90-007 P-166

Applications of Fourier Transform Infrared Spectrometry to Studies of Copper Corrosion Under Bacterial Biofilms

Gill G. Geesey
Philip J. Bremer

Department of Microbiology
California State University
Long Beach, California

ABSTRACT

Copper pipework has been reported to undergo pitting corrosion. The corrosion reactions appear to involve the activities of microorganisms embedded in the overlying biofilm. Attenuated total reflection Fourier transform infrared spectroscopy provided a useful approach to evaluate the types of microbial metabolites that accumulate at the metal surface. Specific strains of bacteria cause deterioration of copper metal surfaces during growth and biofilm development under static and flowing bulk phase conditions. Acidic polysaccharide components of the exopolymers excreted by the surface-associated bacteria promote oxidation of the copper surface.

INTRODUCTION

Copper and copper alloys have been used extensively for many years for the fabrication of materials that are exposed to corrosive environments. Until recently, corrosive reactions associated with copper water pipes were viewed as a series of electrochemical reactions that depended primarily upon the properties of the metal surface and the bulk aqueous phase (Campbell, 1950; Lucey, 1975; Mattson and Fredriksson, 1968; Shalaby et al., 1989). Little attention was given to biologically-mediated reactions that occurred at the solid/liquid interface. A cursory examination of the corrosion products and a knowledge of the chemistry of the bulk aqueous phase was considered sufficient to characterize copper corrosion in freshwater systems.

In the past five years, there has been an increase in the number of failures of copper tubing in industrial water systems that involve the formation of pits that perforated the wall of potable water pipes within a short period of time. The reaction(s) appears to be different from the classical Type 1 and Type 2 pitting corrosion (Fischer et al., 1987). Unlike Type 1 pits described by Lucey (1975), those in water lines in hospitals in such diverse locations as Hellersen, West Germany, Dundee, Scotland, and Riyadh, Saudi Arabia contained no CuCl deposit adjacent to the base metal, and in every instance, large numbers of bacteria were found within the pit and associated corrosion deposits (Keevil et al., 1988). The carbon film described by Campbell (1950) appeared to be contributed by bacteria rather than by residual drawing lubricant introduced during tube manufacture.

When a clean metal surface comes in contact with natural or industrial waters, it is rapidly colonized by microorganisms. The adherent bacteria tolerate the "antimicrobial" effects of the metal and replicate over the surface to produce a biofilm containing very high cell densities. The biofilm and its associated microbial activities have been proposed to exert an influence on the underlying surface that may be quite unique from that of the overlying bulk aqueous phase. In the case of metal surfaces, the adherent microbial population has been implicated in surface corrosion (Costerton and Geesey, 1985; White et al., 1985; Nivens et al., 1986). However, the precise role of the microorganisms in the corrosion reactions is not known at this time.

Our understanding of the mechanisms by which biofilm populations influence or alter the properties of the surfaces with which they associate has been limited in the past by difficulties in determining the activities of bacteria that are attached to surfaces. It has been postulated that the activities of biofilm bacteria depend on the maintenance of the structural integrity of the biofilm (Hamilton and Characklis, 1989). The physical relationships between different bacterial cells and between bacterial cells and the surface with which they associate are most commonly maintained by extracellular polymeric material produced by the bacteria (Geesey et al., 1977). These polymers form a matrix that influences the diffusion of molecules within the biofilm. Thus, the chemical conditions within the biofilm and at the surface are often quite different from those in the overlying bulk fluid (Costerton and Geesey, 1985).

An improved level of understanding of biologically-influenced corrosion of submerged metal surfaces can be achieved by evaluating the chemical properties of an undisturbed biofilm. This requires that analysis be carried out in situ with the biofilm in a hydrated state. Analysis should also be conducted in such a way as to minimize interference from the bulk aqueous phase. During the past five years we have used Attenuated Total Reflectance Fourier Transform Infrared (ATR/FT-IR) spectroscopy to characterize interactions between surfaces submerged in an aqueous medium and microbial products adsorbed from the bulk aqueous phase. It has become apparent that this technique not only provides new insight into chemical changes that occur within a developing biofilm on a submerged surface but also offers

useful information about the stability of the underlying surface. In this paper, we describe how ATR/FT-IR spectroscopy can be used to obtain a better understanding of the relationship between biofilm processes and the corrosion of underlying copper surfaces.

CHARACTERIZATION OF CHEMICAL SPECIES ADSORBED TO SOLID SURFACES SUBMERGED IN AQUEOUS ENVIRONMENTS

Application of internal reflection spectroscopy to biofilm characterization developed from work using Multiple Attenuated Internal Reflection (MAIR) infrared spectroscopy to characterize the nature of the conditioning film that adsorbed to clean surfaces following submersion in natural waters (Baier and Loeb, 1971; Loeb and Neihof, 1975). Baier (1973) and DePalma and Baier (1978) demonstrated that a thin, proteinaceous film typically 100–200 angstroms in thickness appeared within the first ten minutes of exposure to natural waters and caused the first modification of the initial surface condition. Their results indicated that the same amount of organic material adsorbs to the surfaces of a wide range of material. In natural waters, the source of this material was suggested to be humic substances present at part per million concentrations. It is this organic conditioning film rather than the original surface that microorganisms adhere to during colonization (Costerton et al., 1978; Baier, 1980).

