METHODS FOR INSIGHT INTO MECHANISMS OF MICROBIALLY INFLUENCED METAL CORROSION

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SUMMARY

Microbially influenced corrosion (MIC) produces areas of preferential metal loss and microbes exacerbate this process by generating acids, chelating metals, and inducing areas of differential cathodic activity. Ultrasensitive methods for determining the microbial biomass, community structure, and nutritional status based on signature biomarker patterns of polar lipid fatty acids can be used to identify specific groups of microbes important in MIC. Monocultures compared for corrosion activity in a compartmented test apparatus permit experiments where the microbial metabolic byproducts are automatically taken into account.

INTRODUCTION

Microorganisms influence corrosion in the environment through complex consortia that form biofilms. The combination of physiologically different microbial types in close association greatly increases the metabolic versatility of the complex. This versatility probably determines the principal type of corrosion observed since it appears that most known 'corroders' grow best on the relatively simple substrates which are provided by other bacteria. The problem in understanding the activities of these consortia comes from the inability of classical microbiological methods to define the biofilm community structure and metabolic activities. Viable plate count methods require

quantitative recovery from the substratum and media that universally allows microbial growth. The first is difficult and the second nonexistant. Direct microscopic examinations using immunological probes allow identification of specific microbes have helped significantly in understanding biofilm dynamics leading to MIC (Pope, 1986).

METHODS

In "signature" biomarker analysis of biofilms (White , 1984) cell wall or membrane components restricted to specific subsets of the microbial community can be utilized to define the microbial community structure. Phospholipids are found in membranes of all cells and are quantitatively extracted without the necessity of recovery of the microbes from the substratum. Phospholipids are not utilized as storage molecules in eubacteria and breakdown rapidly with the loss of cell viability (Nichols et al., 1985). Analysis of phospholipid ester-linked fatty acids (PLFA) by gas chromatography and mass spectrometry (GC/MS), particularly noting the position and configuration of double bonds, branching and cyclopropane ring formation allows identification of specific groups of microbes at levels of a few hundred cells (Odham et al., 1985). A sampling area of at least 1 cm2 is required for extraction when the film biomass is low. Endogneous storage polymers such as poly beta-hydroxy alkanoate (PHA) or extracelluar polysaccahride glycocalyx, allow an estimation of starvation stress (White, 1986). The redox level for facultative anaerobic bacteria can be correlated with the ratio of the respiratory benzo- to naptho-quinones (Hedrick and White, 1987).

Unfortunately these methods are destructive to the biofilm and provide information of the average composition of the biofilm at the specific time of extraction. Non-destructive biofilm analysis by

Fourier transform infrared spectrometry (FT/IR) using attenuated total reflectance (ATR) attachments allows nondestructive examination of the living biofilms. With the microscopic probe, areas of biofilm 10-20 um in diameter can be analyzed thereby providing spatial resolution.

Corrosion is an electrochemical process where the dissolution of metal is controlled not only by charge transfer but diffusive processes. The analysis of such processes have concentrated mainly upon weight loss and polarization methods. Weight loss experiments are problematic in that removal of corrosion products from carbon steel (typically used in bacterial corrosion studies) surfaces is difficult to reproduce. No mechanistic interpretation is possible. Polarization analyses, on the other hand, allow a limited description of the corrosion processes and a simple calculation to obtain the corrosion rate in the form of the current density Icorr. Usually this follows the Stearn-Geary equation :

$$Icorr. = \frac{ba.bc}{2.303\ (ba+bc)\cdot Rp}$$

where ba and bc are the anodic and cathodic Tafel slopes respectively, and Rp is the polarization resistance. Since Rp is defined as the slope of a current/voltage plot when I=0, a scan through the corrosion potential gives a value for Rp. However, direct current (DC) plots may be expected to modify both biological and chemical surface films due to the drastic change in potential which is usually in the order of several hundred millivolts. To counter this, relatively nondestructive techniques such as AC impedance analysis, galvanostatic coupling and 'noise' monitoring were developed.

The AC impedance technique applies low amplitude sinusoidal waveforms (potentiostatic or galvanostatic) over a range of frequencies to a sample under test. The phase shifts of current to potential may be

calculated in terms of the impedance. A value for Rp may be obtained from low frequency impedance data (Gabrielli, 1984) and in conjunction with other frequency impedances may be interpreted to give an idea of the predominating type(s) of overall corrosion mechanism.

