

p-147

Role of bacterial exopolymers in the deterioration of metallic copper surfaces*

Gill G. Geesey,* Marc W. Mittelman,* Teki Iwaoka,** Peter R. Griffiths**

Microbial exopolymers that anchor cells to surfaces exhibited saturation binding of copper ions in aqueous suspensions. Copper-binding by the exopolymer isolated from a freshwater sediment bacterium was strongly influenced by pH. The exopolymer also promoted deterioration of a metallic copper film submerged in an aqueous environment. Microcolonies of the exopolymer-producing bacterium, when attached to submerged copper metal surfaces, can form copper concentration cells with adjacent areas in a biofilm containing less reactive exopolymers. These concentration cells can be maintained by the acid-producing activities of the biofilm microflora.

Introduction

MICROBIAL FILMS OF VARIED COMPOSITION AND THICKNESS develop on virtually every type of metallic surface in contact with aqueous environments. The metabolic reactions carried out by certain types of microorganisms residing in biofilms promote corrosion.^{1,2} Some biofilms, however, contain microorganisms whose metabolic activities appear to impede corrosion.³ While a few microbiologically related corrosion reactions are now fairly well understood, our knowledge of many other potentially important reactions induced by microbial metabolism is very limited.

Recent studies on dissolved metal interactions with bacterial exopolymers have promoted consideration of their importance to corrosion-type reactions. Specific attention was focused on reactions that may occur within a hydrated biofilm of bacteria attached to a metallic surface. The results suggest that exopolymers, which promote attachment and biofilm development on a metal surface, also promote the deterioration of the surface. This phenomenon may arise from the influence exopolymers exert on the distribution and speciation of the metal at the solution-surface boundary where corrosion reactions occur.

Methods

The bacterium used in this study was isolated from metal-laden sediments of the Fraser River at New Westminster, British Columbia, Canada.⁴ Although it has not yet been positively identified, "pseudomonas-like" properties were dis-

played when the isolate was subjected to a battery of biochemical tests.⁵ The bacterium did not produce H₂S. Cells grown in broth cultures were washed free of organic nutrients in the growth medium, and their exopolymers were obtained by blending for 60 s in the presence of EDTA. The polysaccharide component of the isolated exopolymers was purified by a procedure that removed protein, DNA, and lipid material. Details of the bacterial growth conditions and isolation and purification of the exopolymer have been described previously.⁶

Metal-binding assays were performed with highly purified exopolymer by equilibrium dialysis according to the procedure outlined by Mittelman and Geesey.⁵ Exopolymer (2.4 µg carbohydrate in 100 µL distilled water) was dialyzed against an equal volume of copper solution (concentration range, 0.79 to 63 µM) prepared by dissolving electroanalytical grade copper metal in Ultrex-grade nitric acid.⁽¹⁾ Dialysis was performed at 4 °C until equilibrium was reached (approximately 36 h). At equilibrium, the total metal concentration on each side of the dialysis membrane was determined by flameless atomic absorption spectrometry. The difference in total copper concentration between the 2 compartments provided an estimation of the quantity of copper bound to exopolymer. Details of the metal-binding determination have been presented elsewhere.⁵

Exopolymer accumulation at the surface of submerged metallic copper films and film deterioration was determined by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). A germanium, circular internal reflection element (Ge-IRE), purchased from Barnes-Spectrattech, Stamford, Connecticut, was coated with a thin film of metallic copper by vacuum evaporation and positioned in an "open boat" cell containing either distilled water or a 1% suspension of a crude preparation of the exopolymer in distilled water. The IR spectra of the bulk solutions were obtained by substituting a zinc selenide (ZnSe) IRE for the copper-coated Ge IRE. Accumulation of exopolymer at the surface of the copper-coated Ge IRE was determined by calculating the ratio of the intensities of the polysaccharide absorption band (1080 cm⁻¹) and the water absorption band (1640 cm⁻¹) according to the method of Iwaoka et al.⁷ Deterioration of the copper film was determined by monitoring the water absorption band at 1640 cm⁻¹ during exposure of the Cu-coated IRE to a crude preparation of the exopolymer. Spectra were obtained with a Nicolet 60-SX Fourier transform infrared spectrometer. Details of the procedure for coating the IRE and performing the ATR-FTIR spectroscopic analyses have been described by Iwaoka et al.⁷

* Presented during CORROSION/85, Paper No. 297, NACE, Houston, TX, 1985.

