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Laboratory-Based Model of Microbiologically Induced Corrosion of Copper

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The interactions of bacteria isolated from corroded copper coupons on thin films of copper evaporated onto germanium internal reflection elements were evaluated nondestructively in real time by attentuated total reflectance Fourier transform infrared spectroscopy. The films were stable in the presence of flowing or static sterile culture medium. When exposed to and colonized by the bacterium CCI 8, the copper thin film corroded. Corrosion was enhanced under quiescent conditions. In conjunction with corrosion of the copper thin film was an increase in the concentration of polysaccharide material at the copper-biofilm interface. A different bacterium (CCI 11) did not corrode the copper thin film, and the establishment of this bacterium on the copper surface prevented corrosion of the thin film by CCI 8.

Recent studies have indicated that pitting corrosion of copper tubing is occurring with increasing frequency in water distribution systems in Europe (4, 5, 19). In some instances, the pitting has lead to tube perforation and the subsequent failure of the water distribution system, but in other cases, through-the-wall perforations have not been reported.

Examination of the pits has revealed that they are different from those of the well-documented forms of copper pitting corrosion known as type I (22) and type II (24). Type I corrosion generally occurs in cold, hard water, with pits forming under a deposit of $Cu(OH)(CO_3)_2$ or $Cu(OH)(SO_4)_2$, while type II corrosion occurs in hot (>60°C), soft water, with pits forming under a deposit of precipitated $CaCO_3$. In contrast, the new type of pitting corrosion has occurred in both hot and cold water lines carrying soft water with a low pH. Although the mechanism of the corrosion process has not been elucidated, bacterial biofilms have been implicated (4, 19). Demonstration and characterization of this type of surface phenomenon under controlled conditions, however, have been difficult because of a lack of analytical techniques that can analyze interfacial reaction occurring beneath biofilms in a nondestructive manner (20).

Attenuated total reflection infrared spectroscopy has been used extensively to obtain information on substances at solid-liquid interfaces (12). A modification of this technique involves the deposition of a thin film of metal onto the surface of the internal reflection element (IRE). If the metallic film is thin enough, the energy transmission of the IRE remains sufficiently high to allow the detection of substances at the liquid-metal interface (13, 14).

The application of attenuated total reflection infrared spectroscopy–Fourier transform infrared spectroscopy (FT-IR) to studies of corrosion became apparent when it was shown that the intensity of the water absorbance band at 1,640 cm^{-1} was extremely sensitive to changes in the thickness of the Cu film, as differences of as little as 3 to 4 Å (0.3 to 0.4 nm), equivalent to two to three atomic layers of copper, produced detectable changes in the absorbance band at 1,640 cm^{-1} (2, 13, 17). Attenuated total reflection infrared spectroscopy–FT-IR therefore provided a sensitive, nondestructive means to evaluate the corrosion of thin metal films submerged in aqueous environments.

We previously reported (7, 8) that a biofilm bacterial species (CCI 8) isolated from a corroded Cu coupon promoted the deterioration of a Cu thin film under both batch and continuous culture conditions, while another species (CCI 11) did not promote the corrosion of a Cu thin film under batch culture conditions. This paper expands the previous research and describes the interactions of these two bacterial species growing as a biofilm on a Cu surface exposed to flowing culture media.

MATERIALS AND METHODS

Culture techniques. The bacteria used in this study, designated CCI 8 and CCI 11, were isolated from corroded copper coupons that had been exposed to flowing municipal water. Both bacteria are rod-shaped, gram-variable, facultative anaerobes. The bacteria were cultured in a defined culture medium (DCM) consisting of 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of K_2HPO_4, 0.2 g of $NaNO_3$, 0.2 g of sodium succinate, 0.5 g of sodium citrate, 5 g of sucrose, 10 mg of pyridoxol hydrochloride, 20 mg of thiamine-HCl, 10 mg of asparagine, 10 mg of L-lysine, 10 mg of L-cysteine, 10 mg of L-ornithine, 10 mg of L-glutamic acid, 2.4 mg of $CoCl_2 \cdot 6H_2O$, 2.5 mg of $CuSO_4 \cdot 5H_2O$, 2.4 mg of $Ca(NO_3)_2 \cdot 4H_2O$, 2.8 mg of $FeSO_4 \cdot 7H_2O$, 1.7 mg of $MnSO_4 \cdot 2H_2O$, and 2.9 mg of $ZnSO_4 \cdot 7H_2O$ dissolved in 1 liter of double-distilled water. The pH of the medium was adjusted to 7.0, and the medium was sterilized by membrane filtration (0.45-μm-pore-size filter, Millipore Corp.).