In galvanostatic coupling two identical electrodes are separated by a 0.2 uM membrane which permits the passage of metabolites but not bacteria. The electrodes are connected by via a "zero" resistance ammeter (Gerchakov et al., 1986; Little et al., 1986) and provide a value for the net current generated by the difference in rate of dissolution of metal.

Electrochemical noise is a technique where the oscillations in potential of a corroding system are monitored as a passivation layer is sequentially broken and repassivated. The larger amplitude oscillations imply that pitting is a significant process (Dawson et al., 1983; Hepburn et al., 1986) and is a common phenomenon during MIC.

RESULTS AND DISCUSSION

An obvious mechanism of MIC is the generation of strong mineral acids by microbial metabolism. Concrete sewers in which volatile reduced sulfur compounds diffuse into aerated areas encourage the growth of the sulphuric acid producing Thiobacilli. These organisms generate sulfuric acid and the concrete degenerates. These bacteria are difficult to culture in some cases, grow slowly, and may be present in small numbers. Fortunately they contain a wealth of unique PLFA which can be readily detected and rapidly identified (Kerger 1986). The use of PLFA biomarkers analyzed by GC/MS has shown excellent correlations with the degree of biodeterioration of concrete both in test devices and in the field (Kerger et al., 1987).

Studies with organic acid producing facultative heterotrophic microbes which produce acids like acetic acid when grown under conditions of limiting oxygen, have shown MIC estimated by both DC potentiodynamic and AC impedance methods (Dowling et al., in press). Traces of these organisms on the surface of metal coupons were detected by analysis of PLFA. Other studies carried out at IFREMER have shown that cathodic protection even at high impressed currents cannot prevent corrosion from occurring in environments with high numbers of sulphate-reducing bacteria. Figure 1 shows a complex plane impedance plot after being cathodically protected for two months. The frequency decades are also listed. Figure 2 shows evolution of a similar plot with time until one month had elapsed. Thereafter the cathodic protection was removed and the corrosion rate accelerated until a passivation film was formed.

Microbes in natural biofilms occur in consortia. Gaylarde and Johnson (1982) showed a combination of Vibrio anguillarum and Desulfovibrio vulgaris, under anaerobic conditions, induced greater weight loss than either organism alone. Similar experiments in which aerated seawater was used in a continuous flow system confirmed the increased corrosion of the mixed culture and showed that the combination of heterotrophic, facultative acid-producing Vibrio could generate an anaerobic niche that sulfate-reducing bacteria require for growth (Dowling et al., 1986). The addition of bacteria with different physiological activities to artificial systems, with the coupons exposed to media that is continuously flowing, offer an excellent experimental tool to help define the mechanisms of MIC. Corrosion in these systems could be monitored by the non-destructive techniques like AC impedance or electrochemical noise.

The ability to accurately define the microbes constituting a biofilm involved in MIC can be important in defining the mechanism. Careful analysis of sulfate-reducing bacteria has shown that the lactate-utilizing Desulfovibrio contain significant quantities of unusual PLFA (Boon et al., 1977; Edland et al., 1985) that can be readily differentiated from the acetate utilizing Desulfobacter type of sulfate-reducing bacteria (Taylor and Parkes, 1983; Dowling et al. 1986). These biomarkers have been found in the biofilms formed on titanium and stainless steel pipes which have been exposed to pumped Atlantic seawater.

Desulfovibrio contains hydrogenases and is believed to facilitate corrosion by the classical mechanism of cathodic depolarization (Von Wolzogen Kuhr and Van der Vlugt, 1934). Desulfobacter does not contain hydrogenase yet in co-culture with facultative heterotrophs shows facilitation of corrosion (Dowling et al., 1986). Clearly there are other mechanisms besides cathodic depolarization involved in MIC with the SRB's. There is evidence however, that monocultures of Desulfovibrio probably facilitate corrosion to a greater degree than monocultures of Desulfobacter (Booth and Tiller, 1968).

Many bacteria produce extracellular polymers that condition the microenvironment at surfaces and induce irreversible adhesion. Microscopic examination of biofilms of the heterotrophic Vibrio natriegens showed that increased corrosion rates of stainless steel corresponded to the elaboration of extracellular polysaccharide polymer by the biofilm (Nivens et al., 1986). The examination of the coupons with FT/IR correlated bacterial growth with the appearence of adsorption at 1660 cm^{-1} (Amide I of the bacterial proteins) whereas the exopolymer maximal adsorption at 1440 cm^{-1} was found to be a calcium-polysaccharide. Rapid increases in the corrosion rate corresponded with

increases in the 1440 cm^{-1} absorption not the bacteria themselves (1660 cm^{-1}). Removing the bacteria by sonication had little effect on the corrosion rate whereas removing the exopolymer decreased the corrosion rate to control levels. In these experiments it proved possible to show reversible MIC could result from nonuniform deposition of extracellular polymer.