* California State University, Long Beach, CA 90840.

** University of California, Riverside, CA 92521.

⁽¹⁾ Registered trade name of J. T. Baker Chemical Co., Phillipsburg, New Jersey.

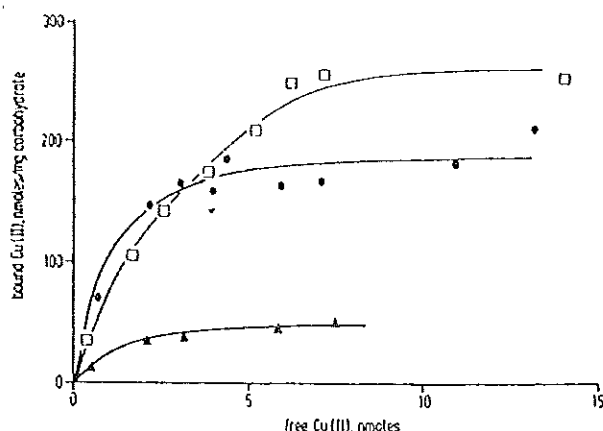


FIGURE 1 — Freundlich plot of copper-binding by highly-purified preparation of exopolysaccharide at pH 7.0 (Δ), 6.0 (●), and 5.0 (□).

Results

Cells of a bacterium isolated from metal-laden river sediments produced exopolymers that mediated their attachment to copper surfaces submerged in aqueous solutions. The exopolymers also promoted the formation of cell aggregates in solution and a slime layer on the inner wall of swirling glass culture flasks. The aggregates and surface film disappeared as the culture passed into late stationary phase. The surface film could also be easily removed from the sides of culture flasks by increased shear force.

Exopolymer material released and isolated from the cells by a method that minimized contamination by intracellular components exhibited the capacity to bind free cupric ion when dialyzed against dissolved copper concentrations ranging from 0.79 to 63 μM . Copper-binding by the exopolymer was influenced by pH (Figure 1). Approximately six times more copper was bound at pH 5.0 than at 7.0. Over this pH range, accumulation of dissolved copper within the hydrated exopolymer matrix resulted in the establishment of a copper concentration gradient that did not require the input of energy or the presence of cells.

Fourier transform infrared spectroscopy revealed the isolated exopolymer exhibited the capacity to accumulate at the surface of a copper-coated internal reflection element submerged in an aqueous suspension of the polymer [Figure 2(A)]. The absorption band centered at 1080 cm^{-1} was assigned to the polysaccharide, whereas, the band centered at 1640 cm^{-1} was assigned to water. After 1 h exposure, the liquid boundary layer adjacent to the copper film exhibited a polysaccharide/water absorbance ratio of 0.71. A ratio of 0.57 was obtained when the bulk aqueous phase of the same exopolymer suspension was sampled with an uncoated ZnSe IRE [Figure 2(B)].

The intensity of the water absorption band increased with increased time of exposure of the copper film to the exopolymer suspension (Figure 3). In contrast, no change in the water band intensity was observed when the copper-coated IRE was submerged in polymer-free water.

Discussion

In abiotic environments, aqueous metal chemistry is controlled by a variety of physical and chemical factors. Under the conditions employed in the equilibrium dialysis study above, free Cu(II) ion was the major copper species in solution at pH 5.0.⁸ Copper hydroxides became more important, however, as the pH increased to 7.0.⁹ It is likely that the increased copper binding capacity observed with decreasing pH reflects a preference by the bacterial exopolymer for the free Cu(II) ion. Previous studies revealed the binding of copper resulted in the release of H^+ from the exopolymer.⁵ These observations sug-

