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LOCALIZATION AND SPECIATION OF COPPER IONS IN BIOFILMS ON CORRODING COPPER SURFACES

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ABSTRACT

X-ray absorption near edge structure (XANES) techniques can be used to differentiate Cu^{+1} and Cu^{+2} species within biofilms attached to surfaces. Copper ions could not be demonstrated with XANES within a marine biofilm of *Oceanospirillum* on a corroding copper surface. Furthermore, Cu^{+2} concentration cells do not appear to be a significant mechanism for microbiologically influenced corrosion in marine environments.

Keywords: copper, microbiologically influenced corrosion, polymer, XANES

INTRODUCTION

An aerobic, gram-negative, marine bacterium, *Oceanospirillum*, was isolated from several copper-containing surfaces exposed in marine environments. When grown on copper, the organism produces copious amounts of extracellular polymer and accelerates corrosion of copper metal.[1] The organism with associated polymer has been shown to bind copper ions from solution. Geesey et al.[2] demonstrated that exopolymers produced by adherent bacterial cells promoted deterioration of copper. The authors developed a conceptual model for microbiologically influenced corrosion (MIC) in fresh water that required the formation of exopolymer-bound copper concentration cells. Our experiments were designed to determine whether or not the copper-binding properties of the exopolymer from a marine bacterium were important in the corrosion process. We attempted to detect the presence and valence state of copper ions in a marine biofilm and to relate the spatial distribution of bound copper species with localized corrosion.

Methods and Materials

Biofilms of *Oceanospirillum* were grown on 90:10 copper:nickel foils and on glass slides in batch and semi-batch cultures of nutrient-rich (AVS)[3] and nutrient-deficient (glutamate) seawater[4] media for six and ten weeks, respectively. Cultures maintained in batch cultures were not replenished with nutrients over time while medium in semi-batch cultures was replaced biweekly. Glass slides colonized by *Oceanospirillum* were exposed to separate solutions containing Cu^{+1} and Cu^{+2}. Cu^{+1} in solution was maintained in an anaerobic condition to prevent oxidation to Cu^{+2}. Corrosion rates were determined from polarization curves using POLFIT software.[5] Copper:nickel foils were transitioned from culture medium through filtered seawater to distilled water and examined wet using environmental scanning electron microscopy (ESEM) to document the horizontal distribution of cells and localized corrosion.[6] Thin sections of epoxy-embedded foils were examined with transmission electron microscopy (TEM) and ESEM coupled with energy-dispersive x-ray spectroscopy (EDS) to resolve the relationship between bound metals and cells.

Biofilms were removed from copper substrata and bound copper concentrations determined using atomic absorption spectroscopy (AA) and x-ray photoelectron spectroscopy (XPS).[7] X-ray absorption near edge structure (XANES) was used to determine the speciation of copper within biofilms on copper surfaces.[8] The electrochemical impact of copper concentration cells as defined by Geesey et al.[2] was evaluated using a dual-cell corrosion-measuring device[9] with galvanically coupled 99% copper electrodes in tap water and artificial seawater (3.5%).[10] Identical electrodes were allowed to equilibrate for 16 hours to stabilize the galvanic current. Cu^{+2} was added to one individual half-cell as cupric chloride (0.3 mM) and the resulting current measured. In an additional experiment, the dual cell was used to evaluate the electrochemical significance of a Cu^{+1}/Cu^{+2} concentration cell in artificial seawater. One half-cell was deaerated with bubbling nitrogen while the other half-cell was aerated. Cu^{+1} (0.15 mM) was added to the deaerated half-cell, Cu^{+2} (0.15 mM) to the aerated half-cell, and the resulting galvanic current measured.

