89-006

P-157

PAPER NUMBER

190

CORROSION 89

April 17-21, 1989
New Orleans Convention Center, New Orleans, Louisiana

# EVALUATION OF OCCURRENCE AND RELATIVE CONCENTRATION OF ORGANIC PRODUCTS OF BIOFILM METABOLISM THAT ACCUMULATE AT CORRODING COPPER SURFACES

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## ABSTRACT

Attenuated total reflectance-Fourier transform infrared spectroscopy was used to detect components of the exopolymers elaborated by film-forming bacteria that accumulated on a copper surface. Water was subtracted from spectra in order to detect ir absorption bands characteristic of proteins and other organic molecules that concentrated at the liquid-solid interface. Polysaccharide and protein were detected in the exopolymer fraction on the basis of their characteristic ir absorption bands. Adsorption of polysaccharide to the copper thin film was demonstrated by comparing spectra collected before and after rinsing the surface with distilled water. Time-dependent changes in the concentration of adsorbed species was demonstrated by a change in intensity of the absorption bands. The results of this study suggest that this sampling technique should be useful in identifying corrosive metabolites produced by biofilms which accumulate on submerged metal surfaces.

## INTRODUCTION

Surfaces submerged in aqueous environments develop biofilms of adherent bacteria. The biofilm is composed of a consortium of microorganisms. The microrganisms are anchored to the surface by adhesive exopolymers which they synthesize during surface colonization. The exopolymers restrict the movement of the

bacteria and the diffusion of their metabolic products[1].

The corrosion of metal surfaces which contain biofilms is, in some instances, believed to be promoted by the presence and activities of specific bacteria in the biofilm[2,3,4]. Identification of the products of microbial metabolism that accumulate in the region where the biofilm contacts the metal surface, however, has been difficult. Internal reflection spectroscopy has been used in the past to obtain infrared spectra of species at solid-liquid boundaries. Jakobson[5] described how internal reflection elements could be coated with thin metal films to study reactions at a liquid-metal interface. Iwaoka et al.[6] demonstrated that Fourier transform infrared spectroscopy using cylindrical internal reflection elements provides a means of collecting water-subtracted spectra of organic species adsorbed to copper thin films. In this paper we describe how this sampling procedure is used to characterize components of bacterial exopolymers adsorbed to a copper thin film in an aqueous environment.

## METHODS

Germanium (Ge) internal reflection elements (IRE) were coated with a thin film (3.4 nm) of copper using an Enerjet magnetron sputtering system. Substrates were at ambient temperature. Target material consisted of 99.999% pure copper. A pump-down pressure of $2 \times 1^{-7}$ Torr was achieved before sputtering. Neon working gas was introduced into the sputtering chamber at a pressure of 30 milliTorr. The deposit rate was 0.12 nm $s^{-1}$. A quarts crystal oscillator was used to monitor the Cu thin film thickness while graphite furnace atomic absorption spectroscopy was used for analysis of the Cu thickness on microscope slides for system calibration.

The coated IRE was mounted in an open boat cell positioned in a circular internal reflection beam condenser accessory that was mounted on the optical bench of a Perkin Elmer 1800 Fourier transform infrared spectrometer equipped with a mercury cadmium telluride detector. The optical bench and sample chamber were purged with dry air before acquisition of spectral data.

Expolymer was isolated from cells of a bacterium (FRI) isolated from river sediment according to the procedure of Platt et. al.[7] and introduced to the open boat as a 1% solution in distilled water. Spectra were collected at a resolution of 4 $cm^{-1}$. Spectra of distilled water were collected prior to introduction of the exopolymer solution. Aqueous solutions of the plant polysaccharide gum arabic and the protein bovine serum albumin were prepared at concentrations of 10 and 1%, respectively.