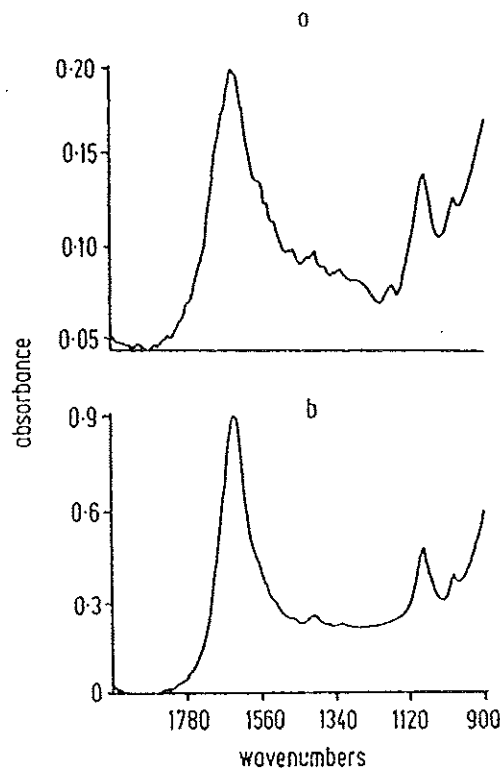


FIGURE 2 — (A) Fourier transform infrared spectrum of a 1% aqueous suspension of a crude preparation of exopolymer sampled with a copper-coated Ge IRE; and (B) a bare ZnSe IRE.

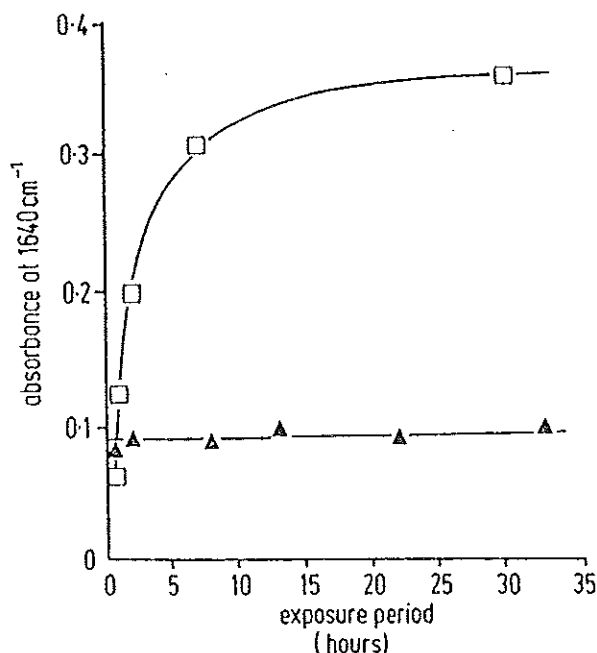


FIGURE 3 — Water absorption at 1640 cm^{-1} during exposure of Cu-coated Ge IRE to a 1% aqueous suspension of a crude preparation of exopolymer (□) and to polymer-free water (Δ).

gest that copper-binding by the exopolymer is cooperative since it leads to a reduction in pH which, in turn, favors formation of the copper species that bind.

Using a Ge IRE like that employed in the present study, Iwaoka et al.⁷ calculated that only the 3 to 4 μm boundary layer adjacent to the IRE is sampled by the evanescent wave of infrared radiation; whereas, a ZnSe IRE of the same geometry samples further into the bulk phase. They also showed that a comparison of the relative intensities of solute and solvent absorption bands obtained with these 2 IREs provided a means to detect solute concentration differences between the bulk phase and the aqueous boundary layer at the Ge IRE surface. The higher polysaccharide/water absorbance ratio obtained with the Cu-coated, Ge IRE compared to that obtained with the uncoated ZnSe IRE in the present study suggests that the exopolymer attained a higher concentration at the copper film-water interface than that present in the bulk aqueous phase. Whether or not the surface accumulation resulted from exopolymer binding to insoluble copper species at the surface and whether or not binding occurred at the same sites on the exopolymer that promoted accumulation of dissolved copper remains to be determined.

