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Richard I. Ray, Jason S. Lee and Brenda J. Little

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IRON-OXIDIZING BACTERIA: A REVIEW OF CORROSION MECHANISMS IN FRESH WATER AND MARINE ENVIRONMENTS

Richard I. Ray[*], Jason S. Lee[*], Brenda J. Little[**]
Naval Research Laboratory
Codes 7332[*]/7303[**]
Stennis Space Center, MS 39529

ABSTRACT

Models for corrosion influenced by iron-oxidizing bacteria (IOB) in fresh water are specific for material/environment combinations, i.e., 300 series stainless steel exposed to oxygenated chloride-containing potable water and carbon steel exposed in oxygenated fresh water ($[Cl^-] \leq 20$ ppb) containing dissolved copper. Reports of IOB influenced corrosion in marine environments have been limited to rusticle formation on shipwrecks. IOB involved in corrosion in fresh water include *Gallionella, Leptothrix,* and *Siderocapsa.* Historically these organisms have also been thought to be active in marine environments. New isolation and molecular identification techniques are demonstrating the presence of novel IOB in both freshwater and marine environments, and expanding our understanding of their potential role in microbiologically influenced corrosion.

Key words: iron-oxidizing bacteria, microbiologically influenced corrosion, freshwater, marine, steel, copper

INTRODUCTION

Iron-oxidizing bacteria (IOB) have been implicated in microbiologically influenced corrosion (MIC) since the 1960's.[1] IOB derive energy from the oxidation of ferrous (Fe^{2+}) to ferric (Fe^{3+}) at/near neutral pH and in some cases the result is the formation of dense deposits of Fe oxides. Most IOB are microaerophilic, requiring low concentrations of oxygen (O_2) for growth. For example, Druschel et al.[2] determined that the maximum O_2 levels associated with growth of the IOB *Sideroxydans lithotrophicus* were 15-50 μM. Because of the requirement for low concentrations of O_2, IOB are often found in association with other microorganisms or in areas where reduced iron is exposed to an aerobic environment. However, IOB contribute substantially to Fe^{2+} oxidation rates in low O_2 environments with a sustained concentration of Fe^{2+}.[3,4] It is difficult to isolate microaerophilic IOB, due to their relatively fastidious requirements for growth. The liquid medium gradient method of Kucera and Wolfe[5] uses opposing gradients of Fe^{2+} and O_2 for culturing IOB. This gradient method allows the microorganisms to grow under their preferred oxygen concentration (diffusing from the top of the test tube), with a continuous source of Fe^{2+} diffusing up from a plug of reduced iron present at the bottom of the tube. A recent modification of Kucera and Wolfe's method[5] by Emerson and Moyer[4] uses agarose to provide a more solid matrix for establishing the O_2 and Fe^{2+} gradients. The IOB form discrete layers of cells in the agarose at their preferred O_2/Fe^{2+} concentrations. As a result of the development of this agarose gradient tube technique, several new isolates of obligatory lithotrophic IOB have been identified, e.g., *Sideroxydans* and *Mariprofundus*.[6]

IOB INFLUENCED CORROSION IN FRESH WATER ENVIRONMENTS

The IOB that have received the most attention in MIC are *Gallionella, Leptothrix,* and *Siderocapsa.* Most of the documented case histories MIC associated with IOB have involved exposure of a 304 or 316 stainless steel to well water or chlorinated drinking water. The corrosion mechanism is under-deposit corrosion or formation of a differential aeration cell.[7-15] Under stagnant conditions, IOB form dense deposits within months, excluding oxygen from the area immediately under the deposit and initiating a series of events that are individually or collectively very corrosive (Figure 1). In an oxygenated environment, the area deprived of oxygen becomes a relatively small anode compared to the large surrounding oxygenated cathode. Metal at the anode dissolves, forming metal cations that undergo hydrolysis and decrease pH. The extent of the pH decrease is determined by the alloy composition.[16] For this reason, underdeposit attack is particularly aggressive on 300 series stainless steels, containing 17.5 to 20% Cr. In addition, chloride (Cl^-) from the electrolyte migrates to the anode to neutralize any buildup of charge, forming metal chlorides that are extremely corrosive. Under these circumstances, pitting involves the conventional features of differential aeration, a large cathode:anode surface area and the development of acidity and metallic chlorides.