Bacteria were grown as aerated batch cultures in 250-ml flasks containing 50 ml of DCM. The cultures were incubated at 25°C and 100 rpm for 3 to 4 days prior to introduction to the Circle cells as described below.

Interactions of CCI 8 on a Cu-coated (7.1-nm Cu) Ge IRE. Two flowthrough micro Circle cells (Spectra-Tech, Stamford, Conn.), each containing a Cu-coated (7.1-nm Cu, nominal thickness) germanium (Ge) IRE, were positioned side by side on the optical bench of a Perkin-Elmer model 1800 FT-IR spectrometer. The technique used to Cu coat the

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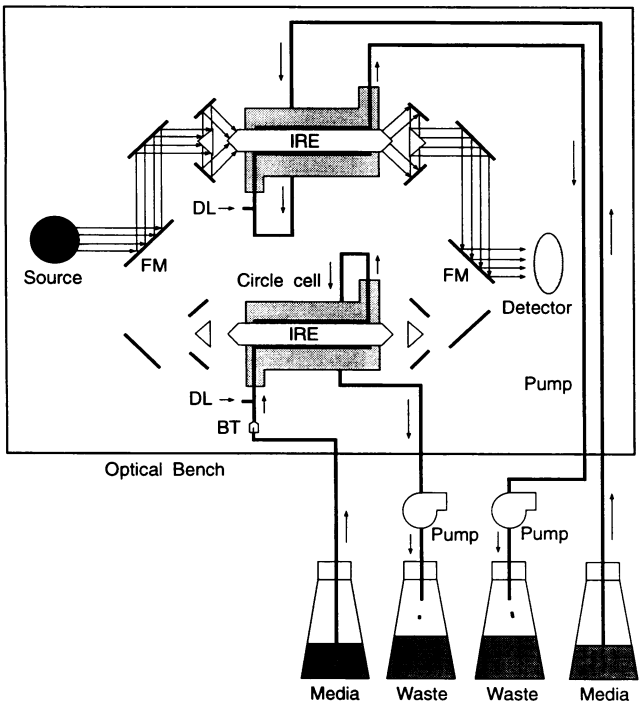

FIG. 1. Schematic diagram showing the position of the Circle cells on the optical bench of the FT-IR spectrometer, the flow of medium to the Circle cells, and the path of the infrared radiation through the Circle cells. BT, medium break tube; DL, dead leg (inoculation port); FP, flip mirror.

IRE as well as the techniques used to characterize the Cu-evaporated surface have been published elsewhere (2).

The Circle cells containing the Cu-coated IREs were sterilized with ethylene oxide. Glassware and silicon tubing were sterilized by autoclaving, and the tubing was aseptically connected to the Circle cells with stainless steel Swagelok fittings. An inoculation port was positioned immediately upstream of one Circle cell, and a medium break tube located upstream of the inoculation port prevented back growth and contamination of the medium reservoir (Fig. 1).

Sterile DCM was pumped from the medium reservoir (500 ml) through the Circle cells with a model 375A Sage peristaltic pump (Orion Research Inc.) at a flow rate of 360 μl h^{-1}, providing a turnover time of 28 min and a Reynolds number of less than 1 in each Circle cell, to achieve laminar flow of medium over the surface of the IRE.

Medium was passed through the Circle cells for 3 h before inoculation to achieve a stable baseline for water absorbance at 1,640 cm^{-1}. After stability was achieved, a 0.3-ml inoculum of a batch culture of CCI 8 (10^8 CFU ml^{-1}) was introduced to the medium flowing into one of the Circle cells. The other Circle cell, which received sterile DCM only, served as an abiotic control.

At intervals over a 417-h period, single-beam spectra were collected alternately from each Circle cell with a synchronized, oscillating mirror. A single-beam spectrum was calculated from 50 averaged interferograms. A ratioed single-beam spectrum was obtained by determining the ratio of a single-beam spectrum collected at a particular time to a background spectrum collected from the respective Circle cell prior to the addition of sterile DCM. FT-IR operating parameters were identical to those reported by Bremer and Geesey (1).

At the end of the experiment, the IREs were removed from both Circle cells, visually examined, and photographed. Biofilm material that had accumulated on the surface of the IRE inoculated with CCI 8 was carefully collected and added directly to an open-boat Circle cell containing a zinc selenide (ZnSe) IRE. The infrared spectrum of the biofilm was ratioed against the spectrum of the ZnSe IRE in the presence of water to obtain the water-subtracted spectrum of the biofilm material.