Chemical characterization of organic films that develop on surfaces submerged in aqueous environments is generally performed on distilled water-rinsed, air-dried films (Nivens et al., 1986; Baier et al., 1983). This approach is destructive in nature and susceptible to artifacts created during the rinse and dehydration steps. The recent availability of Fourier Transform Infrared (FT-IR) spectrometers and improvements in the design of liquid sampling cells for Attenuated Total Reflection (ATR) spectroscopy now permits the acquisition of water-subtracted IR spectra of fully-hydrated material (Braue and Panella, 1987; Hopkinson et al., 1987; Dousseau et al., 1989). Water may be accurately subtracted from a spectrum by virtue of the high signal-to-noise ratio and computer manipulations achieved by these FT-IR instruments (Griffiths and de Haseth, 1986). Iwaoka et al. (1986) showed that the water from fully-hydrated acidic polysaccharide that had adsorbed to a germanium (Ge) cylindrical Internal Reflection Element (IRE) positioned in an ATR CIRCLE CELL (Spectra-Tech, Inc., Stamford, CT) could be subtracted with sufficient accuracy to detect contaminating (1 percent) levels of protein that were present.

Recent studies in our laboratory have revealed that dehydration results in the following: (1) a significant increase in absorbance of compounds adsorbed to the surface and (2) a change in the relative absorption intensities of different chemical species associated with the surface. Using an open boat CIRCLE CELL containing a zinc selenide (ZnSe) internal reflection element (IRE), we compared ATR/FT-IR spectra of hydrated and dehydrated exopolymer from the film-forming bacterium *Alteromonas (Pseudomonas) atlantica*. Dehydration resulted in an increase in the intensity of protein absorption bands relative to carbohydrate absorption bands (Figure 1). Thus, for accurate information on the relative concentrations of chemical species adsorbed to surfaces in aqueous systems, it is important to obtain IR spectra under fully hydrated conditions. This approach also permits evaluation of changes in concentration of various substances at the solid/liquid interface that occur on a submerged surface over time, an option that is not available when the surface is dehydrated prior to analysis.

LONG-TERM STUDIES ON BIOFILMS USING ATR/FT-IR

Past applications of ATR/FT-IR have involved studies of reactions that occur over a relatively short period of time (Gendreau et al., 1981; Winters et al., 1982). Consequently, spectral artifacts contributed by long-term fluctuations in energy throughput and temperature have not been a major problem. The colonization of surfaces by microorganisms and the subsequent development of a biofilm generally requires from several days to weeks before a stable population structure is established under conditions typical of natural and industrial water systems. During this time, fluctuations in instrument performance and temperature can introduce spectral artifacts

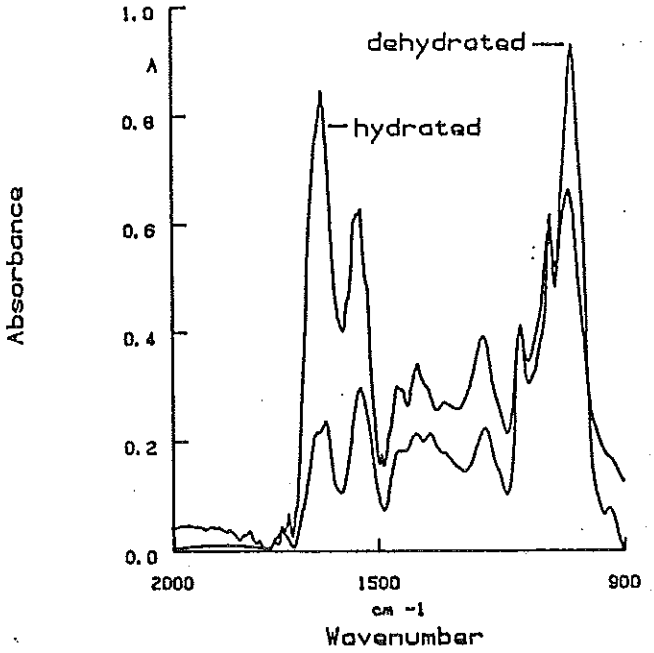

Figure 1. *FT-IR spectra of hydrated and dehydrated expolymer from* Alteromonas (Pseudomonas) atlantica.

that are difficult to subtract when uninoculated control experiments are performed at a time different from the inoculated test experiment, as is the case when one uses ıgle beam FT-IR spectrometer.

These artifacts may be minimized with the use of a double-beam FT-IR spectrometer. Bremer and Geesey (submitted for publication) showed that by alternately focusing the IR beam with a flipper mirror between two CIRCLE CELLS positioned side-by-side in the optical bench of a Perkin Elmer (Model 1800) FT-IR spectrometer, it was possible to collect a spectrum from an uninoculated control CIRCLE CELL and from an inoculated CIRCLE CELL within a few seconds of each other. Fluctuations in radiation energy and temperature were easily subtracted from biofilm spectra when the instrument was operated either in the double beam mode or as two independent single beam instruments. Culture medium components including water were efficiently subtracted from biofilm spectra when an uninoculated medium control was sampled along with the inoculated test system.