In a study to determine the cause of aggressive localized corrosion on carbon steel pilings in Duluth-Superior Harbor, MN and WI, (DSH), Hicks,[17] using the gradient technique described by Emerson and Moyer,[4] isolated an IOB from corroded areas on coupons. He identified the organism as *Sideroxydans lithotrophicus* by sequencing the 16S rDNA. The corroded carbon steel (CS) pilings have an orange rusty appearance (Figure 2). Divers reported that tubercles were randomly distributed from the waterline to approximately 3 m below the surface, an area influenced by ice scouring.[18,19] Tubercles varied in diameter from a few millimeters to several centimeters and when removed, large and often deep pits were exposed (Figure 3a&b). Ray et al.[20] examined tubercles formed in DSH in detail. X-ray diffraction data indicated that tubercles were amorphous Fe oxides surrounded by magnetite (Fe_3O_4). Transmission electron microscopy (TEM) confirmed amorphous Fe oxides in association with bacteria (Figure 4). Tubercles were made up of porous layers or strata (Figure 5). Diatoms, animals with siliceous frustules, colonized the topsides of the tubercles, and an energy dispersive x-ray spectroscopy (EDS) spectrum analysis of the topside indicated the presence of Mg, Al, Si, S, K, Ca and Mn in addition to Fe. The underside of the tubercle, the surface that had been in contact with the metal, was comprised of bacteria with two predominant morphologies: large rod-shaped bacteria (Figure 6a) and long Fe-encrusted filaments (Figure 6b) as identified by environmental scanning electron microscopy (ESEM). Twisted filaments found in the DSH tubercles are typical of sheath-producing microaerophilic IOB, e.g., *Gallionella.* The bacterium is a kidney-shaped cell (not evident in Figure 6b) with an elongated stalk made up of helically wound mineralized fibrils. The underside of the tubercle contained elevated concentrations of S, Sn, Cr and Cu compared to the exterior. Cu was localized at the base of the tubercles and was evident as a greenish sheen on the underside of a tubercle (Figure 7). Gerke et al.[21]

examined five tubercles from a single drinking water distribution system, evaluating, morphology, mineralogy and chemistry. The overall morphology of all five samples was similar - a core (either soft or hard) with a hard shell layer, covered with surface material. They demonstrated that heavy metals were either trapped within the structure or sorbed onto regions of the tubercles. The overall morphology of DSH tubercles was similar to that described by Gerke et al.[21] The possibility that bacteriogenic Fe oxides in DSH tubercles sorbed Cu was considered and discarded. The distribution of Cu in the DSH tubercles was a well-defined layer at the base of the tubercles.

Ray et al.[20] used galvanic couples[22] to represent the following sequential conditions on the surface of the pilings: 1) establishment of localized O_2 concentration cells as a result of tubercle formation, 2) deposition of Cu under anaerobic conditions 3) formation of a galvanic couple between deposited Cu and underlying CS and 4) exposure of the galvanic couple to O_2 when the ice scour disrupts the tubercle. Synthetic lake water (20 ppm or 5.6×10^{-4} M NaCl) containing concentrations of 32, 16, 8, and 0 ppm Cu^{2+} (molar concentrations of 5×10^{-4} M, 2.5×10^{-4} M, 1.25×10^{-4} M, and 0 M, respectively) was prepared from deionized water and reagent-grade crystalline Cu sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$). To examine the effect of [Cl^-], a solution of 10 ppm Cl^- (2.8×10^{-4} M NaCl) with 32 ppm Cu^{2+} was also prepared. [Cu^{2+}] and [Cl^-] were representative of DSH water. CS (chemical composition by %, C, 0.17-0.23; Mn, 0.3-0.6; P max, 0.04; S max, 0.05 and Fe, balance) was machined into discs 1.58 cm (5/8 in.) dia. x 0.158 cm (1/16 in.) thickness and squares 10.2 x 10.2 x 0.32 cm (4 x 4 x 1/8 in.). Prior to exposure to synthetic lake water, coupons were rinsed in acetone, ethanol and distilled water and dried with N_2 gas to removed grease and residual surface debris. Disc shaped CS coupons were mounted separately in electrode holders with a knife-edged polytetrafluoroethylene (PTFE) gasket defining an exposure area of 1 cm^2. The electrode holder fit into a spherical glass corrosion cell, similar to the standard cell detailed in ASTM G-5.[23] A separate 2 L beaker contained one square CS coupon (10 cm x 10 cm x 0.5 cm) along with a saturated calomel electrode (SCE). New coupons were used for each experiment. A salt bridge was fabricated from plastic tubing filled with saturated KCl solution and sealed with glass frits on either end. Electrical conduction was achieved by placing each end of the salt bridge into the two containers. At the onset of each experiment the electrode holder was removed from the corrosion cell, 700 ml of synthetic lake water was added to the corrosion cell and deaerated with bubbled N_2 gas for 1 hr. At the same time, 500 ml of the same solution was added to the 2 L beaker so that 100 cm^2 of the square CS coupons was submerged. The beaker was left open to air. After 1 hr, the electrode holder was placed into the spherical cell so that the entire exposed surface (1 cm^2) of the coupon was submerged in the deaerated solution. The solution was bubbled continually with N_2. The cells were then immediately connected to a computer-controlled potentiostat/zero resistance ammeter (ZRA) where the 1 cm^2 disc coupon was connected to the working electrode (WE) cable, the 100 cm^2 square coupons to the counter electrode (CE) cable, and the SCE to the reference electrode (RE) cable. Each electrochemical cell had its own WE and RE. The two CS coupons were coupled through the ZRA and maintained at the same potential - the couple potential vs. SCE. The current flowing between the two electrodes was recorded every minute over a 24 hr period. In this configuration the 1 cm^2 electrode in the deaerated synthetic lake water represented the anaerobic area under the tubercle and the larger electrode represented the surrounding area exposed to O_2. Positive current indicated electrons flowing from the small disc coupon (anode) to the large square coupon (cathode), i.e., the anode corroded preferentially to the cathode.