RESULTS

XANES spectra for Cu^{+1} and Cu^{+2} ions bound from solution within an *Oceanospirillum* biofilm grown on glass slides were unique (Figure 1). Oxidation of Cu^{+1} bound within the biofilm was not observed during exposure to air. The corrosion rate of copper colonized by *Oceanospirillum* depended on the seawater medium and the rate at which nutrients were replenished. The highest corrosion current densities were measured in nutrient-deficient glutamate medium under semi-batch conditions (Figure 2). Cells in association with copious amounts of polymer were distributed in patchy areas on all surfaces exposed to bacteria in both glutamate and AVS media (Figure 3). Localized intergranular corrosion was documented on surfaces colonized in glutamate medium (Figure 4). Attempts to demonstrate copper bound within *Oceanospirillum* biofilms grown on copper surfaces using TEM/EDS, XANES, XPS and ESEM/EDS were unsuccessful. Small amounts of copper (50 ppb) within biofilms from both media were determined with AA. The addition of 0.3 mM Cu^{+2} as cupric chloride to fresh water in one-half of the dual-cell corrosion measuring device resulted in a maximum galvanic current of 0.4 μAcm^{-2}. Under the same experimental conditions, the addition of 0.3 mM Cu^{+2} as cupric chloride to one half-cell of the dual-cell corrosion measuring device containing artificial seawater, no galvanic current could be measured. Results of galvanic current measurements with differential aeration coupled with copper speciation cells produced a maximum of 1.6 μAcm^{-2} in artificial seawater.

DISCUSSION

Copper alloys are vulnerable to MIC in the form of pitting, crevice or underdeposit attack.[11–13] During seawater exposure, biofilms form on copper surfaces within hours.[14] Bacterial exopolymers are known to bind heavy metals from corroding metal substrata[15] and from solution.[16] Metallic ions associated with biofilms can be solubilized, incorporated into inorganic molecules or adsorbed onto internal or external portions of cells. Metal binding to cell envelopes of gram-negative bacteria,[17] accumulation of copper within intracellular lysosomal structures[18] and immobilization of copper ions by extracellular polymers[19] have been previously demonstrated. Valence states of metal ions associated with biofilms is largely unknown.

Surface analytical tools have been used to resolve questions related to bound metals within biofilms. For example, EDS analyses are excellent tools for demonstrating the presence of metal ions within biofilms but cannot be used to determine the speciation of metal ions. Several investigators are attempting to determine the speciation of bound metals within cultures grown in liquid media using XPS.[20,21] However, XPS cannot be used to evaluate metals bound within biofilms attached to surfaces. High flux x-ray beams produced by synchrotron light sources are useful for probing local environments of metal atoms and can be used to investigate gases, liquids, solids, solutions, and gels. XANES provides information on metal site symmetry, oxidation state, and the nature of the surroundings, and the absorption fine structure (EXAFS) provides details about the type, number, and distances of atoms in the vicinity of the absorber. Several studies have investigated Cu-N, Cu-O and Cu-S bonding in proteins.[22,23] Similar bonding sites are likely to be found in marine biofilms.

The role of bound metals in accelerating MIC has not been clearly defined. Scotto et al.[24] attributed ennoblement of corrosion potential in natural seawater to acceleration of the oxygen reduction reaction by organometallic catalysts formed within biofilms. The presence of organometallic compounds formed between bacterial exopolymers and metals, either from a corroding metal surface or from an electrolyte, has never been demonstrated. One mechanism proposed for MIC of copper-containing alloys is related to the binding capacity of microbial exopolymers. The conceptual model for corrosion proposed by Geesey et al.[2] requires the formation of copper concentration cells in which Cu^{+2} generated from the corroding copper substratum is selectively bound within adjacent exopolymers having differential affinities for Cu^{+2}. In the model, the exopolymers are excreted from two different organisms.

The corrosion rate of copper colonized by *Oceanospirillum* for ten weeks depended on the seawater medium. The highest corrosion rates were measured in semi-batch cultures in nutrient-deficient glutamate. Cells in association with copious amounts of polymer were distributed in patchy areas on all surfaces exposed to *Oceanospirillum*. Attempts to demonstrate copper bound within biofilms grown on copper surfaces using XANES, XPS and ESEM/EDS were unsuccessful. Small amounts of copper (50 ppb) within biofilms from both media were determined with AA. Electrochemical data indicate that a galvanic current is generated in tap water by the formation of Cu^{+2} concentration cells. No current was generated in artificial seawater. In our investigations we were able to document Cu^{+1} and Cu^{+2} bound within biofilms grown on glass slides exposed to media containing the specific ions. Once Cu^{+1} was bound within the biofilm under anaerobic conditions, exposure to air did not result in further oxidation. Galvanic current measurements indicate that differential binding of Cu^{+1} and Cu^{+2} within adjacent aerobic and anaerobic regions within marine biofilms may be a significant mechanism for MIC.