The intensity of the water absorption band at 1640 cm^{-1} has been shown to be a sensitive measure of changes in thickness of copper coatings to IREs.⁷ Thickness variations of a few angstroms or a few atomic layers, in the case of copper films, can be detected. The increased water absorbance observed during exposure of the copper-coated IRE to the bacterial exopolymer suspension indicates the thickness of the copper film decreased. The deterioration of the film was likely the result of the exopolymer since no changes in water absorption were observed over a similar period in the presence of polymer-free water. A similar phenomenon has been reported when a copper film was exposed to an aqueous suspension of the acidic plant polysaccharide, Gum Arabic.⁷ The initial rate of deterioration of the copper surface was greater, however, in the presence of the bacterial exopolymer. Deterioration did not result from initial differences in solution pH since both the bacterial exopolymer and Gum Arabic, as well as the polymer-free water, were adjusted to pH 6.8 before exposure to the IRE.

Although the nature of this surface destruction has not yet been determined, it is possible that high affinity copper-binding by exopolymer concentrated at the IRE surface reduced the dissolved copper concentration in the biofilm to such an extent that dissolution of metallic copper occurred to establish equilibrium with the dissolved copper species.

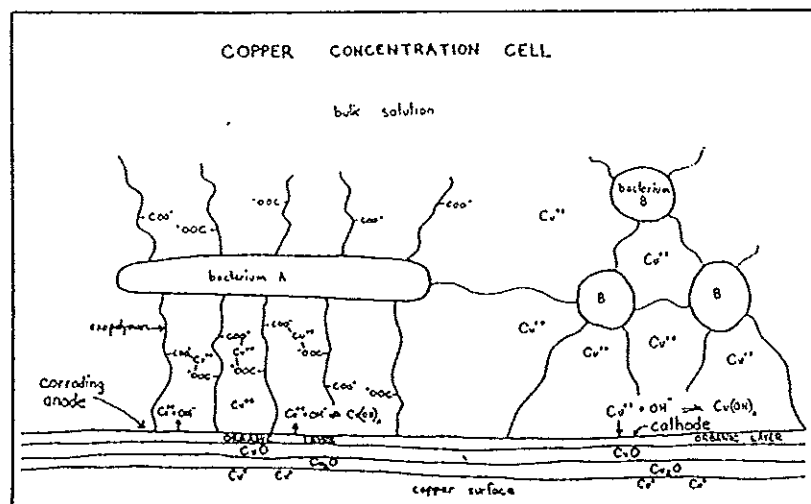
Dissolution would continue until all binding sites are saturated or until the metal is depleted. The plateau observed in Figure 3 may reflect one of these processes.

The exopolymers produced by the freshwater bacterium described in this study formed a hydrated network of extracellular fibers when submerged in aqueous environments. These fibers, like those produced by many aquatic microorganisms, anchored the cells to each other and to copper and glass surfaces and promoted the formation of a biofilm that isolates the surface from the bulk solution. Natural biofilms that develop on metallic surfaces in contact with an aqueous environment inevitably contain a mixed population of exopolymer-producing microorganisms.¹⁰ Since different species of bacteria produce exopolymers with different affinities for copper,⁵ those areas of a biofilm containing bacteria that produce high-affinity, copper binding exopolymers, such as the bacterium described in this report, will likely contain lower concentrations of dissolved free cupric ion than adjacent areas containing microcolonies of bacteria that produce less reactive exopolymers. When the underlying surface is a copper-containing alloy, a copper concentration cell will develop; the area underlying the highest density of cupric ion-binding sites with the strongest affinity for the ion will be anodic to areas underlying exopolymers that are less reactive toward the metal ion [Figure 4(A)]. This condition will persist until all the copper-binding sites on the exopolymers are saturated. At this point, surface metal dissolution and ion diffusion processes become more important in controlling free cupric ion concentration in the biofilm, eventually resulting in a deterioration of the concentration cell. Biofilms saturated with ions of the underlying metal may even promote anodic polarization by serving as a simple diffusion barrier to metal ions migrating from the surface to the bulk solution [Figure 4(B)].

However, the sediment bacterium used in this study, like many found in biofilms, releases acid as an end-product of cell metabolism. As discussed above, acidification leads to enhanced copper-binding by the exopolymer. Thus, biofilms containing such bacteria will likely maintain a copper concentration cell as long as the cells remain metabolically active. Efforts are currently in progress to demonstrate that metal concentration cells develop in biofilms containing bacterial populations which possess exopolymers with different metal-binding capacities.