A biofilm of the marine bacterium *Alteromonas (Pseudomonas) atlantica* produced in batch culture was evaluated by ATR/FT-IR using the optical bench described above. The two CIRCLE CELLS were loaded with ZnSe IREs in order to obtain spectra of only that material present in the 2 um aqueous boundary layer adjacent to the ZnSe surface. A spectrum collected from the inoculated CIRCLE CELL was ratioed using the double beam mode against a spectrum obtained at a similar time from the CIRCLE CELL containing only sterile culture medium. ɜ resulting spectrum yielded a chemical fingerprint of ıne biofilm. By sampling each cell at various intervals after inoculation, changes in the chemical composition of the developing biofilm were monitored (Figure 2). By 75 h, protein absorbing at 1,637 and 1,548 cm^{-1} and polysaccharide absorbing between 1,028 and 1,085 cm^{-1} dominated the biofilm spectrum. The products of biofilm metabolism that accumulated in the 2 um boundary layer adjacent to the IRE closely resembled the crude exopolymer that sloughed off into the menstruum of chemostat cultures of these bacteria (Figure 3). Thus, exopolymer appears to contribute the bulk of the material that accumulates at the biofilm/substratum interface.

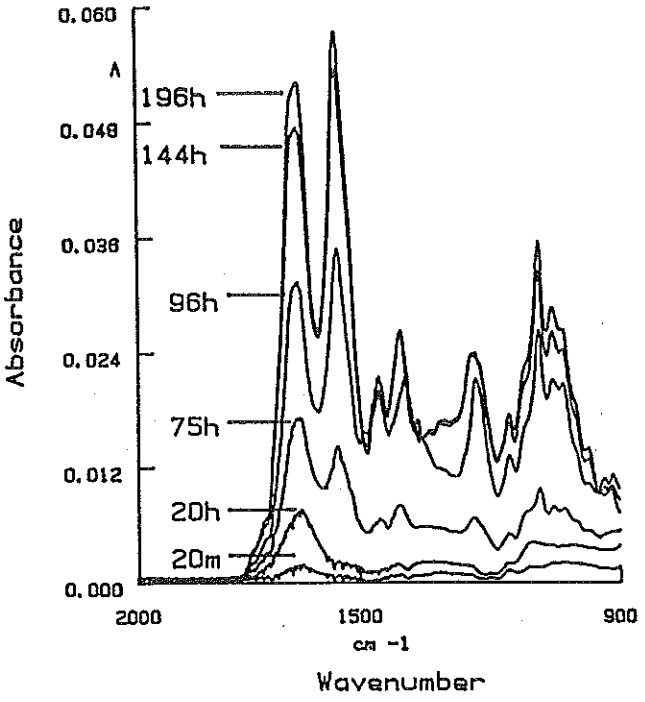

Figure 2. *Double-beam ATR spectra of culture medium-subtracted biofilm produced by a static culture of* Alteromonas atlantica. *Spectra were collected at various times after introduction of culture to one CIRCLE CELL. The other cell was maintained as a sterile culture medium control.*

USE OF ATR/FT-IR IN STUDIES OF CORROSION

The application of ATR/FT-IR to studies of corrosion became apparent when it was shown that IR spectra could be obtained for water and other substances at the solid-liquid interface of a metal-coated IRE submerged in aqueous environments. Iwaoka et al. (1986) described a method to evaporate copper films (1—4 nm thickness) on the surface of a cylindrical Ge IRE. Although transmission electron microscopic examination revealed minor surface irregularities (channels) in these thin copper films (Iwaoka et al., 1986), recent studies by Ishida and Griffiths (submitted for publication) demonstrated that a more uniform surface microstructure could be achieved when the IRE target is positioned at least 11 cm from the filament on which the copper is vaporized during film deposition.

Copper thin films deposited on Ge IREs by either vacuum evaporation or plasma sputtering were found to be stable for many hours in deionized water (Iwaoka et al., 1986; Jolley et al., 1989a). Stability was evaluated by the intensity of the water absorption band at 1,640 cm^{-1} in spectra obtained by ATR/FT-IR. Jolley et al. (1989a) found that less than 5 percent of the copper deposited on a Ge IRE by a magnetron sputtering system was lost during a twenty-four hour period of exposure to deionized water. However, X-ray Photoelectron Spectroscopy (XPS) suggested that some of the Cu^{o} had oxidized to a +1 oxidation state within one hour of exposure to the deionized water (Jolley et al., 1988).

The intensity of the water absorption band at 1,640 cm^{-1} was found to be extremely sensitive to changes in thickness of the thin copper film over a thickness range of 1—4 nm (Iwaoka et al., 1986; Jolley et al., 1989a). Thickness differences of as little as 3–4 angstroms, which are roughly equivalent to 2–3 atomic layers of copper, produced detectable changes in absorbance at 1,640 cm^{-1}. Thus, ATR/FT-IR provides a sensitive, non-

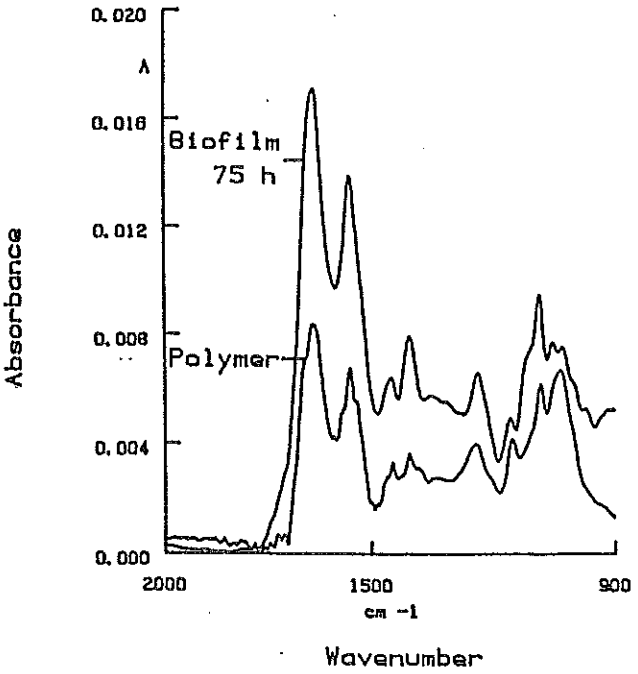

Figure 3. *ATR spectra of* Alteromonas atlantica *biofilm established on a ZnSe IRE after seventy-five hour exposure and the exopolymer isolated from the menstruum of a chemostat culture of the same bacterium.*