The neutral hexose content of the biofilm material was determined by the phenol-sulfuric acid procedure of Dubois et al. (3), and the protein concentration was estimated by the method of Lowry et al. (21).

Influence of other bacteria on the stability of the Cu film. A flowthrough micro Circle cell was set up as described above. Unlike the first experiment, however, this experiment was run with a single Circle cell, so rather than having a separate sterile control, the stability of the Cu thin film was checked by subjecting the Circle cell to flowing sterile DCM for 169 h prior to the inoculation of CCI 11 (0.2 ml of a 10^6-CFU ml^{-1} suspension). An additional inoculum of CCI 11 (1 ml of a 10^6-CFU ml^{-1} suspension) was added to the Circle cell after 334 h, and the flow of medium to the Circle cell was stopped. After 503 h, the flow of medium was resumed and an inoculum of CCI 8 (1 ml of a 10^8-CFU ml^{-1} suspension) was added to the Circle cell. The flow of medium to the Circle cell was stopped after 25 h, and the culture was maintained as a mixed batch culture for an additional 215 h.

Upon termination of the experiment, the IRE was aseptically removed from the Circle cell and visually examined, and the associated bacteria were removed with a moist cotton swab, plated out on solid DCM containing 1.8% agar, and incubated at 25°C for 72 h for colony development.

RESULTS

Stability of a Cu-evaporated thin film. Two Circle cells, each containing a Cu-coated Ge IRE, were aligned on the optical bench of an FT-IR spectrometer and exposed to flowing sterile DCM for 3 h to achieve a stable baseline for water absorbance at 1,640 cm^{-1}. The areas of the water absorption band from the IREs in the front and back Circle cells were 10.43 and 11.6 relative units, respectively. The area of the water absorption band with a bare Ge IRE in water was 46.4 relative units. Deposition of a thin Cu film on the surface of the IREs resulted in 77.5 and 75% reductions in the water absorbance values.

The back IRE was maintained as a sterile control, receiving only sterile DCM over the course of the experiment. The area of the water absorption band in the control Circle cell was constant between 0 and 22 h, increased slightly after 48 h, and was then stable for 150 h before gradually decreasing to a value that was comparable to that at the start of the experiment (Fig. 2A). This result indicated that the Cu thin film did not corrode significantly over a 417-h period in the presence of sterile culture medium.

Interactions of CCI 8 on a Cu-coated (7.1-nm Cu) Ge IRE. Following exposure of the front Circle cell to DCM for 3 h, a suspension of CCI 8 was added to the medium passing into the Circle cell. The stability of the Cu thin film in the presence of CCI 8 was determined by measuring the area of the water absorbance at 1,640 cm^{-1} over a 417-h period (Fig. 2A). From 0 to 330 h, the area of the water absorption band increased to 18.01 relative units, indicating that slight corro-

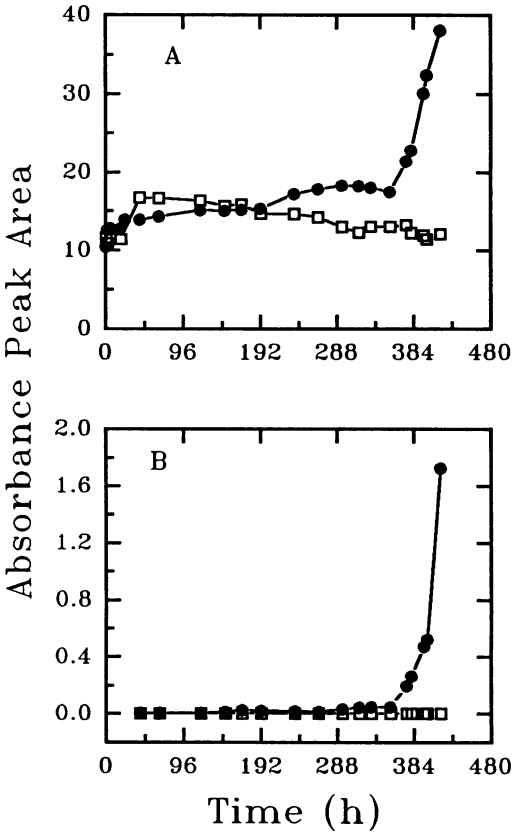

FIG. 2. Absorbances (peak areas) of water at 1,640 cm^{-1} (A) and polysaccharide at 1,062 cm^{-1} (B) in the presence of sterile DCM (□) or DCM inoculated with CCI 8 (●). IREs were exposed to flowing DCM for 330 h and to quiescent conditions thereafter.