In the Ray et al.[20] experiments the magnitude of the galvanic current was related to the amount of copper deposited on the surface, which was directly related to the concentration of dissolved Cu^{2+}. Cu^{2+} precipitated on anaerobic CS surfaces. A positive current established by the galvanic couple between the Cu-coated CS (anaerobic anode) and the larger CS (aerobic cathode) was initially high, but stabilized within a few hours (Figure 8). The positive value of the galvanic current indicated that the anode was preferentially corroding with respect to the cathode. Peak positive galvanic current scaled linearly with solution [Cu^{2+}] (Figure 9) and can be explained by increased anodic kinetics for Cu-coated CS under anaerobic conditions (Figure 10). When O_2 was introduced to the anaerobic anode, the galvanic current reversed to negative values for all solutions spiked with Cu^{2+} (Figure 11). With both coupons exposed to O_2, the Cu-coated CS coupon had a higher corrosion potential that the larger CS coupon due to Cu having a higher redox potential than Fe. However, after 0.5 hr the galvanic current again reversed to positive values indicating that the Cu-coated coupon was again preferentially corroding with respect to the larger CS coupon. Ice scouring breaks tubercles allowing ingress of O_2 and aggressive corrosion. The depth of the aggressive corrosion coincides with the range over which ice scour is reportedly important. The peak galvanic current under both anaerobic and aerobic conditions was related to [Cu^{2+}] over the range of 0 to 32 ppm (Figures 9 & 12).

Accelerated corrosion of CS in contact with Cu in fresh water has been acknowledged since the early 1920's.[24] Several investigators have reported metal-binding, including Cu, by bacterial exopolymers.[25,26] Others have demonstrated that bacterial exopolymeric substances (EPS) rich in uronic acids promote deterioration of metals.[27,28] Geesy et al.[29] described deterioration of a metallic Cu film due to the formation of Cu concentration cells. In their studies, cells of an adherent freshwater bacterium produced EPS capable of binding Cu^{2+}, creating Cu concentration gradients on the surface. Bacteriogenic Fe oxides, made up of intact and/or partly degraded remains of bacterial cells mixed with amorphous hydrous ferric oxides, are formed in response to chemical or bacterial oxidation of Fe^{2+} to Fe^{3+}. Bacteriogenic Fe oxides have reactive surfaces and act as sorbents of dissolved metal ions and enrichments of Pb, Cd, Al, Cu, Cr, Mn, Sr and Zn have been reported.[30-32]

IOB INFLUENCED CORROSION IN MARINE ENVIRONMENTS

Emerson et al.[33] indicated that little is known about marine IOB because the oceans are generally considered aerobic and depleted in Fe^{2+}. However, they identified *Mariprofundus ferroxydans*, a stalk forming marine IOB as a common isolate of Fe-rich microbial mats associated with hydrothermal vents in the deep ocean. A number of other researchers have observed iron stalk and sheath structures at similar sites[33,34] and work investigating the diversity and distribution of these organisms is ongoing.[35]

Ray et al.[36] demonstrated iron-encrusted bacteria associated with tubercles on a corroded weld of 316L stainless steel (UNS S30403) after 10-week exposure to flowing seawater (Figure 13). The term "rusticle" was coined by Ballard[37] to describe rust features covering the wreck of the ocean liner RMS *Titanic*. The wreck has been at a depth of 3,800 m for 95 years at a temperature of 1°C and 6000 psi. He described these features as very fragile reddish brown stalactites of rust hanging down as much as several feet, and speculated this was caused by "iron-eating bacteria".[37] Cullimore[38,39] described rusticles on the *Titanic* as, "a complex of microbial communities within an iron-rich and calcium deficient porous-like home." He proposed a corrosion mechanism whereby IOB "were extracting iron from the steel of the ship and then exporting that iron into the oceanic environment as red dust and yellow colloids." He further observed more rusticle-type growth in 1998 than in 1996. Stoffyn-Egli and Buckley[40,41] studied the mineralogy and microbiology of rusticles recovered from the *Titanic* and concluded that bacteria caused the precipitation of an outer shell of lepidocrocite (γ-FeO(OH) and that the interior of the rusticle was euhedral goethite (α-FeO(OH)) crystals. In addition to IOB they identified sulfate-reducing bacteria (SRB) as responsible for rusticle formation. They proposed a theoretical mechanism for rusticle formation that included SRB, reducing conditions on a small scale within rust flakes and the co-existence of minerals with different redox potentials. Herdendorf et al.[42] described similar formations on the wreck of the SS *Central America*, a wooden steamer with iron machinery that has been on the floor of the North Atlantic Ocean in 2200 m water for 144 years (5.6 mg/L O_2). *Leptothrix* and *Siderocapsa* were tentatively identified as the organisms causing the rusticles based on light microscopy evidence.

CONCLUSION

A review of the literature on IOB influenced corrosion in fresh and marine waters demonstrates the complexity of potential mechanisms and the need to understand specific microorganism/metal/ environment interactions. There are no explanations for tubercle formation in fresh water and rusticle formation in marine waters by the same organism.