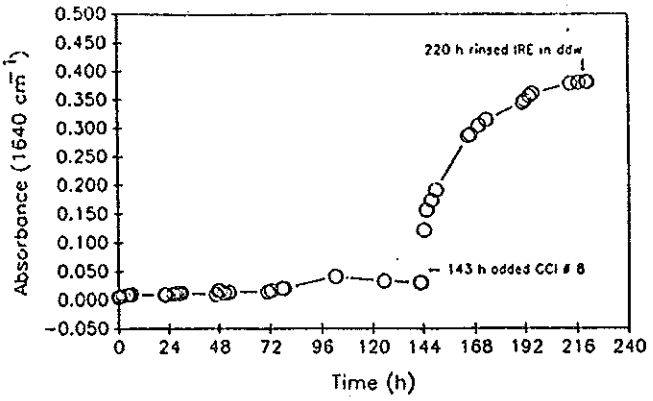

Figure 4. *Intensity of water absorption band from ATR spectra obtained in presence of a copper-coated Ge IRE. During the first 143 hours, the CIRCLE CELL contained a static suspension of sterile culture medium. The culture medium was then replaced with a logarithmic phase culture of CCI #8. The bacterial culture was replaced with distilled water after 220 hours.*

destructive means to evaluate corrosion of thin metal films submerged in aqueous environments.

DETERIORATION OF THIN COPPER FILMS IN THE-PRESENCE OF BACTERIA

The stability of copper thin films in the presence of bacteria was evaluated by ATR/FT-IR spectroscopy. A thin (7.2 nm) film of copper was evaporated under vacuum onto a Ge IRE, which was then installed in an open boat CIRCLE CELL. The cell was sterilized by ethylene oxide and positioned in the optical bench of the double beam FT-IR spectrometer as described previously. Sterile culture medium was aseptically introduced into the CIRCLE CELL and the intensity of the water absorption band at 1,640 cm^{-1} was monitored at intervals over 144 h. During this time the water absorbance increased slightly from 0.005 to 0.030 absorbance units (Figure 4). The sterile culture medium was then carefully replaced with a three-day culture (2 ml of 10^8 colony forming units ml^{-1}) of CCI #8 bacterium. This bacterium originally was isolated from a corroded copper coupon exposed to flowing tapwater. Exposure of the copper thin film to the bacterial culture (pH 6.95) under static conditions resulted in an immediate increase in the intensity of the water absorption band from 0.03 to 0.14 absorbance units (Figure 4). The initial increase in water absorption was followed by a slower but steady increase over the subsequent seventy-seven hour period, indicating that the bacteria or their metabolic products had destabilized the copper film. The destruction of the copper thin film was not the result of physical disturbance during removal or addition of the liquids as there was little change in water absorbance when the bacterial suspension was replaced with distilled water (Figure 4). The intensity of the water absorption band at 1,640 cm^{-1} in the presence of the uncoated IRE was 0.56 absorbance units. Thus, there was still sufficient copper on the IRE to evaluate physical disturbance when the distilled water was applied. The increase in water absorption during exposure of the copper-coated IRE to the bacterial culture was due to degradation of the copper thin film.

Another Ge IRE was coated with a thin film (6.7 nm) of copper and positioned in a clean CIRCLE CELL. The entire assembly was sterilized and then mounted in the optical bench of the FT-IR spectrometer as described above. Sterile culture medium was introduced and IR spectra were collected over 117 hours. Similar to the first study, the water absorption intensity increased only slightly from 0.004 to 0.01 absorbance units over the five-day period (Figure 5). The sterile culture medium was then replaced with a three-day culture of the bacterial isolate CCI #11 (10^6 colony forming units ml^{-1}, pH 6.55) that had been recovered from a corroded copper coupon submerged in flowing tapwater. Only a slight increase (from 0.01 to 0.05 absorbance units) in water absorbance was observed during seventy-seven hour exposure of the IRE to this bacterial isolate (Figure 5). The results suggest that, unlike CCI #8, CCI #11 had little effect on the stability of the copper thin film. It is possible that the difference was due to the hundredfold lower cell density of CCI #11 as compared to CCI #8. It seems unlikely, however, that the size of the initial

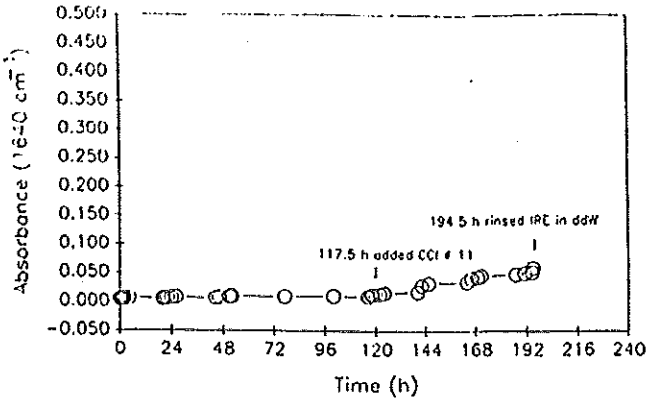

Figure 5. *Experiment conducted similar to that described for Figure 4 except that the sterile culture medium was replaced with a logarithmic phase culture of CCI #11.*

bacterial inoculum is the rate limiting step in copper corrosion. Microscopic examination showed that copper thin films exposed to cultures of CCI #11 or CCI #8 were rapidly colonized when the suspended cell concentration was 10^6 cells/ml. As in the first study, there was no significant change in water absorbance when the culture of CCI #11 was replaced with distilled water in the CIRCLE CELL (Figure 5). Thus, some but not all bacteria promote the deterioration of copper thin films.