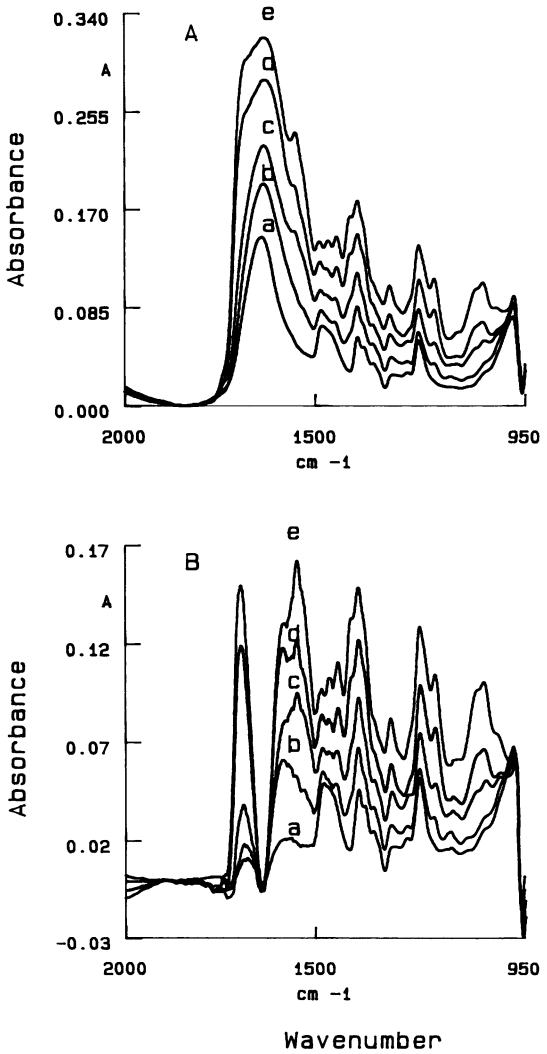

FIG. 3. Spectra obtained at intervals following exposure to an inoculum of the bacterium CCI 8. Shown are ratioed single-beam spectra (A) and water-subtracted ratioed single-beam spectra (B) for sterile DCM just prior to inoculation (time zero) (a), 330 h after inoculation (b), 380 h after inoculation (c), 400 h after inoculation (d), and 417 h after inoculation (e). Note the appearance of the polysaccharide band at 1,062 cm^{-1} beginning at 380 h.

sion of the Cu thin film had occurred. After 330 h, the flow of sterile DCM into the Circle cells was stopped, and both Circle cells were maintained under stagnant flow conditions. Spectra obtained from the sample Circle cell shortly after quiescent conditions were established (396 h) showed a dramatic increase in the area of the water absorption band to 38.04 relative units (Fig. 2A). This result indicated that there was a rapid decrease in the average thickness of the Cu thin film on the Ge IRE.

In conjunction with the increase in the water absorption band area, there was an increase in an absorption band at 1,062 cm^{-1} in spectra obtained from the sample Circle cell after 354 h (Fig. 2B and 3A). The 1,062-cm^{-1} absorption band was not detected in spectra obtained from the sterile, control Circle cell (Fig. 2B). The absorption band at 1,062 cm^{-1} has been reported to correspond to the C—O stretch of polysaccharides (6, 10).

In the ratioed spectra obtained from the Circle cell inoculated with CCI 8, an absorbance band at 1,550 cm^{-1} was detected in a spectrum taken after 374 h. To clearly distinguish the absorption band at 1,550 cm^{-1}, it was necessary to mathematically subtract the strong absorbance band of water from the spectra with interactive difference software. The resulting spectra are shown in Fig. 3B. Except for revealing the absorption bands at 1,690 and 1,550 cm^{-1}, the water-subtracted spectra were practically identical to the original spectra. The areas of the major absorption bands present in the spectra were calculated and tabulated (Table 1).

The infrared absorption bands were tentatively assigned to compounds at the surface of the IREs. The absorption band at 1,699 cm^{-1} was considered to correspond to the presence of carboxylic acids (23). The absorption bands at 1,550 and 1,307 cm^{-1} were assigned to the protein amide II and amide III bands, respectively. Problems in achieving accurate subtraction of water at 1,640 cm^{-1} during corrosion of the Cu thin film made it difficult to recover the amide I band at 1,650 cm^{-1}. The absorption bands at 1,229 and 1,062 cm^{-1} were both considered to be due to the presence of bacterial polysaccharide at the metal surface, the 1,229 cm^{-1} absorption band having been reported to correspond to the asymmetrical stretching of polysaccharide phosphate groups (26).

