EVALUATION OF BIOFILM MICROORGANISMS IN COPPER CORROSION

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Biofilms contain a consortium of physiologically distinct microorganisms bound together by a polymeric matrix. Our understanding of the complex processes mediated by these microorganisms has been limited by problems encountered in sampling at interfaces. It is important to be able to distinguish between processes that occur in the biofilm from those that occur in the bulk aqueous phase. It is also crucial to be able to study these processes without disturbing the structural integrity of the biofilm during analysis. The biofilm should be evaluated in situ in a hydrated state by techniques that apply no unnatural conditions to the biofilm. Finally, biofilm analysis should be non-destructive so that repeated sampling can be carried out.

The conditions imposed above can be satisfied by attenuated total reflectance Fourier transform infrared spectroscopy (ATR/FT-IR). Biofilms are grown on a metal-coated cylindrical internal reflection element (IRE) which is positioned in a Circle cell mounted in the optical bench of a Fourier transform infrared spectrometer. When infrared radiation is focused through the IRE, most of the radiation is reflected internally and the resulting signal collected by a detector. A portion of the radiation in the form of an evanescent wave penetrates the surface of the IRE into the surrounding environment. The depth of penetration varies depending upon the thickness of the thin metal film and the refractive indices of the IRE and the surrounding environment. In the case of a bare germanium IRE in water, the depth of penetration ranges from 400-900 nm depending on the wavelength of the radiation.

IREs were coated with 7 nm thick films of copper by vacuum evaporation (Iwaoka et al., 1986) and positioned in the Circle cells as described below. It was determined that copper films of this thickness permitted sufficient transmittance of ir radiation to detect chemical species at the metal/liquid interface. It was also determined that slight (2-3 angstroms) changes in the thickness of the copper film could be detected by changes in the intensity of the water absorption band at 1640 cm-1 (Iwaoka et al., 1986). As the thickness of the copper thin film on the IRE decreased, the intensity of the water absorption band increased (Fig. 1). This feature was used to evaluate the integrity of the copper surface during colonization by bacterial cells.

In the studies presented below, a Perkin Elmer, Model 1800 FT-IR spectrometer containing a flipper mirror that directs the radiation from the source alternately into 2 Circle cells, located side by side in the optical bench, was used to study biofilms which developed on a copper coated IRE in one of the Circle cells. The other copper-coated IRE in another Circle cell served as a sterile control to eliminate artifacts introduced to the biofilm spectra caused by energy and temperature fluctuations in the instrument. The radiation was collected in a liquid nitrogen-cooled, mercury-cadmium-telluride detector and an absorption spectrum generated by computer-controlled software.

Broth medium that supported the growth of bacteria was pumped from a reservoir through a media break tube, past an inoculation port, through the 2 Circle cells and finally to waste. The flow rate was set at 500 uL hr-1. A diagram of the assembled apparatus is shown in Fig. 2. Experiments were initiated by collecting spectra of the sterile culture medium in each Circle cell. One Circle cell was then inoculated with a bacterium (CP-8) that was isolated from a corrosion deposit on copper tubing from a failed heat exchanger. The bacterial cells were pumped through the Circle cell with

sterile culture medium so that only those bacteria that attached to the copper thin film on the IRE were retained in the Circle cell. Spectra were collected at similar intervals in both the inoculated (sample) and uninoculated (reference) Circle cells. At each sampling interval, the spectrum collected from each Circle cell was ratioed against that obtained from each cell prior to inoculation. Changes in water absorption band intensity in the inoculated Circle cell caused by the presence of the bacteria could then be compared with those obtained under sterile conditions. This approach also permit identification of chemical components contributed by the bacterial cells colonizing the copper thin film.