CONCLUSIONS

XANES appears to be an excellent technique for detecting copper ions and their speciation *in situ* within biofilms. Based on surface analytical and electrochemical data, it is unlikely that the formation of Cu^{+2} concentration cells is a mechanism for MIC of copper alloys in marine environments. The electrochemical impact of Cu^{+2} concentration cells varies with the electrolyte and may be significant in fresh water systems and on surfaces that have adjacent aerobic and anaerobic areas within biofilms.

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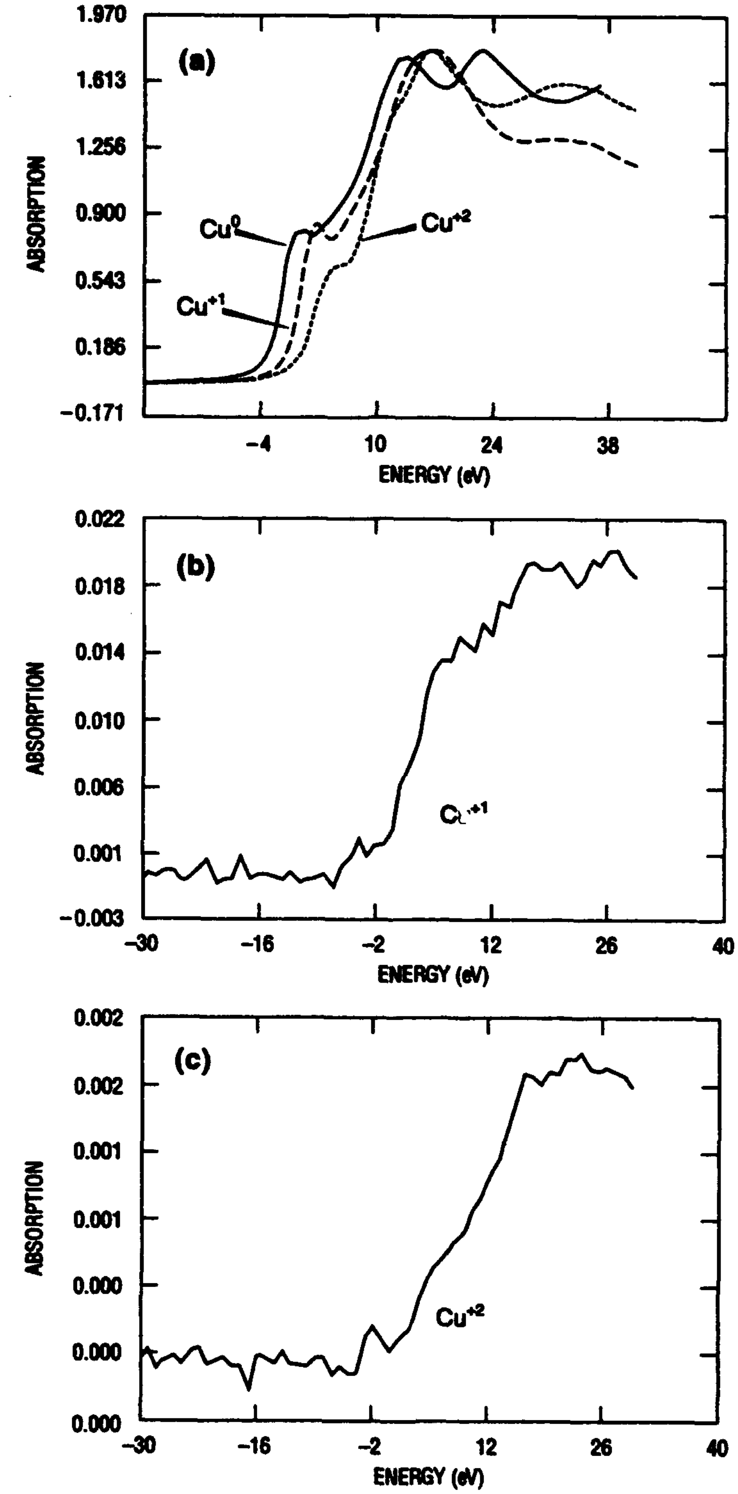

FIGURE 1 – XANES spectra (a) copper foil (Cu^0), cuprous oxide (Cu^{+1}), and cupric oxide (Cu^{+2}); (b) Cu^{+1} bound from solution within a biofilm of *Oceanospirillum* grown on a glass slide; and (c) Cu^{+2} bound from solution within a biofilm of *Oceanospirillum* grown on a glass slide.