To verify that changes had occurred in the concentrations of compounds corresponding to microbial metabolites at the

TABLE 1. Areas of selected absorption bands from water-subtracted spectra in Fig. 3B and area of the water absorption band at 1,640 cm^{-1} from the spectra in Fig. 3A

Time (h)	Area at the following wavenumber (cm^{-1}):					
	1,692	1,640	1,550	1,307	1,229	1,062
374	0.927	11.04	4.115	0.089	0.291	0.217
380	1.318	12.35	4.550	0.107	0.535	0.305
396	4.106	19.64	6.061	0.146	1.337	0.729
400	5.04	21.93	6.769	0.164	1.720	0.894
417	6.316	27.61	8.472	0.204	2.620	1.608

copper surface during corrosion of the film, we compared the areas of their absorption bands with that of water (Table 2). This was found to be necessary, since deterioration of the copper film increased the depth of penetration of the infrared radiation into the surrounding medium and any microbial metabolite that had accumulated in this extended sampling region would contribute an additional absorbance that could be interpreted as an increase in the concentration of that metabolite within the original sampling region adjacent to the copper film (17).

The ratio of the area of the water absorption band (1,640 cm^{-1}) to the areas of the polysaccharide absorption bands (1,229 and 1,062 cm^{-1}) decreased from 354 h (the time of the initial appearance of the polysaccharide absorption bands) to the termination of the experiment at 417 h. This result indicated that the concentration of polysaccharide at the Cu surface increased over this period.

The areas of the amide II and amide III absorption bands relative to the area of the water absorption band increased only slightly between 374 and 417 h, suggesting that, in contrast to the concentration of polysaccharide, the concentration of protein decreased at the Cu surface during the time that corrosion was observed. This result suggested that corrosion of the Cu film correlated more closely with polysaccharide accumulation than with protein accumulation.

The accumulation of biofilm material on the IRE was confirmed by visual inspection of the surface of the inoculated IRE upon removal from the Circle cell after 417 h (Fig. 4). Biofilm material was clearly visible on regions of the sample IRE. Furthermore, the Cu thin film beneath the biofilm was discolored. In contrast, the IRE removed from the control Circle cell was clean and shiny and appeared to be biofilm free.

The biofilm material associated with the inoculated IRE was carefully collected and added directly to an open-boat Circle cell containing a ZnSe IRE. An infrared spectrum of this material (Fig. 5) was similar to those obtained from the undisturbed biofilm on the Cu-coated Ge IRE. This result

TABLE 2. Ratios of the areas of selected absorption bands from water-subtracted spectra to the area of the water absorption band

Time (h)	Ratio of the area at the following wavenumber (cm^{-1}):			
	1,640/1,550	1,640/1,307	1,640/1,229	1,640/1,062
374	2.7	123.7	37.8	174.4
380	2.7	115.4	23.1	40.5
396	3.2	134.5	14.7	26.9
400	3.2	133.7	12.8	24.5
417	3.2	135.3	10.5	17.7

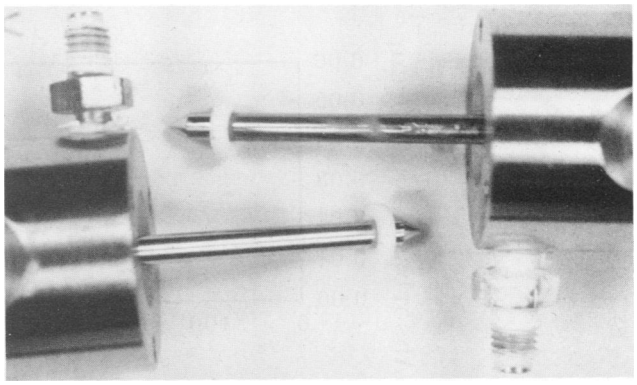

FIG. 4. Cu-coated Ge IRE after exposure for 417 h to either sterile DCM (bottom) or DCM inoculated with the bacterium CCI 8 (top). Note the accumulation of biofilm on the latter.

indicated that the portion of the biofilm material in contact with the surface of the Cu thin film was similar in composition to the bulk of the biofilm material. The major absorption bands have tentatively been assigned to reflect absorbances by the following moieties: lipids (1,716 cm^{-1}), protein (amide I [1,666, 1,652, and 1,636 cm^{-1}] and amide II [1,553 and 1,543 cm^{-1}]), and polysaccharide (1,262 and 1,077 cm^{-1}). That the biofilm material contained polysaccharide and protein was confirmed by colorimetric tests carried out on the isolated biofilm material which revealed a carbohydrate concentration of 30.2 ± 2.5 μg of glucose equivalents ml^{-1} and a protein concentration of 179.6 ± 1.5 μg of protein ml^{-1}.