## RESULTS

Attenuated total reflectance Fourier transform infrared spectroscopy was employed to identify products of microbial metabolism that accumulate under biofilms on a metallic copper surface submerged in an aqueous environment. When infrared

radiation is reflected internally through a germanium element that is submerged in water, an evanescent wave of radiation penetrates the element surface into the surrounding aqueous phase. An ir spectrum can be obtained for the molecular species in the aqueous environment near the surface of the element (Fig. 1). The depth of penetration of the evanescent wave is dependent upon the refractive indices of the internal reflection element, the medium surrounding the element, and the wavelength of the radiation. The shallower the depth of penetration, the narrower the lense of water that is sampled. The depth of penetration of ir radiation at 1640 $cm^{-1}$ through a germanium IRE into water is approximately 350 nm (Fig. 2). The depth of penetration increases to approximately 850 nm if a zinc selenide IRE is used. Since the depth of penetration increases with increasing wavelength (decreasing wave number), the sampling depth for molecular species such as polysaccharides, which absorb at lower wavenumbers, is somewhat greater than that of water (Fig. 2).

The depth of penetration can also be reduced by depositing a thin metal film over the surface of the IRE. A copper thin film with a thickness of up to 35 angstroms will permit penetration of an evanescent wave of radiation capable of producing a water absorption spectrum (Fig. 3). Films of between 10 and 20 angstroms in thickness provide a water absorption intensity that is 20-40% of a bare Ge IRE. A consequence of this phenomenon is the extreme sensitivity of the water absorption band to differences in thickness of the thin metal film. Detectable differences in water absorption accompany film thickness differences of 2-3 angstroms, which in the case of copper, corresponds to approximately 2-3 atomic layers.

When exopolymer of the biofilm-forming bacterium FRI is suspended in water and introduced into a CIR cell containing a Ge IRE coated with a copper thin film, exopolymer accumulation at the surface of the film was readily detected by ATR-FTIR (Fig. 4). The resulting spectrum was dominated by the strongly-absorbing water band centered at 1640 $cm^{-1}$. An absorption band centered at 1050 $cm^{-1}$ corresponds to the C-O stretching vibration of the sugars in the polysaccharide component of the exopolymer. The identity of the 1050 $cm^{-1}$ absorption band was corraborated by comparison to the spectrum of the polysaccharide gum arabic (Fig. 5).

When the contribution of water is subtracted from the spectrum of the FRI exopolymer, the difference spectrum reveals absorption bands centered at 1540 and 1650 $cm^{-1}$ which correspond to the amide I and amide II vibrations of a protein component (Fig. 6). The identity of these absorption bands was verified by comparison to the water-subtracted spectrum of a 1% aqueous suspension of the protein bovine serum albumin (Fig. 7).

That the polysaccharide material detected at the solid-liquid boundary is adsorbed to the copper thin film was also demonstrated by ATR-FTIR. Comparision of the intensity of the polysaccharide absorption band collected after exposure of the IRE to a solution of gum arabic for 17 days with the intensity of absorption after rinsing and resubmerging the IRE in distilled water, demonstrated that approximately 50% of the polysaccharide initially present (shown in Fig.5) was retained following

the rinse treatment (Fig. 8).

Changes in the concentration of biomolecules at the surface of the copper thin film was also demonstrated by ATR-FTIR. Spectra obtained after exposure of the IRE to a 1% aqueous solution of bovine serum albumin for 5 min and 18 hr yielded amide I and amide II absorbances that increased with time of exposure (Fig. 7). We have also observed an increase in the intensity of the polysaccharide absorption band with increasing time of exposure of a gum arabic solution to a copper thin film (Geesey et al., 1987).

## CONCLUSIONS

These results demonstrate that ATR-FTIR should be a useful sampling approach to detect specific products of biofilm metabolism that accumulate at the underlying surface. The sampling procedure does not interfer with the integrity of the biofilm and therefore should be useful in evaluating changes in product concentration that may occur during biofilm development. Since the path of the ir radiation does not significantly penetrate the bulk aqueous phase, only features of the 500 nm-thick aqueous boundary layer adjacent to the surface are recognized. The technique should be useful in identifying corrosive microbial products that accumulate in biofilms on metal surfaces.

## ACKNOWLEDGMENT

This work was supported by funds from California State University, Long Beach, by funds from the Department of Energy Contract No. DE-AC07-76ID01570, and by funds from the National Science Foundation (ECE-8701462).