As sterile culture medium was pumped through both Circle cells, there was small gradual increase in the intensity of the water absorption band at 1640 cm-1 in the sample Circle cell and an abrupt initial increase followed by a small gradual decrease in the reference (uninoculated) Circle cell over a 330 h period (Fig. 3). When the flow of culture medium into the 2 Circle cells was stopped after 330 H, the intensity of the water absorption band in the sample Circle cell increased rapidly after an initial lag, indicating rapid corrosion of the copper film (Fig. 3). In contrast, the intensity of the water absorption band in the uninoculated Circle cell remained unchanged when the flow was stopped, indicating that no corrosion had occurred on the copper film in the absence of the bacteria (Fig. 3). When the IRE was removed from the sample Circle cell, a thick biofilm was observed, whereas the IRE recovered from the uninoculated Circle cell appeared clean and showed no evidence of bacterial contamination. These results demonstrate the corrosive action of bacteria that colonize copper surfaces.

Examination of the ir spectra during copper film degradation in the sample Circle cell revealed the presence of a polysaccharide absorption band at 1062 and 1082 cm-1 (Fig. 4). Polysaccharides of bacterial and plant origin have been previously shown to promote corrosion of thin copper films (Gessey et al., 1987; Jolley et al, 1989). It is possible, therefore, that the degradation of the copper thin film in these studies was promoted by the polysaccharide exopolymers produced by the bacterial cells attached to the copper surface. These studies demonstrate the utility of ATR/FT-IR spectrometry in evaluating the corrosion of metallic copper surfaces under microbial biofilms exposed to flowing aqueous media. Further research using this approach should help identify the microbiological reactions that promote copper corrosion.

ACKNOWLEDGEMENT

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Iwaoka, T., Griffiths, P.R., Kitasako, J.T., Gessey, G.G. 1986. Copper-coated cylindrical internal reflection elements for investigating interfacial phenomena. Appl. Spectrosc. 40(7):1062-1067

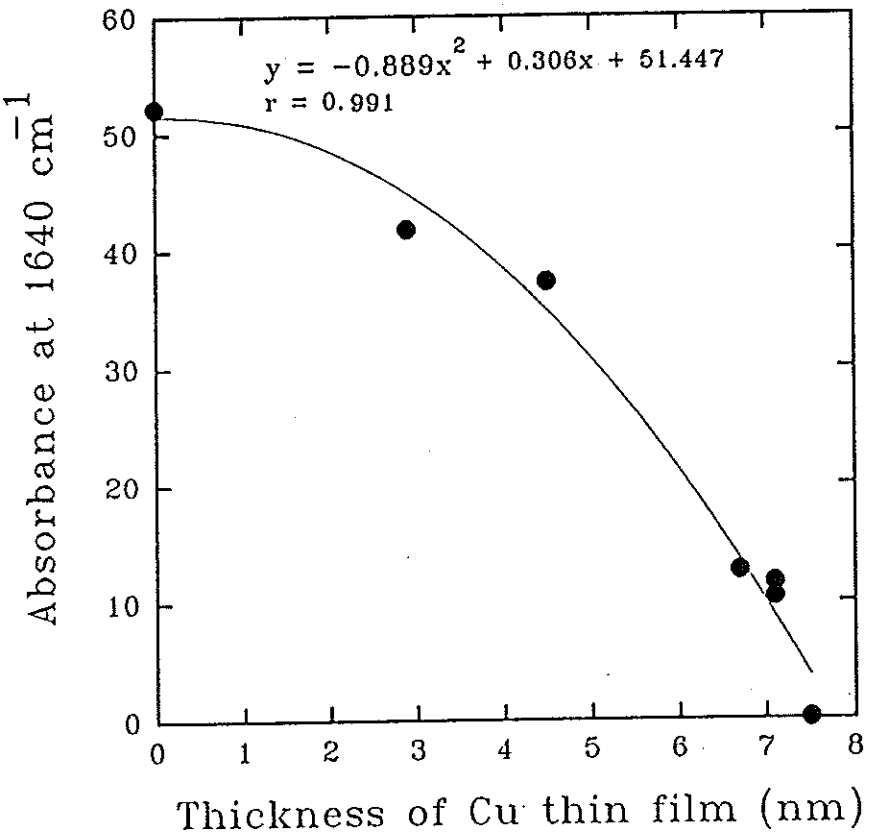

FIG. 1. Relationship between copper film thickness and water absorbance at 1640 cm-1