Influence of other bacteria on the stability of the Cu film. The effect of another bacterium, CCI 11, on a Cu film was evaluated in a manner similar to that described above for CCI 8. The stability of a Cu film in the absence of bacteria was evaluated by allowing sterile DCM to flow through the Circle cell for 169 h prior to the inoculation of CCI 11. During this time, the intensity (peak height) of the water absorption band at 1,640 cm^{-1} remained stable (Fig. 6).

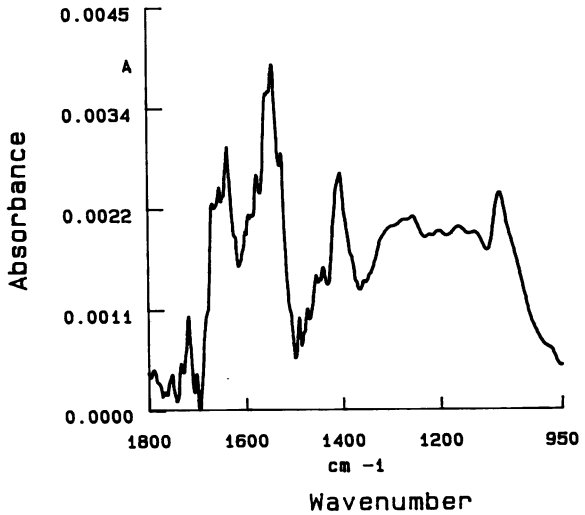

FIG. 5. Spectrum of the biofilm material removed from a Cu-coated Ge IRE 417 h after inoculation with the bacterium CCI 8.

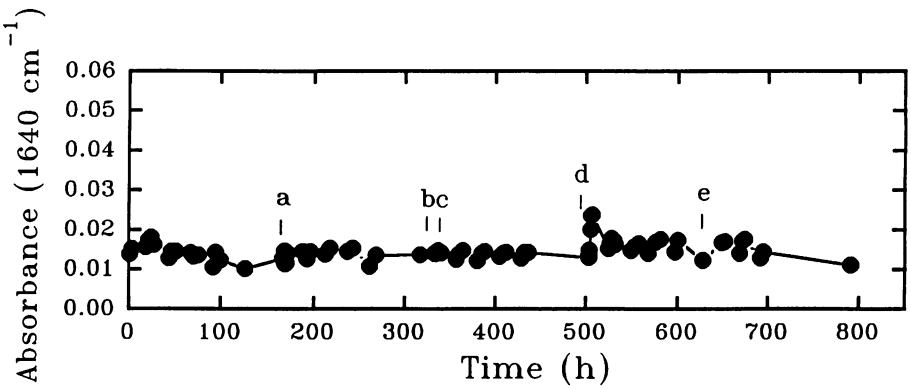

FIG. 6. Intensity of the water absorption band from spectra obtained from a Cu-coated (6.7-nm Cu) Ge IRE exposed to sterile flowing DCM and subsequently inoculated with CCI 11 and CCI 8. a, 169 h, inoculation with CCI 11; b, 334 h, reinoculation with CCI 11; c, 336 h, cessation of the flow of medium; d, 503 h, addition of CCI 8, restarting of the flow of medium; e, 628 h, cessation of the flow of medium.

After 169 h, an inoculum of CCI 11 was added to the DCM flowing through the Circle cell and spectra were obtained at intervals for an additional 165 h. The continued stability of the water absorption band intensity (peak height) over this time indicated that the Cu film remained intact during exposure to this bacterium. To ensure that the bacteria had sufficient opportunity to attach to and colonize the Cu film, we added a larger inoculum of CCI 11 at 334 h and stopped the flow of the medium to the Circle cell after the inoculum had entered the Circle cell (336 h). During the subsequent 167-h period (Fig. 6, interval c-d) of quiescent conditions, no change in the intensity (peak height) of the water absorption band was observed, indicating that the Cu film was stable in the presence of a pure culture of CCI 11.