The influence of bacteria on the stability of the copper thin film was also evaluated under conditions of laminar flow using ATR/FT-IR spectroscopy. Two copper-coated Ge IREs were positioned in different flow-through CIRCLE CELLS and sterilized by ethylene oxide. The cells were positioned side-by-side in the optical bench of the FT-IR spectrometer and connected to a reservoir of sterile culture medium with sterile silicone tubing as described previously (Bremer and Geesey, submitted for publication). Culture medium was pumped through the CIRCLE CELLS with a peristaltic pump at a flow rate of 500 ul/h, which provided a turnover time of 21 min, a dilution rate of 3 and a Reynolds number of less than 1 in each CIRCLE CELL. One of the CIRCLE CELLS was inoculated with CCI #8 while the other was maintained as a sterile control. Unlike the static culture experiment described previously where the copper thin film was exposed to a bacterial inoculum containing a high cell density (10^8 cells/ml), the copper thin films in this experiment were exposed to low ($<10^6$ cells/ml) concentrations of bacteria in the bulk aqueous phase.

The stability of the copper thin film was evaluated by monitoring changes in peak area of the water absorption band at 1,640 cm^{-1} over time. Single beam IR spectra were collected during flow of the liquid through each CIRCLE CELL and ratioed against their respective background spectrum obtained in air. When the water absorbance stabilized such that values obtained within two successive 30 min sampling periods were the same, an inoculum of CCI #8 was introduced into one of the CIRCLE CELLS over a 2.5 h period. The increase in water absorbance during this time reflected the destabilizing effect the bacterial inoculum exerted on the copper thin film, since the water absorbance in the uninoculated CIRCLE CELL remained relatively constant over the same time period (Figure 6). Unlike the results of the previous static culture experiment, no significant change in water absorbance was observed after flow was stopped for 64.5 h to promote bacterial adhesion to and colonization of the inoculated copper-coated IRE (Figure 6). When flow was restored, water absorbance increased dramatically in the inoculated CIRCLE CELL indicating that the copper thin film had experienced further deterioration (Figure 6). In contrast, water absorbance in the uninoculated CIRCLE CELL decreased slightly during the period that flow was resumed (Figure 6). This decrease is likely due to the establishment of a cuprous oxide film on the metal surface. Other studies have demonstrated that the water absorbance in uninoculated CIRCLE CELLS exposed to flowing, sterile culture medium does not change significantly over time periods typical of the experiments described above (Bremer and Geesey, submitted for publication).

There was visual evidence of pitting corrosion on the IRE exposed to bacteria when it was removed from the CIRCLE CELL at the end of the experiment (Figure 7). No visual evidence of pitting corrosion was observed on the sterile, copper-coated IRE after it was removed from the CIRCLE CELL when the experiment was terminated.

The results of these flow experiments corroborated results obtained previously under static conditions that

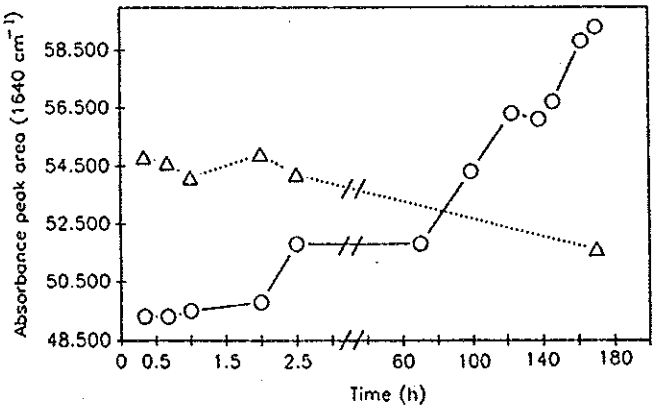

Figure 6. *Intensity of water absorption band of ATR spectra obtained from two CIRCLE CELLS containing copper-coated Ge IREs exposed to flowing culture medium. One cell was inoculated under flow conditions with a suspension of CCI #8. After 2.5 h, flow to both cells was stopped to allow time for bacterial colonization of the inoculated copper-coated IRE. After 64.5 h, flow of sterile culture medium was resumed to both cells for an additional 103 h. Circles and triangles represent water absorption from inoculated and sterile CIRCLE CELLS, respectively.*

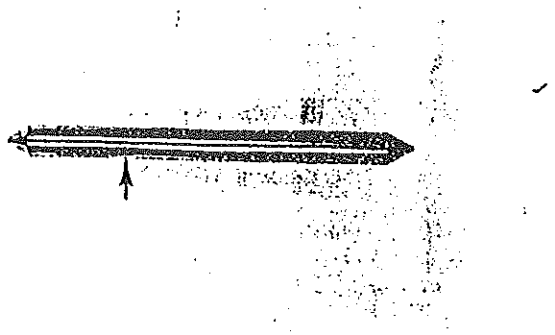

Figure 7. *IRE after removal from inoculated CIRCLE CELL at end of experiment. Note discoloration of the IRE surface where a pit has developed (arrow).*

bacteria or their metabolic products, not culture medium components, promoted deterioration of the copper surface. The data also indicate that the flow conditions employed in the experiment had no deleterious effect on the copper thin film.