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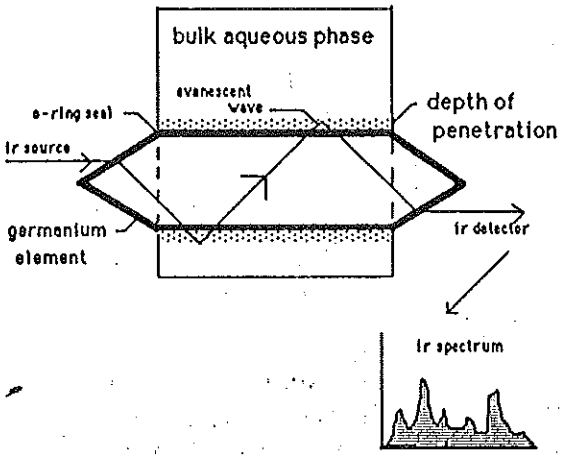


FIGURE 1 - Schematic diagram of circular internal reflection element in open boat cell

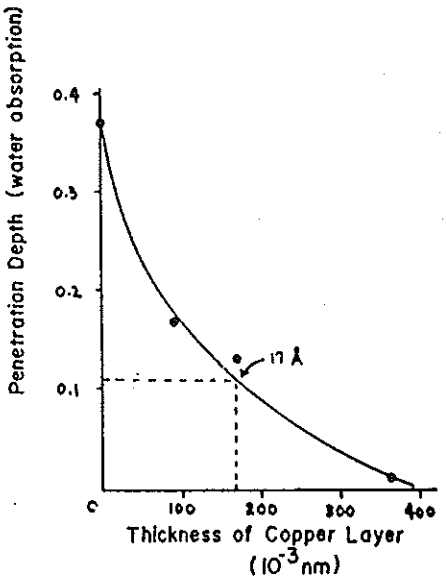


FIGURE 3 - Relationship of thickness of copper film on Ge IRE to penetration depth of evanescent wave in water

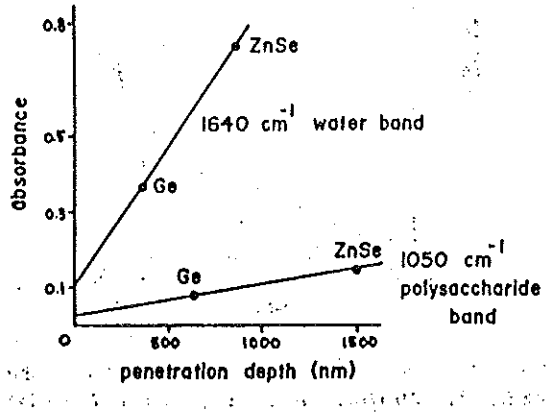


FIGURE 2 - Penetration depth of evanescent wave in water with Ge and ZnSe IREs

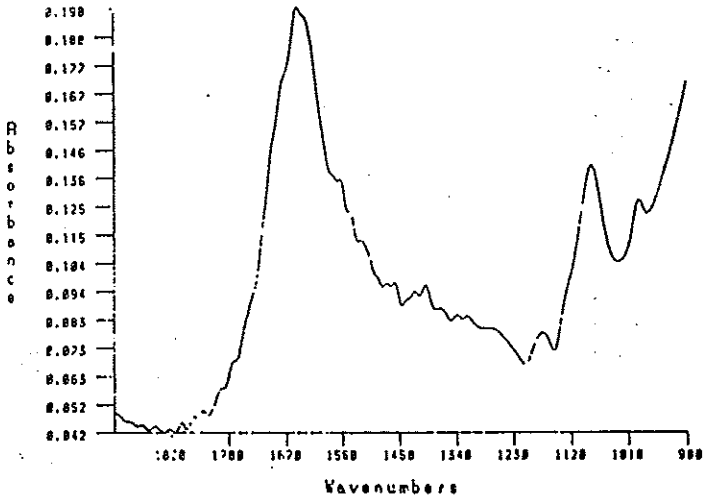


FIGURE 4 - Attenuated total reflectance spectrum of exopolymer from the bacterial isolate, FRI