The direct demonstration of facilitation of corrosion by bacterial polymers has been demonstrated elegantly (Geesey 1986). It is possible to follow the chemical changes in living biofilms by using the ATR attachment. In experiments using metal sputter-coated crystals (ATR) the application of acidic extracellular polymers purified from the bacteria were shown to significantly increase the rate of metal loss. Further studies by Geesey and colleagues with surface chemical techniques like Auger and PMS spectroscopy has showed that copper becomes oxidized as it is bound by bacterial extracellular polymers.

The study of MIC is obviously still in its infancy. As new non-disturbing techniques such as FT/IR, biomarkers and transitory-type electrochemical analyses become more widely used the mechanisms involved in MIC will be better explained.

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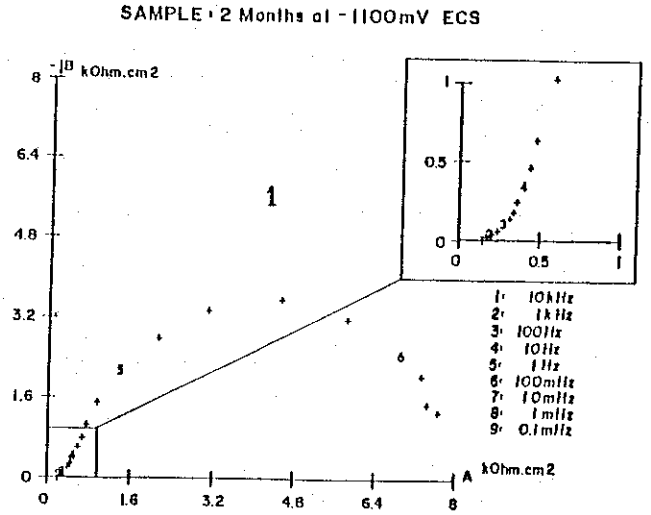

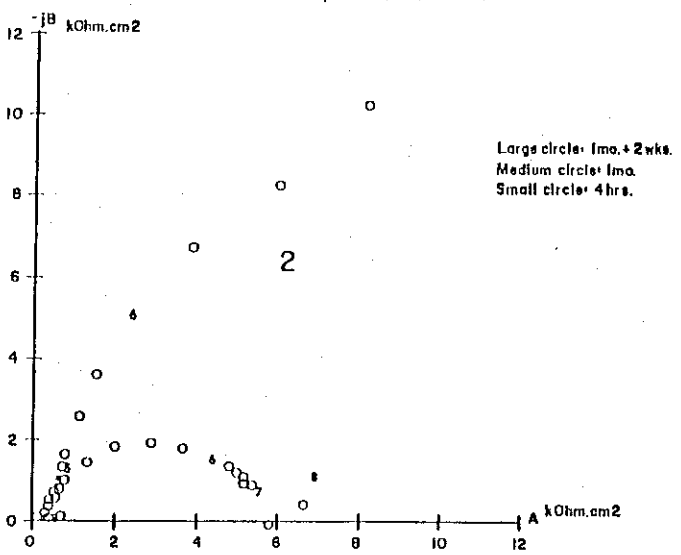

Figure 1: This is an example of a complex plane impedance diagram. Here a 6x6 cm E24 carbon steel coupon was immersed in mud with high numbers of sulphate-reducing bacteria and cathodically protected at -1100 mV/Ag.AgCl. The data was obtained after an immersion time of two months. The semicircle describes a single capacitive loop to approximately 10 mHz. The extrapolated low frequency intercept on the descending (right arm) of the semicircle to the X axis gives a value for Rp, while the high frequency intercept (left arm) provides the uncompensated resistance. This tells us that there is still a significant rate of corrosion which is dominated by a single process, probably the transfer charge resistance. No diffusion phenomena are evinced which would imply that the cathodic protection has successfully prevented the formation of a passive film.

Figure 2: Three plots are shown of coupons similar to those of figure 1 where cathodic protection is at -1100 mV/ECS. After four hours immersion a very small semicircle (fast corrosion) was demonstrated. After 1 month the corrosion is slower (Rp 6KOhms.cm2) however when the cathodic protection is cut, corrosion occurs quickly and formes a passivation film after a further 2 weeks and Rp increases massively (large semicircle).