291

Current Opinion in BIOTECHNOLOGY

Reprinted from Volume 2, 1991

CB
CURRENT
BIOLOGY

Other titles available in the Current Opinion series

Current Opinion in Anaesthesiology
Current Opinion in Cardiology
Current Opinion in Cell Biology
Current Opinion in Gastroenterology
Current Opinion in Immunology
Current Opinion in Infectious Diseases
Current Opinion in Lipidology
Current Opinion in Neurology & Neurosurgery
Current Opinion in Obstetrics & Gynecology
Current Opinion in Oncology
Current Opinion in Ophthalmology
Current Opinion in Orthopaedics
Current Opinion in Pediatrics
Current Opinion in Psychiatry
Current Opinion in Radiology
Current Opinion in Rheumatology

Available soon

Current Opinion in Dentistry
Current Opinion in Genetics & Development
Current Opinion in Neurobiology
Current Opinion in Structural Biology
Current Opinion in Urology

Subscription information

Current Opinion in Biotechnology (ISSN 0958-1669) is published bimonthly by Current Biology Ltd. Each volume consists of six issues of approximately 160 pages.

Subscription rates (Volume 1 and 2, 1990 and 1991, eight parts)

Personal:		Institutional:	
USA and Canada only	US$350	USA and Canada only	US$667
Japan	85,000 yen	Japan	127,000 yen
India	10,390 Rs	India	10,390 Rs
All other countries	£200	All other countries	£400
Cost per single issue	£60		

Orders should be placed with a bookseller or subscription agency, or sent to Current Biology Ltd: *In the USA:* Current Biology Ltd, 20 North Third Street, Philadelphia, PA 19106, USA. *In India*: Mohenjodaro, 29 Sundar Nagar Market, New Delhi 110003, India. *Rest of the world:* Current Biology Ltd, 34–42 Cleveland Street, London, W1P 5FB, UK. Send notices of changes of address to the publisher at least 8 weeks in advance, including both old and new address.

POSTMASTER: Send address changes to *Current Opinion in Biotechnology,* Current Science Ltd, 20 North Third Street, Philadelphia, PA 19106.

Article reprints are available through Current Biology's reprint service. For information contact David Myers at (215) 574-2250.

Printed in the USA on acid-free paper.

ISSN 0958-1669

Biocorrosion

Michael J. Franklin and David C. White

University of Tennessee, Knoxville, Tennessee, USA and Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA

Recent studies have demonstrated that mechanisms of microbiologically influenced corrosion are dependent on the biofilm populations as well as the metal substrata, and that this type of corrosion represents a local phenomenon in terms of both microbial biofilm distribution and activity, and electrochemical activity. The combined approaches of electrochemists, material scientists and microbiologists, using new on-line methods, will continue to provide elegant new insights into the complex interactions between multispecies biofilm consortia and metal surfaces.

Current Opinion in Biotechnology 1991, **2**:450–456

Introduction

Replacement costs and maintenance shut down caused by microbiologically influenced corrosion (MIC) of metal structures are problems in many industries. In addition, the corrosion of long-term waste storage containers by MIC is environmentally hazardous. MIC studies have been initiated in order to better understand this costly and potentially environmentally catastrophic problem. This understanding should lead to more rational countermeasures for MIC.

Studies of the mechanisms of MIC represent a new area of biotechnology. Several traditionally disparate disciplines must be combined in order to elucidate the complexities of microbial interactions with metal substrata. Some of the most important developments in MIC research over the past year have resulted from combinations of nondestructive analytical and electrochemical methods to characterize microbial biofilms and corrosion reactions.

The reader is referred to several detailed reviews of MIC, including proposed mechanisms of MIC [1••], the microbial ecology of biofilms as it relates to MIC [2••] and the electrochemical techniques used to study MIC [3••]. In this review, the results generated using newly developed electrochemical and analytical techniques for MIC studies will be discussed.

Non-destructive electrochemical MIC analysis

Mansfeld and Little [3••] have reviewed the electrochemical techniques used to study MIC. Large amplitude polarization techniques have limited use in MIC studies because of the destructive nature of the applied currents to the microbial biofilms. As a result, several investigators have used techniques which require only small applied potential (or none at all), to study the electrochemistry of the biofilm–metal interface. Among the techniques recently used (in combination with biofilm analyses) are open circuit potential (OCP) analysis, electrochemical impedance spectroscopy, the dual-cell technique and the scanning vibrating electrode technique.