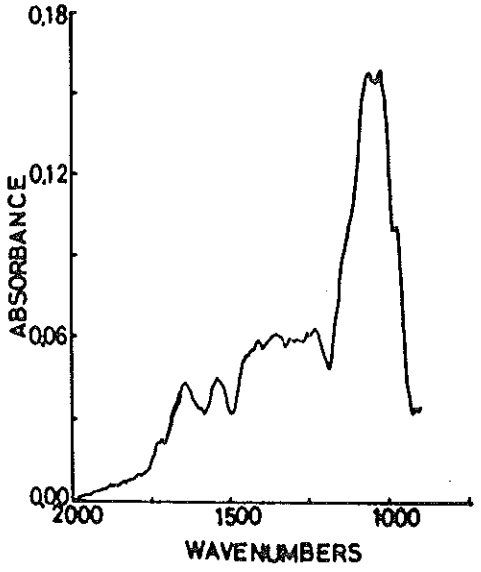


Figure 5 - Water-subtracted spectrum of a 10% gum arabic solution after 17 days exposure to Ge IRE (from Geesey et al., 1987)

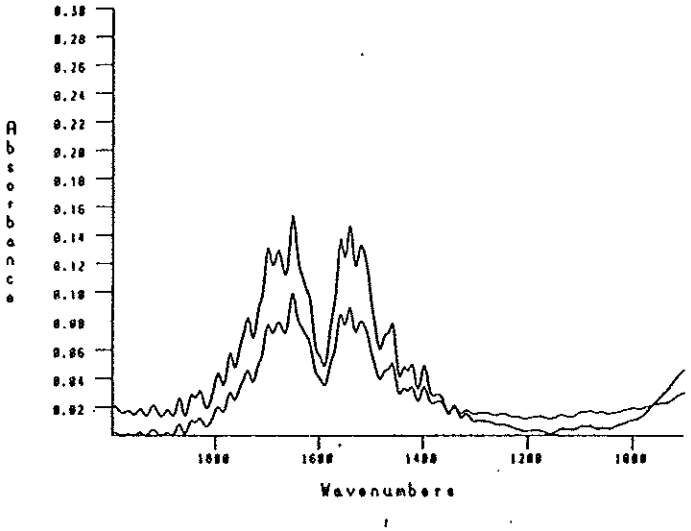


Figure 7 - Water-subtracted spectra of bovine serum albumin. Spectrum with lowest absorbance at 1650 $cm^{-1}$ was collected after 5 min exposure. Spectrum with highest absorbance at 1650 $cm^{-1}$ was collected after 18 hr.

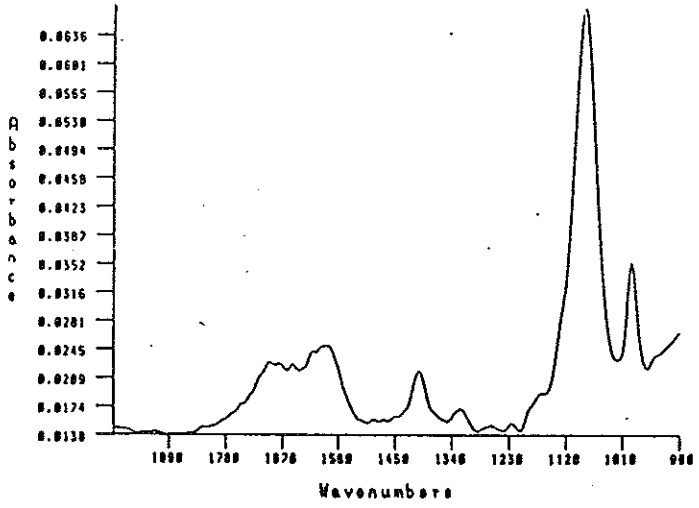


Figure 6 - Water-subtracted spectrum of FRI exopolymer

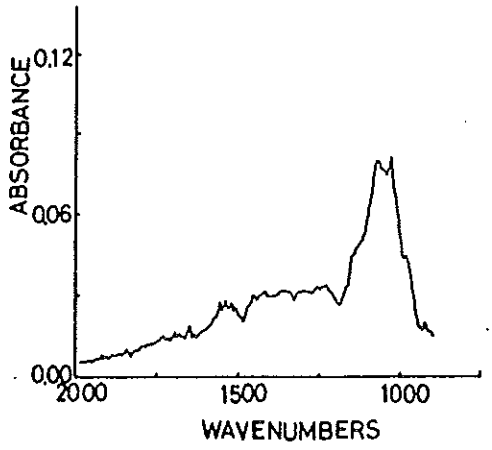


Figure 8 - Water-subtracted spectrum of gum arabic adsorbed to copper thin film after rinsing with distilled water (from Geesey et al., 1987)