The difference in response of the copper thin film to cultures of CCI #8 under the static conditions applied in the two experiments described above is possibly due to differences in bacterial cell or excretory product concentration in the bulk aqueous phase. The relatively low bacterial concentrations ($<10^6$ bacteria/ml) introduced to the CIRCLE CELL in the flow experiment caused no detectable deterioration of the copper thin film over a 64.5 h contact period, whereas the higher bacterial concentrations (10^8 cells/ml) in the bulk aqueous phase of the previous static culture experiment caused rapid destruction of the film. These results suggest that corrosion in the static experiment may not have been promoted by a biofilm per se but rather by a high concentration of corrosive bacterial products, which had access to the metal surface, in the bulk aqueous phase. That a biofilm was not responsible for the immediate and rapid deterioration of the copper film upon inoculation of the IRE in the static experiment is supported by the 64.5 h delay in copper corrosion following inoculation in the flow experiment. Nevertheless, once a biofilm develops, as was the case during the later stages of the flow experiment, it may promote destabilization of the underlying metal surface by concentrating corrosive metabolites synthesized by bacteria on the metal surface.

INVOLVEMENT OF BACTERIAL EXOPOLYMERS IN COPPER CORROSION

Exopolymers secreted by biofilm bacteria have been reported to destabilize copper thin films submerged in aqueous environments. When crude acidic exopolymer isolated from a freshwater sediment bacterium (FRI) was introduced as a neutralized (pH 6.5) 1 percent aqueous suspension to a CIRCLE CELL containing a copper-coated Ge IRE, there was a rapid increase in intensity of the water absorption band in IR spectra obtained by ATR/FT-IR spectroscopy (Geesey, Iwaoka and Griffiths, 1987). Similar effects were achieved with acidic plant polysaccharides, alginic acid and gum arabic. Jolley et al. (1989a) determined by atomic absorption spectroscopy that as much as 41 percent of the copper thin film deposited on a Ge IRE was removed during six-hour exposure to a neutralized 2 percent suspension of alginic acid in deionized water, while exposure of the IRE to pure water removed less than 0.1 percent of the copper thin film. Acidic exopolymer from *Alteromonas (Pseudomonas) atlantica*, when prepared as a 0.5 percent suspension in artificial seawater, removed 6 percent of the copper film deposited on an IRE over a similar period of time. Artificial seawater alone removed 3.5 percent of the copper film (Jolley et al., 1989a). These removal rates corresponded roughly to estimates based on the percent change in intensity of the water absorption band at 1,640 cm^{-1} in ATR/FT-IR spectra. The results indicate that metallic copper is sensitive to a wide range of acidic exopolymers, including those produced by fouling bacteria.

The appearance of absorbance bands corresponding to polysaccharide in the ATR/FT-IR spectra following exopolymer addition to the CIRCLE CELL suggests that the exopolymers had adsorbed to the copper thin film (Iwaoka et al., 1986; Jolley et al., 1989a). Adsorption was demonstrated by the increased ratio of the intensity of the carbohydrate absorption band at 1,050 cm^{-1} to that of the water absorption band at 1,640 cm^{-1} on copper coated versus uncoated IREs (Iwaoka et al., 1986). It is likely that destabilization of the copper thin film resulted from direct interaction with the acidic polysaccharide molecules.

Results from several studies suggest that copper is oxidized by acidic polysaccharides. XPS studies have shown that some of the copper deposited on IREs exposed to gum arabic and alginic acid was oxidized to Cu^{+2}, copper from thin films exposed to *Alteromonas colwelliana* exopolymer (BCS) was oxidized to Cu^{+1}, and copper from thin films exposed to *Alteromonas (Pseudomonas) atlantica* exopolymer displayed little oxidation and remained as Cu^{0} (Jolley et al., 1988; Jolley et al., 1989b).

Auger depth profiles of copper thin films exposed to various acidic polysaccharides and exopolymers from biofilm bacteria verified ATR/FT-IR and atomic absorption spectroscopic results that copper had been removed from the surface. The copper signal of the films exposed to alginic acid, gum arabic and BCS was less than that obtained from copper films exposed to pure water; the initial Ge signal (contributed by the IRE) of copper-coated IREs exposed to these polymers was greater than that obtained from those exposed to pure water (Jolley et al., 1988; Jolley et al., 1989b). The overall rate of copper removal based on Auger depth profile results of alginic acid > gum arabic > BCS > *Alteromonas (Pseudomonas)*

atlantica exopolymer was consistent with results obtained by ATR/FT-IR.

On the basis of the data presented, deterioration of copper surfaces colonized by microbial biofilms is likely due to interactions between copper ions in equilibrium with metallic copper and the exopolysaccharides excreted by the adherent microorganisms. Acidic polysaccharides, including those secreted by biofilm-forming microorganisms, have been shown to possess high affinity binding sites for copper ions (Mittelman and Geesey, 1985; Geesey and Costerton, 1986; Jang et al., 1990). It has been proposed that the complexation of copper ions by the polysaccharides reduces the free metal ion concentration at the metal surface and promotes further ionization of metallic copper in order to establish equilibrium conditions (Geesey and Costerton, 1986; Geesey et al., 1986). A result of copper ion complexation by acidic polysaccharides from some biofilm bacteria is the liberation of hydrogen ions (Mittelman and Geesey, 1985). The resulting increase in acidity within the biofilm is likely to promote further dissolution of metallic copper.