Summary

Cells of an adherent freshwater bacterium produced exo-



Continued

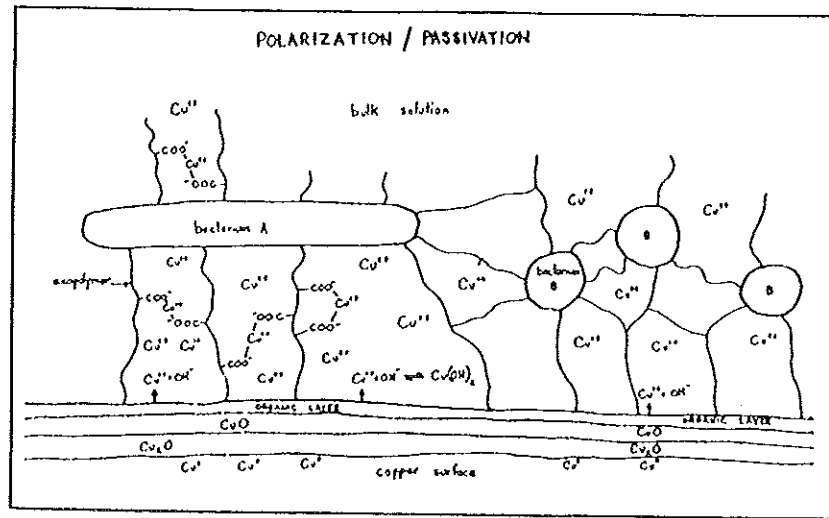


FIGURE 4 — (A) Schematic representation of proposed copper concentration cell established on a copper surface coated with a biofilm of a mixed microbial flora. Exopolymers of a microcolony of Bacterium A possess high-affinity Cu(II)-binding sites. Free Cu(II) ion concentration in the water retained by the biofilm in the vicinity of Bacterium A microcolony would be expected to be less than that in the water around the microcolony of Bacterium B during biofilm growth. (B) Deterioration of copper concentration gradient between areas delineated by microcolonies of Bacterium A and B due to saturation of copper-binding sites on exopolymers of Bacterium A.

polymer that mediated adhesion to surfaces. ATR-FTIR revealed that the exopolymer promoted deterioration of metallic copper and exhibited saturation-binding of dissolved copper that was strongly influenced by pH. The results suggest that exopolymers of microbial biofilms influence the equilibrium of dissolved and metallic copper species with which they are in contact and, under certain circumstances, promote the development of copper concentration cells on an underlying copper-containing surface.

References

1. J. D. A. Miller, *Microbial Aspects of Metallurgy*, p. 61, 1970.
2. C. O. Obuekwe, D. W. S. Westlake, F. D. Cook, J. W. Costerton, *Appl. Environ. Microbiol.*, Vol. 41, p. 766, 1981.
3. R. J. Soracco, L. R. Berger, J. A. Berger, L. A. Mayack, D. H. Pope, E. W. Wilde, *CORROSION/84*, Paper No. 98, NACE, Houston, TX, 1984.
4. G. G. Geesey, L. Borstad, P. M. Chapman, *Water Res.*, Vol. 18, p. 233, 1984.
5. M. W. Mittelman, G. G. Geesey, *Appl. Environ. Microbiol.*, Vol. 49, p. 846, 1985.
6. R. M. Platt, G. G. Geesey, J. D. Davis, D. C. White, *Can. J. Microbiol.*, Vol. 31, p. 675, 1985.
7. T. Iwaoka, P. R. Griffiths, J. T. Kitasako, G. G. Geesey, (submitted for publication).
8. J. O. Nriagu, *Copper in the Environment. Part I. Ecological Cycling*, p. 89, 1979.
9. R. D. Guy, C. L. Chakrabarti, *Can. J. Chem.*, Vol. 54, p. 2600, 1976.
10. D. S. Marszalek, S. M. Gerchakov, L. R. Udey, *Appl. Environ. Microbiol.*, Vol. 38, p. 987, 1979.