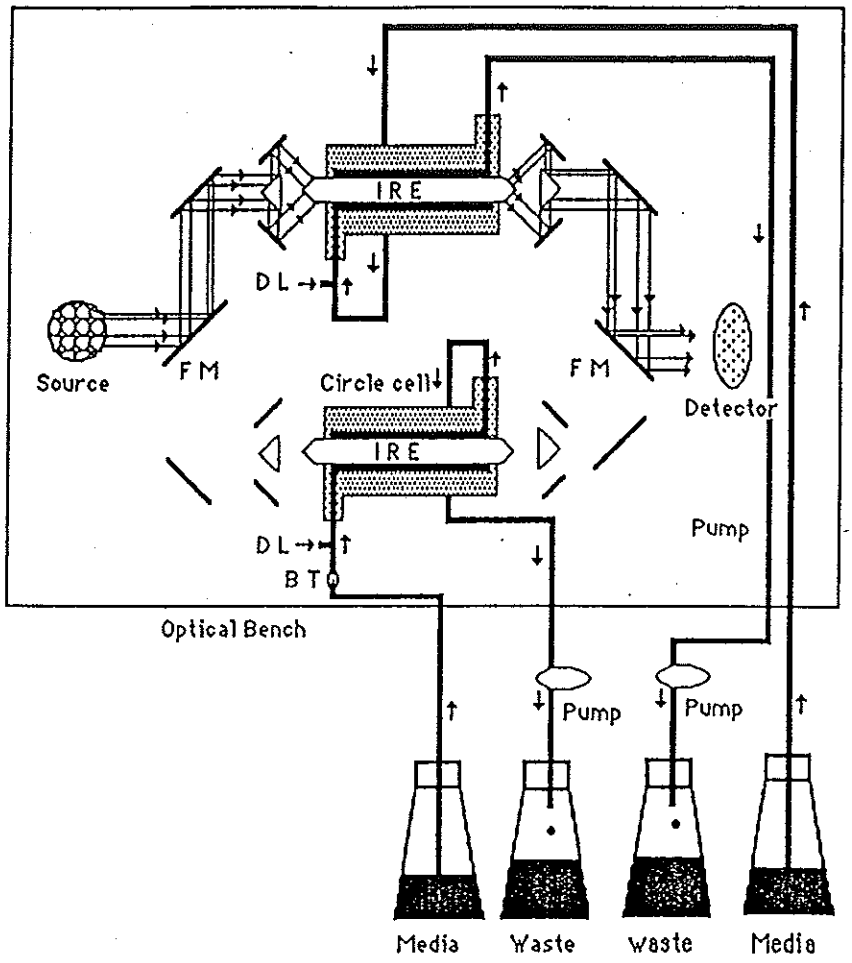

FIG. 2. Diagram of Circle cells positioned in optical bench containing flipper mirror (FP) and media delivery tubing containing media break tube (BT) and deadleg inoculation port (DL).