Acid production by bacteria has been considered one of the possible mechanisms of microbially enhanced corrosion of metals (Pope et al., 1984). Dissolved, low molecular weight acids such as acetic acid have received the greatest attention in this regard (Little et al., 1986). Acidic polysaccharides, however, possess properties that make them particularly important agents of metal corrosion. The inter-ligand distance of ionizable groups on polysaccharides, such as alginic acid and exopolysaccharide of *Alteromonas (Pseudomonas) atlantica*, range from 4–8 angstroms (Jang et al., 1989). Therefore, the concentration of acidic groups associated with exopolysaccharides immobilized at or near the metal surface is likely to be greater than that achieved by diffusable, low molecular weight acids excreted by some bacteria.

Acidic exopolysaccharides appear to be one of the most common metabolic products of surface-associated bacterial populations. Their widespread existence appears to stem from their participation in the adhesion of biofilm microorganisms to surfaces (Costerton et al., 1987). The results presented above suggest that acidic exopolysaccharides may also play an important role in copper corrosion.

CONCLUSION

The application of non-destructive, analytical techniques such as ATR/FT-IR spectroscopy to the studies of wetted metal surfaces containing microbial biofilms should advance our understanding of the microbial reactions that directly influence metal corrosion. As technology for thin film deposition of metal alloys on surfaces progresses, it should be possible to extend the types of studies described here for copper to materials such as copper-nickel and stainless steels, which are also susceptible to biologically influenced corrosion.

ACKNOWLEDGMENTS

The research presented in this report was supported by grants from California State University at Long Beach, the International Copper Institute and the National Science Foundation (DMR-8900417).

REFERENCES

Baier, R. E. 1973. Influence of the initial surface condition of materials on bioadhesion. In: *Proc. 3rd International Congress on Marine Corrosion and Fouling*, ed. R. F. Acker, Evanston, IL: Northwestern University Press.

Baier, R. E. 1980. Substrata influences on the adhesion of microorganisms and their resultant new surface properties, In: *Adsorption of Microorganisms to Surfaces* eds., G. Bitton and K.C. Marshall, pp. 5–104, New York: Wiley.

Baier, R. E. and Loeb, G. I. 1971. Multiple parameters characterizing interfacial films of a protein analogue, polymethylglutamate. In: *Polymer Characterization: Interdisciplinary Approaches*, ed. D. D. Craver, pp. 7–96, New York: Plenum.

Baier, R. E., Meyer, A. E., DePalma, V. A., King, K. W. and Fornalik, M. S. 1983. Surface microfouling during the induction period. *J. Heat Transfer*, 105:618–624.

Braue, E. H. Jr. and Pannella, M. G. 1987. Consistency in circle cell FT-IR analysis of aqueous solutions. *Appl. Spectrosc.* 41:1057–1066.

Campbell, H. S. 1950. Pitting corrosion in copper water pipes caused by films of carbonaceous material produced by manufacture. *J. Inst. Metals* 77:345–356.

Costerton, J. W. and Geesey, G. G. 1985. The microbial ecology of surface colonization and of consequent corrosion. In: *Biologically Induced Corrosion*, ed. S.C. Dexter, p. 223–232. Houston, NACE Publications.

Costerton, J. W., Geesey, G. G. and Cheng, K. -J. 1978. How bacteria stick. *Sci. Am.* 238:86–95.

Costerton, J. W., Cheng, K. -J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M. and Marrie, T. J. 1987. Bacterial biofilms in nature and disease. *Ann. Rev. Microbiol.* 41:435–464.

DePalma, V. A. and Baier, R. E. 1978. Microfouling of metallic and coated metallic flow surfaces in model heat exchange cells. In: *Proceedings of the OTEC Biofouling and Corrosion Symposium*, ed. R. Gray, Washington, D.C.: Department of Energy.

Dousseau, F., Therrien, M. and Pezolet, M. 1989. On the spectral subtraction of water from the FT-IR spectra of aqueous solutions of proteins. *Appl. Spectrosc.* 43:538–542.

Fischer, W., Hannsel, I. and Webster, G. 1987. Status report-INCRA Project 404, Markische Fachhochschule, Fachbereich Physikalische Technik, Iserlohn, West Germany.

Ford, T. E., Maki, J. S. and Mitchell, R. 1987. The role of metal-binding bacterial exopolymers in corrosion processes. *Paper No. 380, Annual meeting of the National Society of Corrosion Engineers*, San Francisco, CA.

Geesey, G. G. and Costerton, J. W. 1986. The microphysiology of consortia within adherent bacterial populations. In: *Pro-*

ceedings of the 4th International Symposium on Microbial Ecology, pp. 238–242. Ljubljana, Yugoslavia.

Geesey, G. G., Iwaoka, T. and Griffiths, P. R. 1987. Characterization of interfacial phenomena occurring during exposure of a thin copper film to an aqueous suspension of an acidic polysaccharide. *J. Colloid Interface Sci.* 120:370–376.

Geesey, G. G., Mittelman, M. W. Iwaoka, T. and Griffiths, P. R. 1986. Role of bacterial exopolymers in the deterioration of metallic copper surfaces. *Materials Performance*, 25:37–40.