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FIGURES

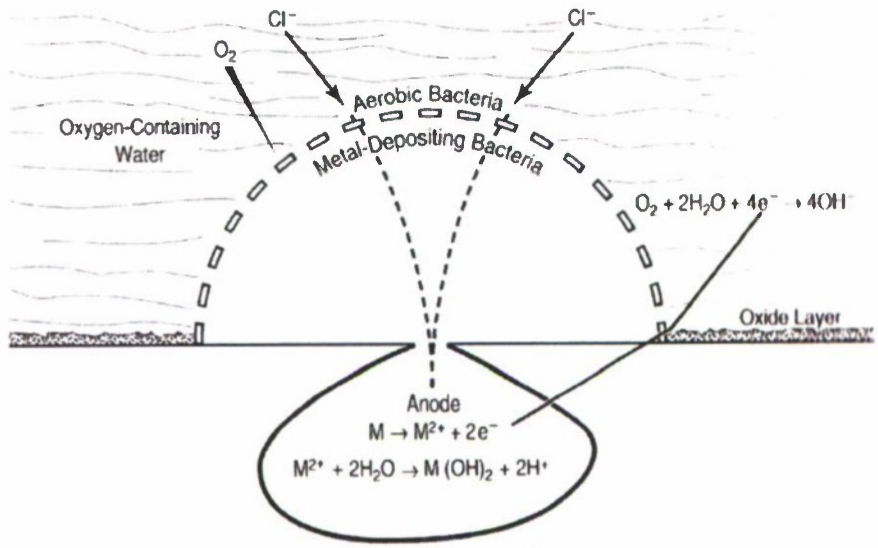

Figure 1. Possible reactions under tubercles created by metal depositing bacteria.

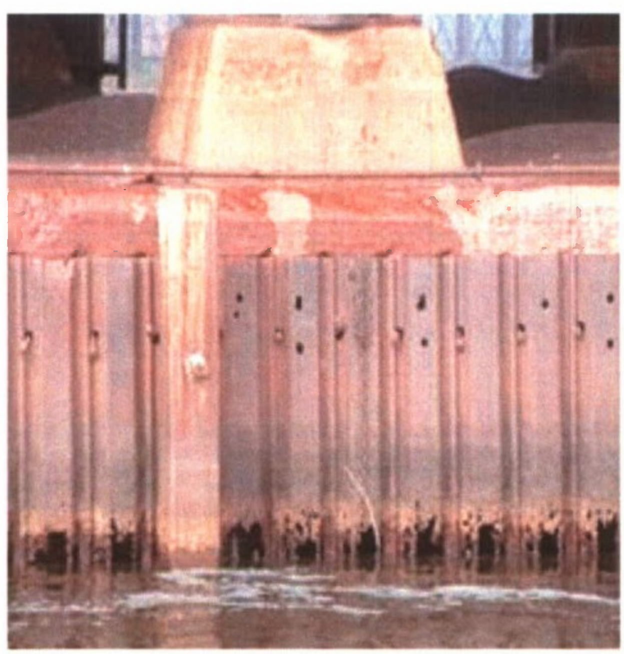

Figure 2. DSH piling with visible perforation at the water line.*

* Photographs reproduced with permission from Gene Clark, Wisconsin Sea Grant Program.

a)

b)

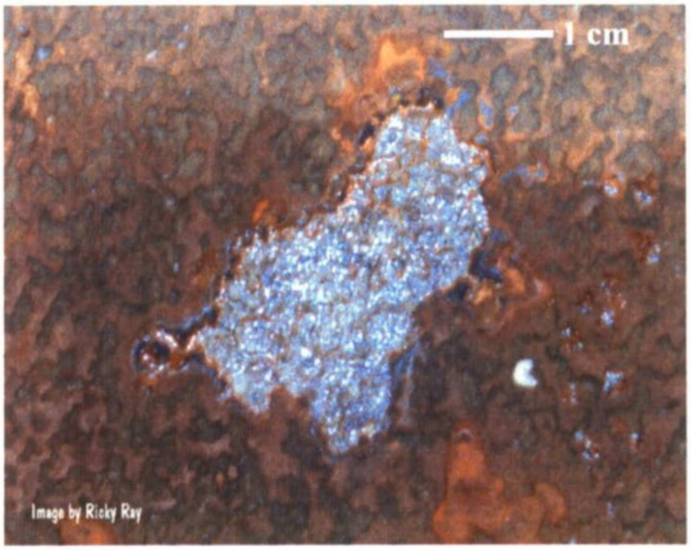

Figure 3a&b. a) Image of tubercle on CS surface and b) after physical removal of tubercle exposing pits underneath the tubercle.