Influence of CCI 11 on the corrosive action of CCI 8. The effect of CCI 8 on the stability of the Cu film after preexposure to CCI 11 was evaluated by reestablishing the flow of DCM through the Circle cell and introducing an inoculum of CCI 8 (Fig. 6, point d). A small increase in the water absorbance was observed immediately following the introduction of CCI 8, but the absorbance returned to the previous level within a few hours and remained there for an additional 125 h under flowing conditions. Even after the flow was suspended (Fig. 6, point e), no change in the water absorbance was observed. These results indicated that prior exposure of the Cu film to CCI 11 neutralized the corrosive action of CCI 8.

Evidence that bacteria had colonized the surface of the Cu film over the duration of the experiment described above was obtained by evaluation of the infrared spectra collected at various intervals. Spectra obtained after 502 h (when the Cu film was exposed to a pure culture of CCI 11) and 791 h (when the Cu film was exposed to both CCI 11 and CCI 8) revealed absorbance peaks at 1,261, 1,097, and 1,030 cm^{-1} characteristic of polysaccharides (Fig. 7A). These results demonstrated that compounds characteristic of the introduced bacteria and unique from those present in the original culture medium were accumulating on the surface of the Cu film. The difference spectrum obtained by subtracting the spectrum collected at 502 h from that collected at 791 h reflected the differences in the microbial populations colonizing the Cu film at these different times (Fig. 7B). The difference spectrum revealed that the biofilm present on the Cu film surface at 791 h contained more compounds with absorbance peaks at 1,261 and 1,097 cm^{-1} than did the biofilm present at 502 h. These additional compounds were

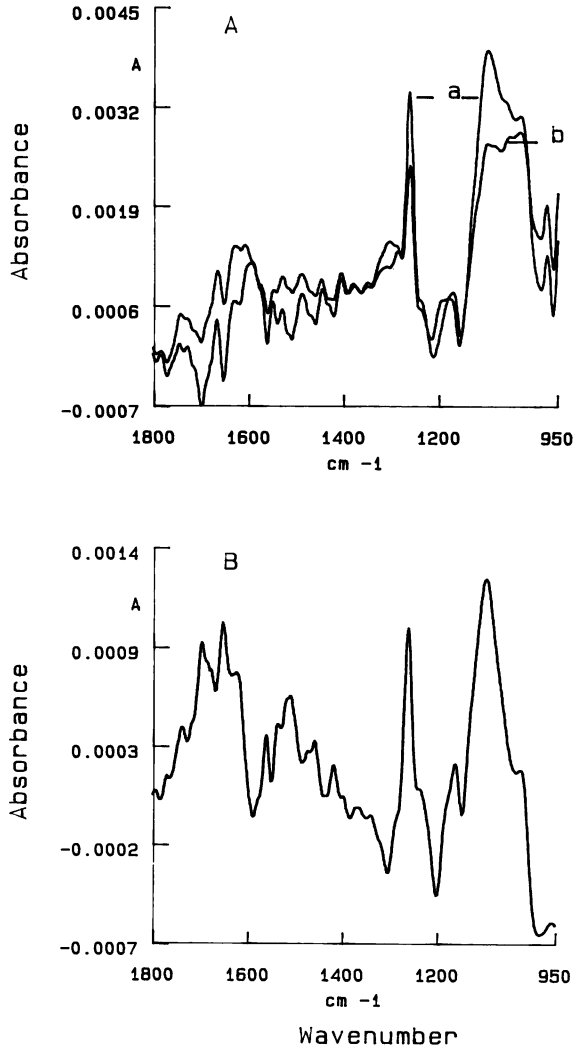

FIG. 7. (A) Spectra obtained from a Cu-coated (6.7-nm Cu) Ge IRE after 503 h (a) and 791 h (b). (B) Difference spectrum obtained by subtracting the spectrum at 502 h from the spectrum at 791 h.

likely contributed by CCI 8, which was introduced prior to 791 h but not at 502 h. Infrared spectra of a biofilm comprised of CCI 8 alone (Fig. 3B) supported this view.

Confirmation that both CCI 8 and CCI 11 had colonized the surface of the Cu film was obtained from culture studies. Although it was not possible to obtain accurate bacterial numbers from plating experiments because of the aggregation of cells within the biofilm that was recovered from the Cu film at the end of the experiment, colonies of both bacteria were obtained. The ratio of the number of CFU produced by CCI 8 to the number of CFU produced by CCI 11 was 1.4.