Geesey, G. G., Richardson, W. T., Yeomans, H. G., Irvin, R. T. and Costerton J. W. 1977. Microscopic examination of natural sessile bacterial populations in an alpine stream. *Can. J. Microbiol*, 23:1214-1223.

Gendreau, R. M., Winters, S., Leininger, R. I., Fink, D., Hassler, C. R. and Jakobsen, R. J. 1981. Fourier transform infrared spectroscopy of protein adsorption from whole blood: *ex vivo* dog studies. *Appl. Spectrosc.* 35:353–357.

Griffiths, P. R. and de Haseth, J. A. 1986. *Fourier transform infrared spectroscopy*, New York: Wiley. 656 pp.

Hamilton, W. A. and Characklis, W. G. 1989. Relative activities of cells in suspension and in biofilms. In: *Structure and Function of Biofilms*, eds. W. G. Characklis and P. A. Wilderer, pp. 199–219. New York: Wiley.

Hopkinson, J. H., Moustou, C., Reynolds, N. and Newberry, J. E. 1987. Applications of attenuated total reflection in the infrared analysis of carbohydrates and biological whole cell samples in aqueous solution. *Analyst* 112:501–505.

Iwaoka, T., Griffiths, P. R., Kitasako, J. T. and Geesey, G. G. 1986. Copper coated cylindrical internal reflection elements for investigating interfacial phenomena. *Appl. Spectrosc.* 40:1062–1065.

Jang, L. K., Harpt, N., Grasmick, D., Vuong, L. N. and Geesey, G. G. 1990. A two-phase model for determining the stability constants for interactions between copper and alginic acid. *J. Phys. Chem.* 94:482–488.

Jang, L. K., Quintero, E., Gordon, G., Rohricht, M. and Geesey, G. G. 1989. The osmotic coefficients of the sodium form of some polymers of biological origin. *Biopolymers*, 28:1485–1489.

Jolley, J. G., Geesey, G. G., Hankins, M. R., Wright, R. B. and Wichlacz, P. L. 1988. Auger electron spectroscopy and X-ray photoelectron spectroscopy of the biocorrosion of copper by gum arabic, BCS and *Pseudomonas atlantica* exopolymer. *J. Surface Interface Anal.* 11:371–376.

Jolley, J. G., Geesey, G. G., Hankins, M. R., Wright, R. B. and Wichlacz, P. L. 1989a. *In situ*, real time FT-IR/ATR study of the biocorrosion of copper by gum arabic, alginic acid, bacterial culture supernatant and *Pseudomonas atlantica* exopolymer. *Appl. Spectrosc.* 43:1062–1067.

Jolley, J. G., Geesey, G. G., Hankins, M. R., Wright, R. B. and Wichlacz, P. L. 1989b. Auger electron and X-ray photoelectron spectroscopic study of the biocorrosion of copper by alginic acid polysaccharide. *Appl. Surface Sci.* 37:469–480.

Keevil, C. W., Walker, J. T., McEvoy, J. and Colbourne, J. S. 1988. Detection of biofilms associated with pitting corrosion of copper pipework in Scottish hospitals. In: *Proceedings of the Biodeterioration Society Meeting on Biocorrosion*, p. 99–117, Paris.

Little, B., Wagner, P., Gerchakov, S. M., Walch, M. and Mitchell, R. 1986. The involvement of a thermophilic bacterium in corrosion processes. *Corrosion*, 42:533–536.

Loeb, G. I. and Neihof, R. A. 1975. Marine conditioning films. In: *Applied Chemistry at Protein Surfaces, Adv. Chem. Ser. No. 145*, ed. R. E. Baier, pp. 31–335, Washington, D.C., American Chemical Society.

Lucey, V. F. 1975. Pitting corrosion of copper in potable water. *Werkst. Korros.* 26:185–192.

Mattsson, E. and Fredriksson, A. M. 1968. Pitting corrosion in copper tubes-causes of corrosion and counter measures. *Br. Corros. J.* 3:246–257.

Mittelman, M. W. and Geesey, G. G. 1985. Copper-binding characteristics of exopolymers from a freshwater sediment bacterium. *Appl Environ. Microbiol.* 49:846–851.

Nivens, D. E., Nichols, P. D., Henson, J. M., Geesey, G. G. and White, D. C. 1986. Reversible acceleration of the corrosion of AISI 304 stainless steel exposed to seawater induced by growth and secretions of the marine bacterium *Vibrio natriegens*. *Corrosion* 42:204–210.

Pope, D. H., Duquette, D. J., Johannes, A. H. and Wayner, P. C. 1984. Microbiologically influenced corrosion of industrial alloys. *Materials Performance*, 23:14–18.

Shalaby, H. M., Al-Karafi, F. M. and Gouda, V. K. 1989. A morphological study of pitting corrosion of copper in soft tap water. *Corrosion* 45:536–547.

White, D. C., Nivens, D. E., Nichols, P. D., Mikell, A. T., Kerger, B. D., Henson, J. M., Geesey, G. G. and Clarke, C. K. 1985. Role of aerobic bacteria and their extracellular polymers in the facilitation of corrosion: use of Fourier transforming infrared spectroscopy and "signature" phospholipid fatty acid analysis. In: *Biologically Induced Corrosion*, ed. S.C. Dexter, pp. 233–243. Houston: NACE Publications.

Winters, S., Gendreau, R. M., Leininger, R. I. and Jakobsen, R. J. 1982. Fourier transform infrared spectroscopy of protein adsorption from whole blood: II. *ex vivo* sheep studies. *Appl. Spectrosc.* 36:404–409.