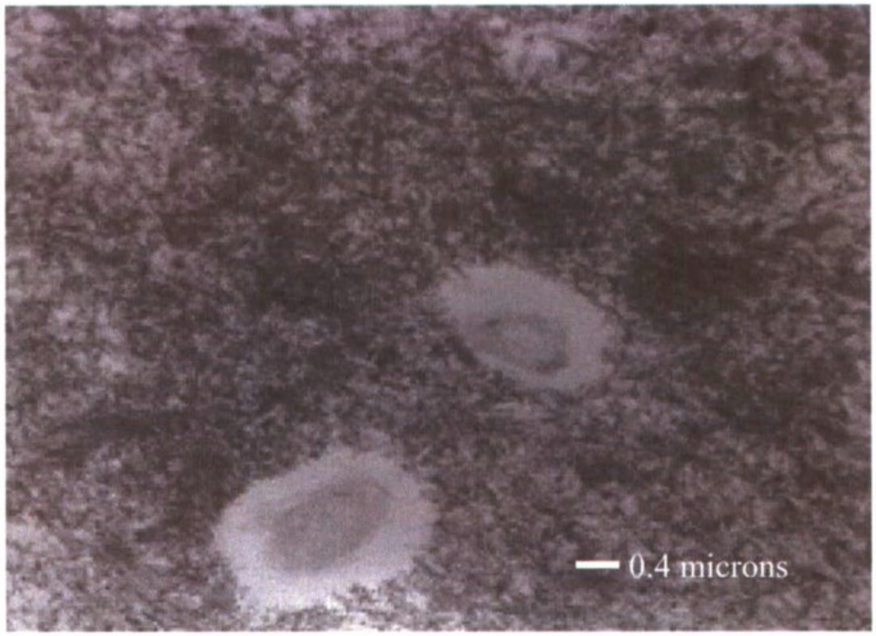

Figure 4. TEM micrograph of tubercle interior in cross-section, showing two bacteria (1.2 micron diameter) surrounded with amorphous iron oxides.

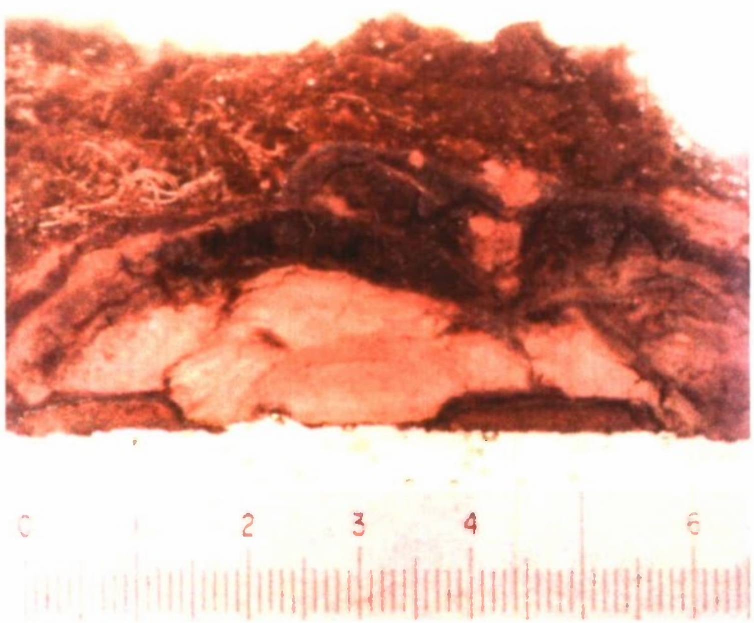

Figure 5. Cross-section of resin-embedded DSH tubercle, showing layers of material within the core. The scale bar indicates 0 – 6 cm.

a)

b)

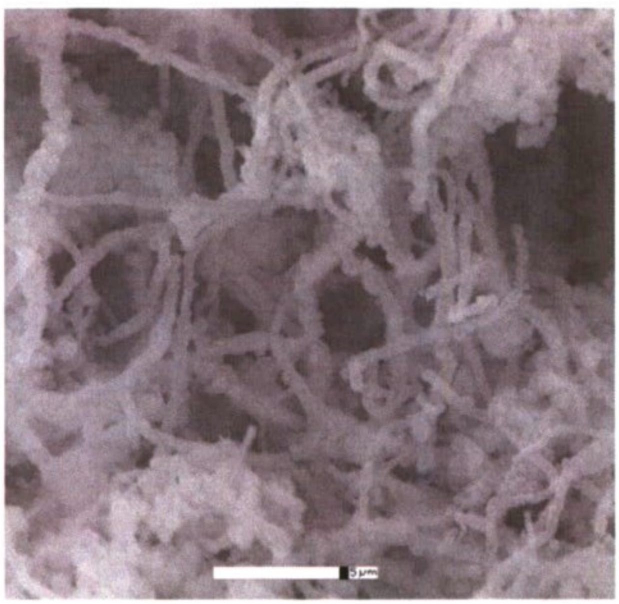

Figure 6a&b. ESEM micrographs from the underside of a tubercle indicating the presence of (a) large rod-shaped bacteria and (b) long iron-encrusted filaments.

Figure 7. Planar view of the underside of a tubercle. Green sheen is due to accumulation of Cu.