DISCUSSION

The results suggested that under the conditions used in these experiments, Cu films are stable in the presence of sterile DCM; however, the addition of the bacterium CCI 8 to the medium flowing into a Circle cell containing a Cu-coated IRE resulted in the corrosion of the Cu thin film, the rate of which appeared to be enhanced under quiescent conditions. Minor fluctuations that occurred in the area of the water absorption band in a Circle cell receiving sterile DCM were likely to be due to the formation and dissolution of a copper oxide film on the surface of the Cu-coated IRE.

In spectra obtained from the intact biofilm of CCI 8 (Fig. 3A), the spectral region between 1,620 and 1,740 cm^{-1} was considered to contain features contributed by lipid carboxylic acids (1,680 to 1,720 cm^{-1}), the protein amide I absorption band (1,650 cm^{-1}), and the dominant water absorption band (1,640 cm^{-1}). Because of increasing corrosion of the Cu thin film with time, the volume of water sampled by the infrared beam continually changed, making it difficult to accurately subtract the contribution of water to the spectra. The end result was that in the water-subtracted spectra (Fig. 3B), some detail in the vicinity of the water absorption band (1,600 to 1,660 cm^{-1}) was lost. The absorption band most affected was the protein amide I absorption band, which generally occurred in conjunction with the protein amide II absorption band, as seen in the spectrum obtained from the isolated, dehydrated biofilm (Fig. 5); this spectrum clearly depicted the presence of both the amide I and the amide II absorption bands.

Despite the loss of the amide I absorption band, the water-subtracted spectra provided a good indication of what was accumulating on the surface of the IRE. To verify this assumption and to confirm that the absorption band at 1,550 cm^{-1} in the water-subtracted spectra was due to the presence of protein, we compared the area of the amide II absorption band (1,550 cm^{-1}) with that of another absorption band that was considered to correspond to the presence of protein, the amide III absorption band (1,307 cm^{-1}). As the ratio of the areas of these two absorption bands remained constant with time (the ratios at 374, 380, 396, 400, and 417 h were 46.2, 42.5, 41.5, 41.2, and 41.5, respectively), it was considered that the absorption band at 1,550 cm^{-1} corresponded to that of amide II. Thus, the subtraction of water did not appear to affect the absorption bands between 2,000 and 1,000 cm^{-1}, other than those such as amide I, which occurred in the immediate vicinity of the water absorption band.

The results presented in Fig. 2A and B indicated that the accumulation of polysaccharide on the Cu surface may be related to the corrosion of the Cu. Previous authors have reported that polysaccharides vary greatly in their ability to corrode Cu: some polysaccharides have a negligible effect, while others promote rapid corrosion (7, 10, 13, 16–18). The corrosion of Cu by polysaccharides has been suggested to involve the reaction of the copper with acidic groups present in exopolysaccharides of many aquatic bacteria, such as those associated with uronic acids and pyruvylated sugars. Acidic polysaccharides, including those secreted by biofilm-forming microorganisms, have been shown to possess high-affinity binding sites for Cu ions (9, 15, 25). It has been proposed that the complexation of Cu ions by polysaccharides reduces the free metal ion concentration at the metal surface and promotes the further ionization of metallic Cu to establish equilibrium conditions (9, 11).

The results of these flow experiments corroborate the results previously obtained under quiescent and flow conditions (7, 8) showing that the bacterium CCI 8 or its metabolic products can promote the deterioration of Cu thin films. The isolate designated CCI 8 had previously been shown to promote the destabilization of a Cu thin film in batch and continuous culture experiments (7). However, the earlier flowthrough experiment was terminated after 180 h, and neither the spectacular increase in the water absorption band nor the increase in the polysaccharide absorption band was recorded. The isolate designated CCI 11 did not corrode the Cu thin film under flow conditions, and this result corroborates the results reported in an earlier paper showing that the colonization of a Cu thin film by CCI 11 does not promote the deterioration of the thin film. Furthermore, CCI 11 appeared to neutralize the corrosive action of CCI 8, although the latter did colonize the Cu thin film.

The infrared spectra obtained (Fig. 7) from the Cu-coated IRE colonized by the bacterium CCI 11 revealed the presence of polysaccharide on the Cu surface. The isolation and characterization of the polysaccharide produced by CCI 11 may help us to understand why colonization of a Cu surface by this bacterium does not result in corrosion, while colonization of a Cu surface by CCI 8 alone does result in corrosion.

ACKNOWLEDGMENTS

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