective FeS film. High amounts of soluble iron prevent formation of protective sulfide layers on ferrous metals. Little et al. (1997) attempted to isolate the impacts of iron and thiosulfate reduction on corrosion of carbon steel by controlling the electron acceptors available in the electrolyte. In the initial experiment, thiosulfate was added to the medium, while in the second it was removed. Substantial electrochemical noise was measured in both cases and both electrodes were pitted.

ESEM examination of carbon steel electrodes revealed extensive bacterial colonization after 1300 h (Fig. 30a). The electrode was macroscopically pitted, and the location of pits coincided with colonies of bacteria (Fig. 30b). EDS analysis of the electrode surface showed that, like goethite and ferrihydrite, the modified surface, while still iron rich, had a complex mixture of elements, including phosphorous, sulfur, and chlorine (Fig. 30c). Mineral replacement reactions were documented by X-ray crystallography. After exposure to *S. putrefaciens* in anaerobic media, surface oxides were converted to ferrous phosphate.

CONCLUSIONS

Microorganisms influence corrosion of metals by both forming and dissolving minerals. Iron and manganese oxide deposition by bacteria make some metals more vulnerable to pitting and crevice corrosion by forcing the corrosion potential above the pitting potential or by initiating a sequence of events that results in underdeposit corrosion. Biomineral dissolution reactions remove passive layers or force mineral replacement reactions that lead to further dissolution. Because of the economic consequences of

corrosion to both industry and military, causal relationships between bacteria and metal corrosion products have been investigated. Recently developed techniques including environmental scanning electron and X-ray microscopies make it possible to determine spatial relationships between microorganisms within biofilms and minerals as they are formed, dissolved, or replaced.

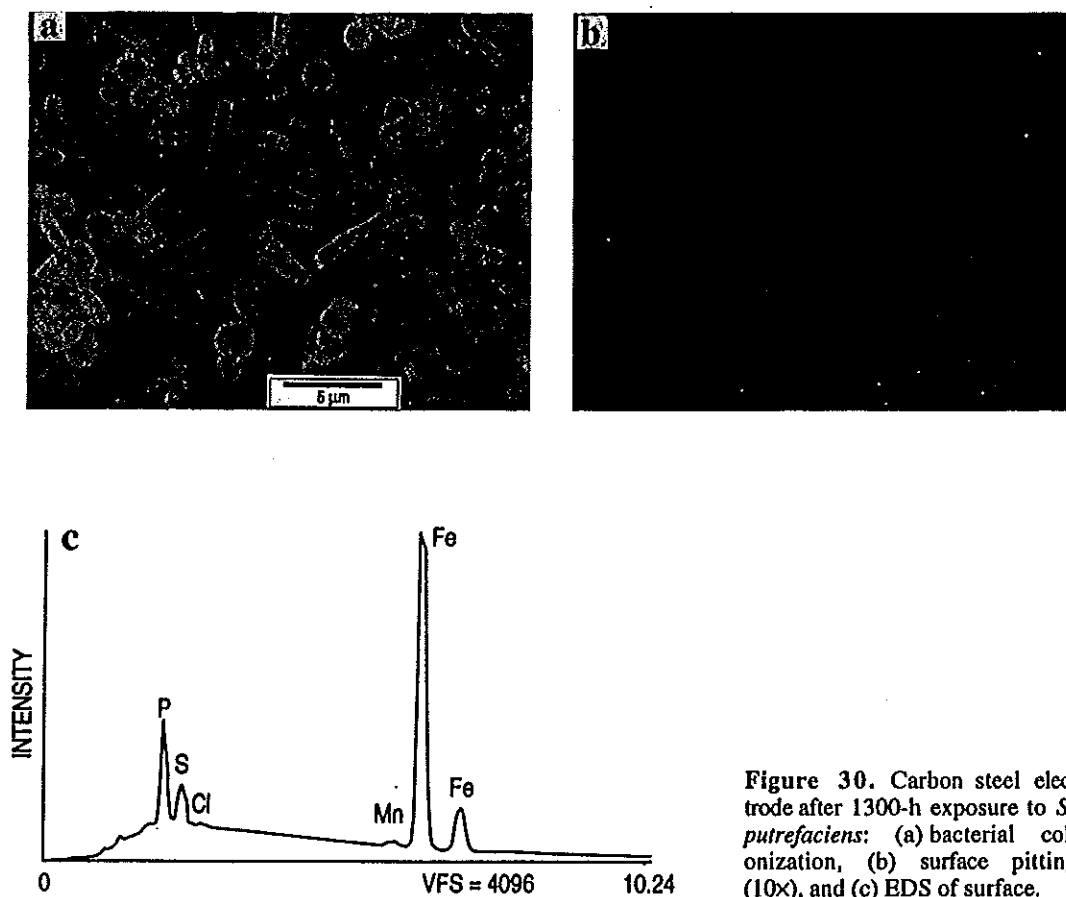


Figure 30. Carbon steel electrode after 1300-h exposure to *S. putrefaciens*: (a) bacterial colonization, (b) surface pitting (10 $\times$), and (c) EDS of surface.

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. As a consequence, microorganisms within biofilms produce minerals and mineral replacement reactions that are not predicted by thermodynamic arguments based on the chemistry of the bulk medium. For that reason, minerals within corrosion products can often be used as fingerprints for microbiologically influenced corrosion. For example, even though the region of stability predicted for mackinawite (tetragonal FeS_{1-x}) using stability diagrams is wholly outside the region defined by surface water Eh/pH conditions, excluding waters influenced by peat bogs, coal mines, volcanic activity and industrial effluents, mackinawite is easily produced from iron and iron oxides in surface waters by consortia of microorganisms that include SRB. The presence of mackinawite in corrosion products formed in shallow water environments, with the exclusions previously delineated, is proof that corrosion was SRB-induced.

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