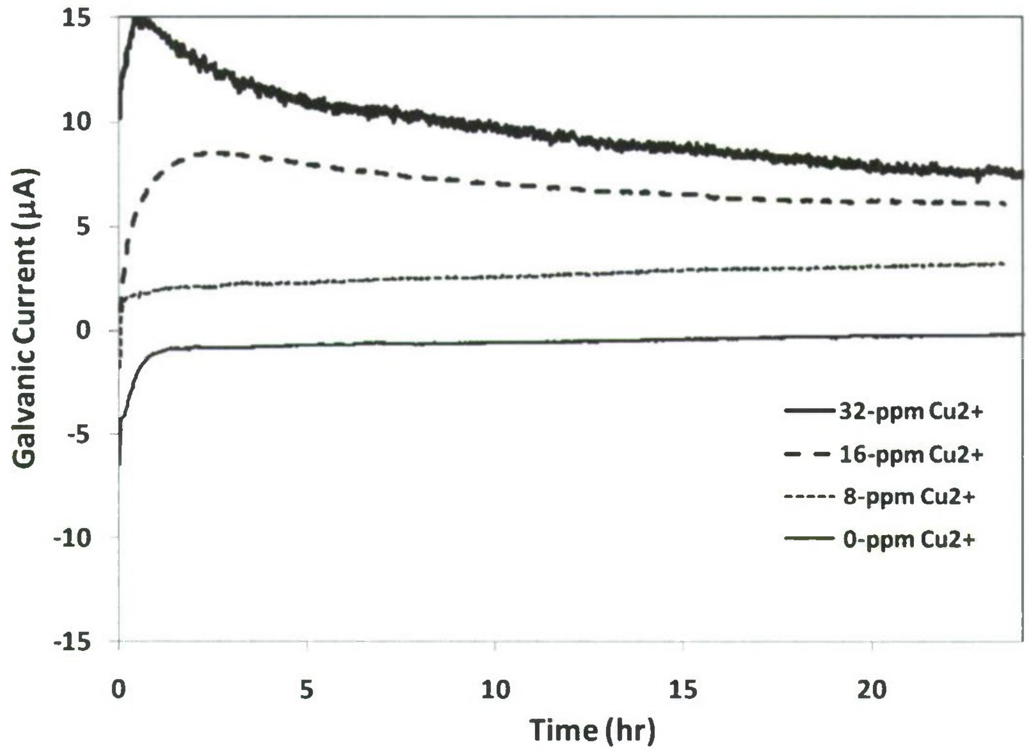

Figure 8. Galvanic current (μA) measured for 24 hr for 1 cm^2 CS coupon in deaerated solutions with 20 ppm Cl^- and different [Cu^{2+}] coupled to 100 cm^2 CS coupon exposed to the same solution under aerated conditions.

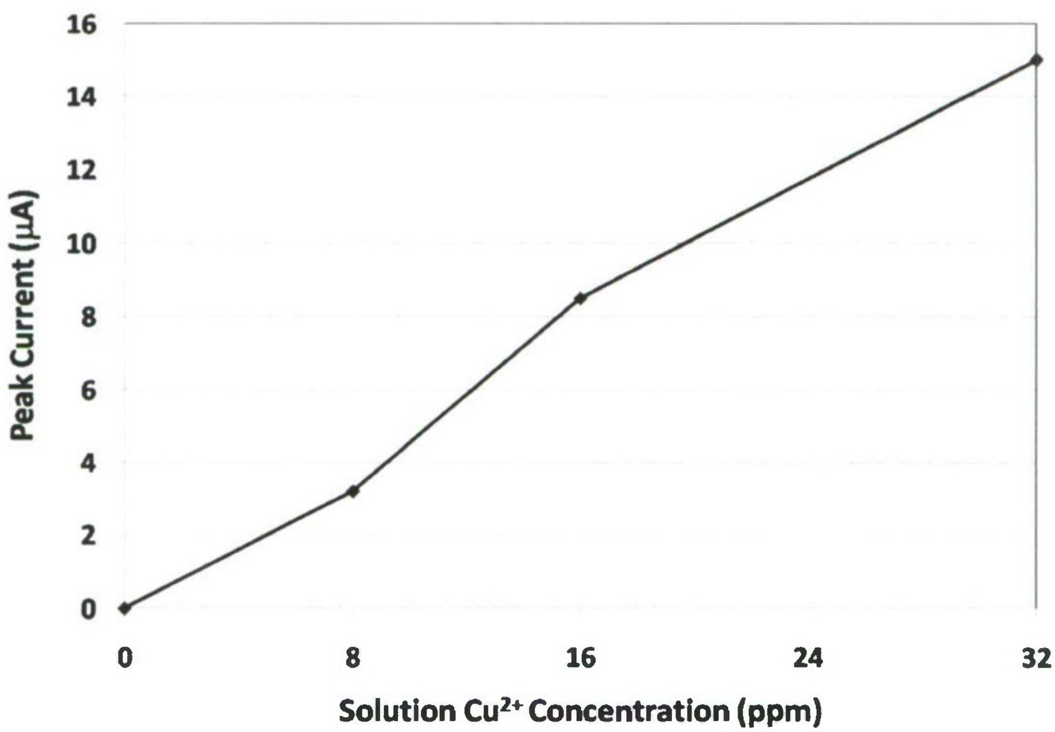

Figure 9. Peak galvanic current (μA) measured during the first 24 hr of exposure under deaerated conditions (for 1 cm^2 coupon) vs. the [Cu^{2+}] in solution.