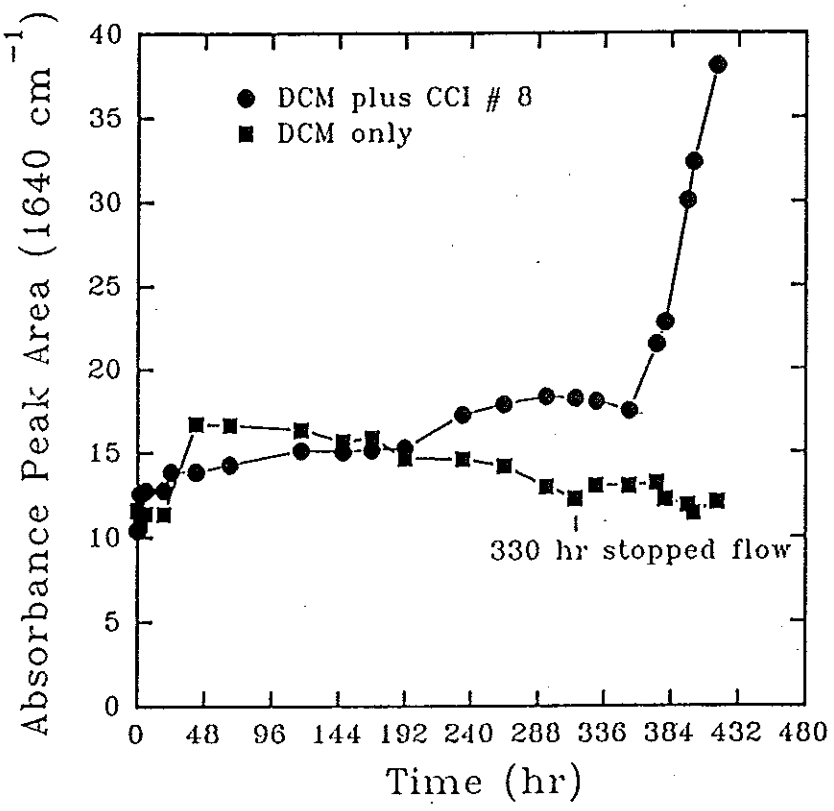

FIG. 3. Intensity of water absorption band (1640 cm-1) as a function of time following inoculation of CP-8 to the sample Circle cell.

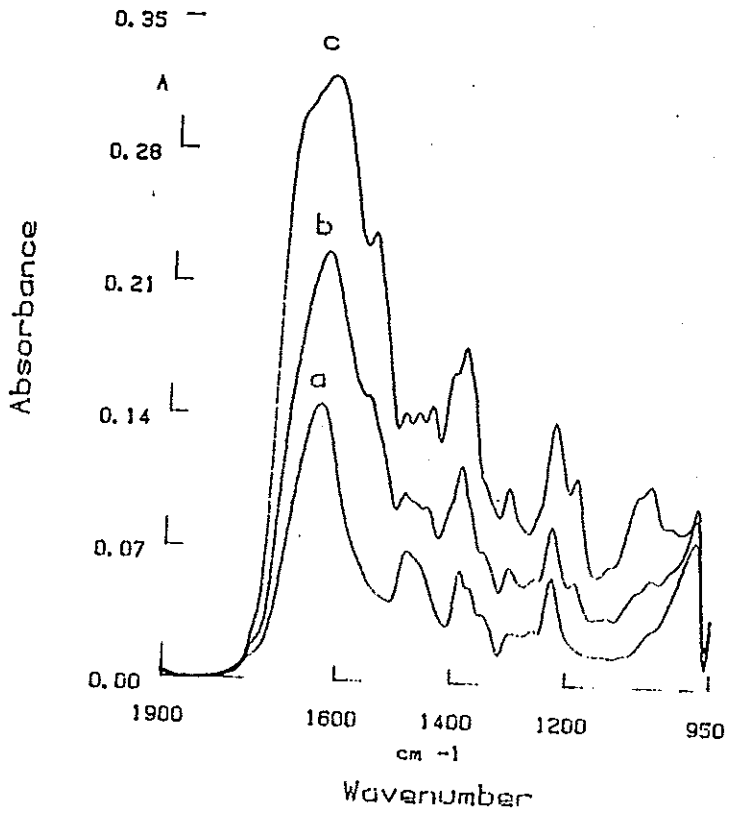

FIG. 4. ATR spectra of collected at various intervals after inoculation of CP-8 to the sample Circle cell; a=sterile culture medium, b=sample cell 380h after inoculation, c=sample cell 417h after inoculation.