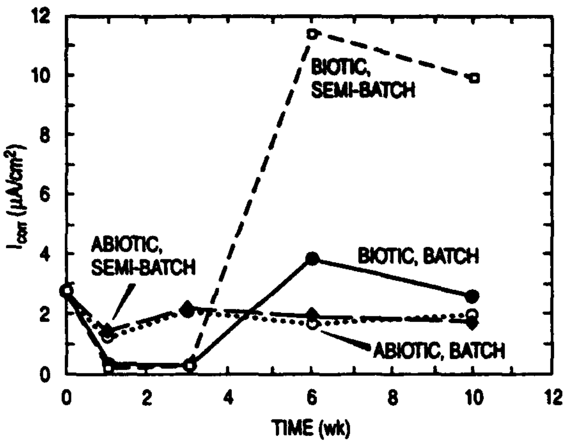

FIGURE 2 – I_{corr} vs time for 90:10 copper:nickel in nutrient-deficient glutamate medium colonized with *Oceanospirillum* compared to abiotic controls for batch and semi-batch cultures.

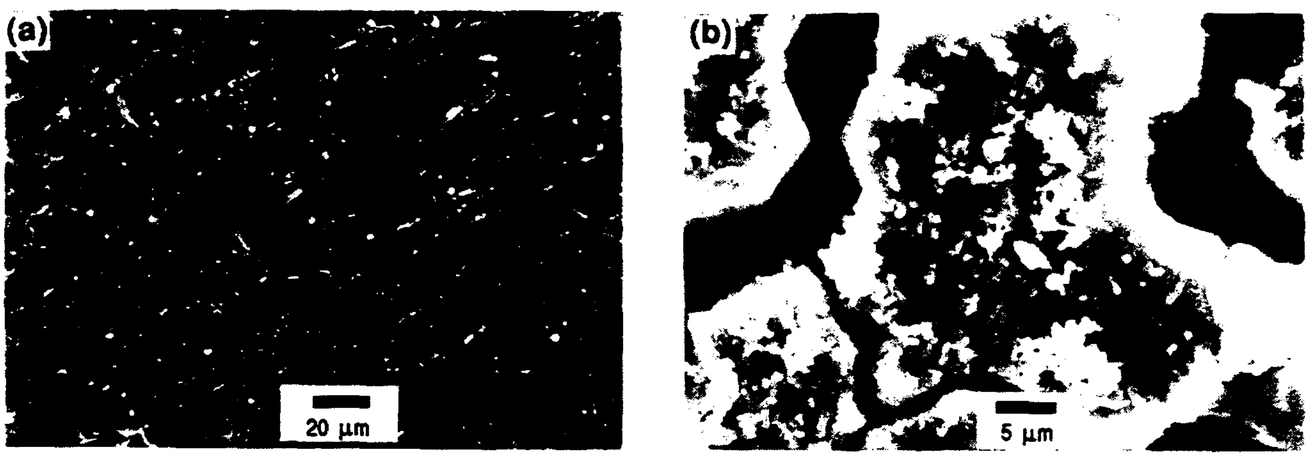

FIGURE 3 – ESEM micrographs of 90:10 copper:nickel surfaces after 10 weeks exposure to glutamate medium under semi-batch conditions (a) abiotic control and (b) *Oceanospirillum* culture.

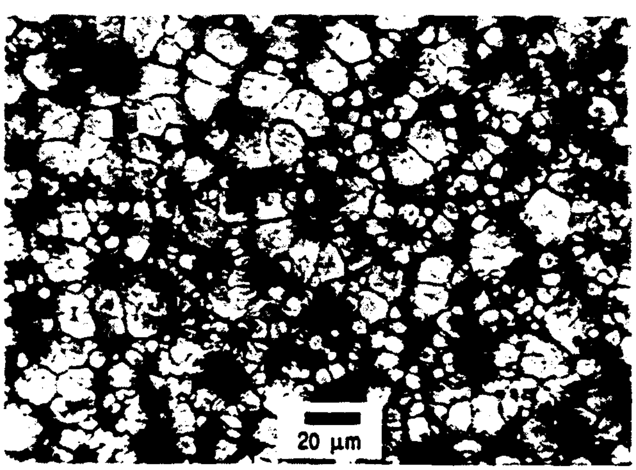

FIGURE 4 – Intergranular corrosion under *Oceanospirillum* biofilm shown in Figure 3b.