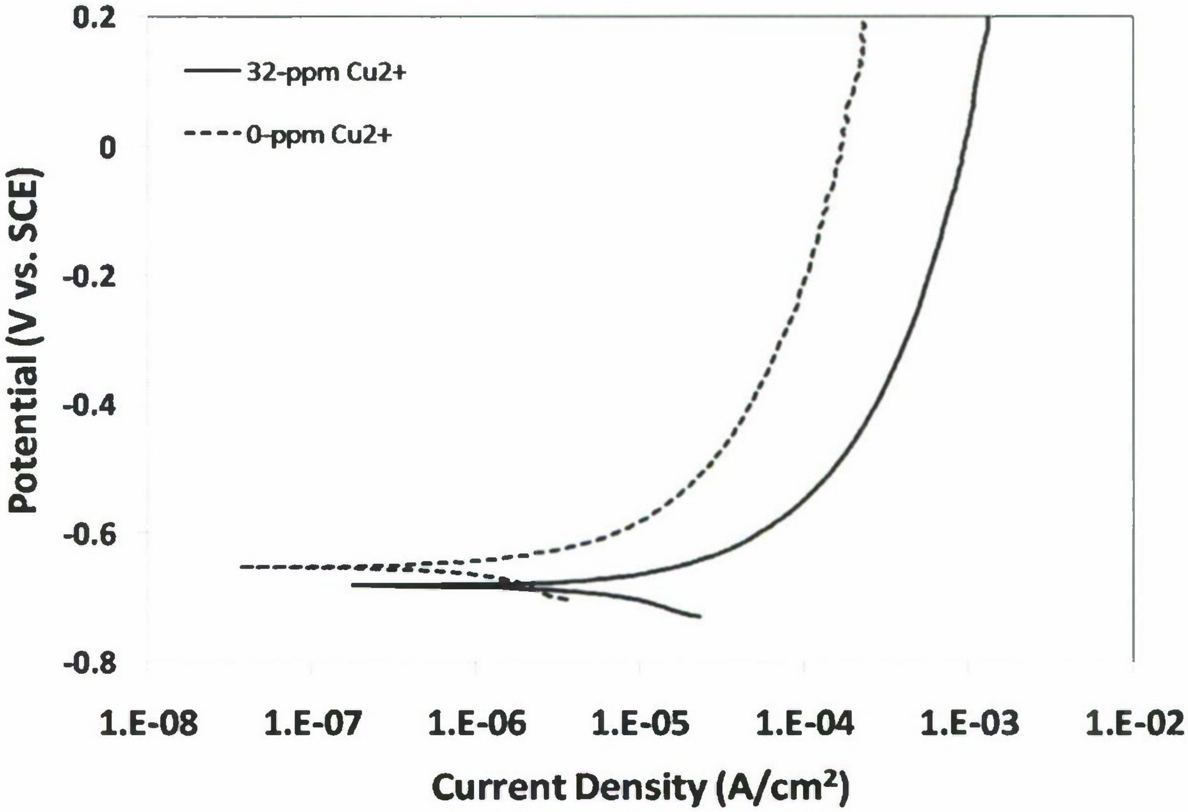

Figure 10. Anodic polarization scans of 1 cm^2 CS coupons after 24 hr exposure in deaerated solutions of 32 and 0 ppm Cu^{2+} with 20 ppm Cl^-.

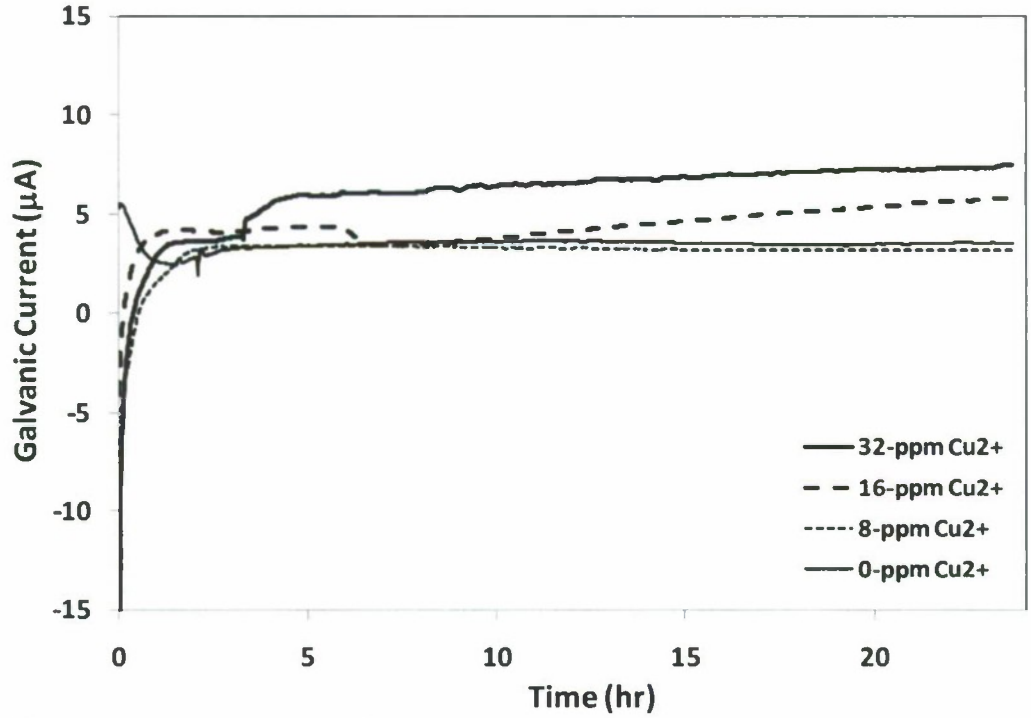

Figure 11. Galvanic current (μA) measured for 24 hr after deaerated solutions (Figure 7) were opened to air.

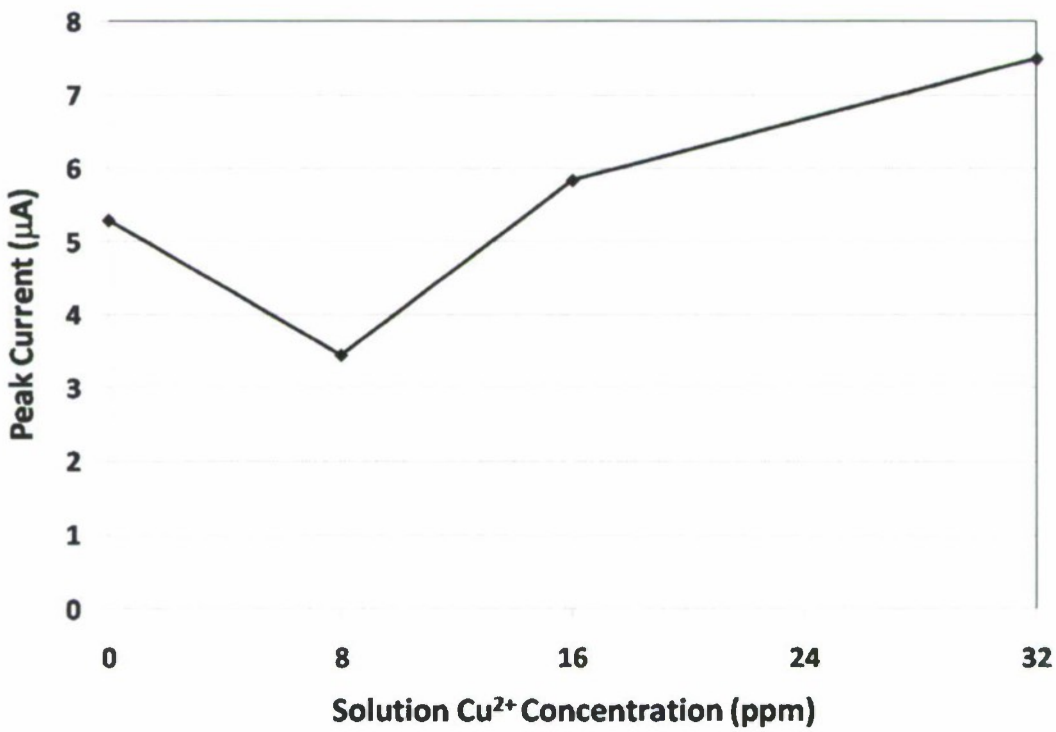

Figure 12. Peak galvanic current (μA) measured during the second 24 hr of exposure under aerated conditions (for 1 cm^2 coupon) vs. the $[Cu^{2+}]$ in solution.

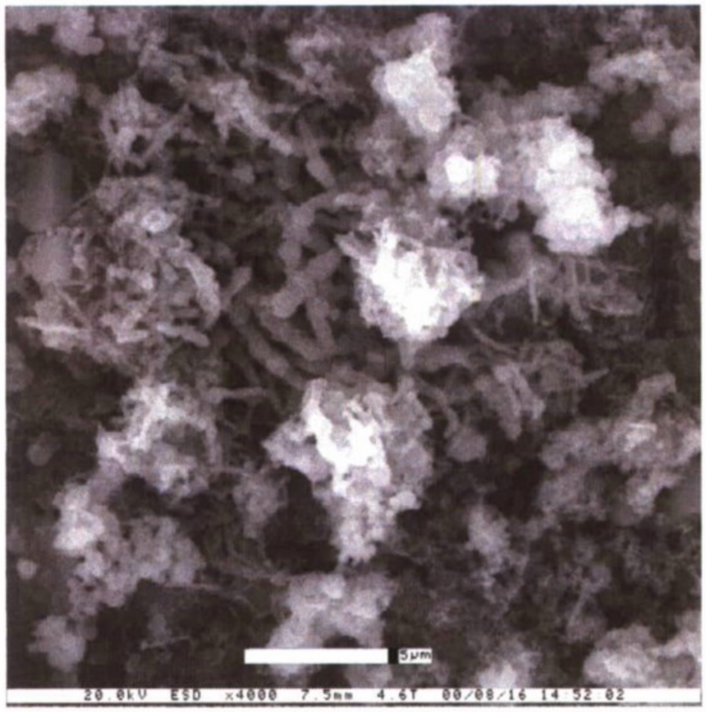

Figure 13. Micrograph of iron-encrusted bacteria associated with tubercles on a corroded weld of 316L stainless steel (UNS S30403) after 10-week exposure to flowing seawater. [36]