Effect of microbial biofilms on metal open circuit potential

The OCP of metal in the aqueous environment is an important parameter in corrosion. The increased susceptibility of stainless steel to pitting or crevice corrosion in natural seawater has been attributed to the increase in the OCP, caused in part by microbial biofilms. Little *et al.* [4••] have used microelectrodes, as well as scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDAX), to evaluate the relationship between the interfacial chemistry and the changes in OCP on stainless steel samples exposed to Gulf of Mexico seawater. An increase in the OCP was observed when the samples exposed to natural flowing seawater were under continuous illumination. SEM and EDAX analyses revealed that the illuminated samples were enriched with thick biofilms primarily composed of diatoms. Microelectrode analysis showed that the surface of the illuminated samples were aerobic (approximately 2 ppm O_2) and the pH at the interfaces was similar to that of the bulk seawater. In contrast, under reduced light conditions, the potential

Abbreviations

ATR-FT/IR—attenuated total reflectance Fourier transform infrared spectroscopy; **CEB**—Center for Environmental Biotechnology; **EDAX**—energy dispersive X-ray analysis; **EIS**—electrochemical impedance spectroscopy; **ESEM**—environmental scanning electron microscopy; **MIC**—microbiologically influenced corrosion; **OCP**—open circuit potential; **PLFA**—phospholipid fatty acid; **QCM**—quartz crystal microbalance; **SCC**—stress corrosion cracking; **SEM**—scanning electron microscopy; **SVET**—scanning vibrating electrode technique.

dropped when the biofilm formed. The biofilms in reduced light conditions were composed primarily of bacteria, and the O_2 concentration at the metal–biofilm interface was below detection limits. The authors propose that the change in stainless steel OCP could be caused by either thermodynamic effects, such as changes in the pH or O_2 concentration, or kinetic effects, such as enhanced catalysis of the O_2-reduction reaction.

Interestingly, no significant change in OCP was observed on stainless steel exposed to Port Hueneme, California seawater under either continuous illumination or reduced light [5••]. This lack of difference may have been because of the very sparse biofilm on the surface of samples, as observed by SEM.

Effect of microbial biofilms on interfacial impedance

The impedance of metal samples exposed to microbial biofilms has been studied using electrochemical impedance spectroscopy (EIS). With EIS, small amplitude sinusoidal signals over a large frequency range (generally from 10 kHz to 5 mHz) are applied to metal samples, and the amplitude and phase shift of the resulting sinusoidal currents are measured. The interfacial impedance is often modeled using electrical circuits containing resistors and capacitors in series and in parallel. The spectra of responses provide information associated with corrosion. For example, EIS can provide values of polarization resistance (which is inversely proportional to the corrosion rate), double layer capacitance and solution resistance. In addition, using EIS analysis, models to study localized corrosion and three-dimensional surface deposits have been developed (see [3••] for review). Recently, EIS has been used, in conjunction with biofilm analyses, to study the effects of both natural and laboratory-generated biofilms on the corrosion of carbon steel and stainless steels [5••,6••,7••].

Mansfeld *et al.* [5••] have used EIS to study corrosion associated with biofilms on stainless steel generated from Port Hueneme, California seawater. These investigators found little change in the polarization resistance of the steel samples over time, and a small change in the double layer capacitance, which could have been caused by the presence of the biofilm. As with the lack of change in the OCP, mentioned above, the lack of change in polarization resistance may have been a consequence of the sparse biofilm that formed on the samples.

Microbial biofilms are often complex stratified structures, containing many physiological types of microorganisms. Aerobic bacteria can create local anaerobic microenvironments which allow for the colonization of steel by strictly anaerobic bacteria, such as sulfate-reducing bacteria. EIS studies have been performed recently in order to determine the effect of changes in community structure of attached bacteria on the corrosion of steel. Jack [6••] used a flow-through freshwater aerobic system to study the differential effects of various combinations of a strict aerobe (*Bacillus* sp.), a facultative anaerobe (*Hafnia* sp.), and a sulfate-reducer (*Desulfovibrio gigas*) on the corrosion of carbon steel. *D. gigas* was able to colonize the surface of the steel, presumably within anaerobic microenvironments produced by the aerobic organisms. Using EIS, the author demonstrated that carbon steel with the consortia containing *D. gigas* had a lower polarization resistance and therefore a faster corrosion rate than steel without the sulfate-reducer. He also showed that the specific combination of the *Hafnia* sp. and *D. gigas* produced greater corrosion than any other combination, including the triculture, even though the total cell number and the number of sulfate-reducers were less than in the triculture. Thus, specific combinations of bacteria are more important in MIC than the total cell numbers.

The biofilm community structures in these studies were analyzed using cluster analysis of the phospholipid fatty acid (PLFA) profiles. The community structure of the laboratory-generated biofilms was compared with biofilms associated with corrosion tubercles, and with biofilms generated by enrichment cultures. It was found that the natural biofilm had more complex PLFA profiles than the laboratory biofilms, and that none of the laboratory-generated biofilms clustered closely with the natural biofilms. However, interesting results were obtained from the cluster analyses. The PLFAs of attached versus bulk-phase bacteria clustered separately, suggesting that either the community or the physiology of the attached bacteria differs from that of the bulk-phase bacteria. In addition, Jack [6••] found that biofilms generated from tubercle enrichments with sulfate-reducer medium clustered most closely to the original corrosion tubercle. Although it is probably not possible to construct laboratory biofilms which simulate natural biofilms, these cluster analyses have provided a means of comparing the laboratory-generated biofilms with natural biofilms.

Dowling *et al.* [7••] have used EIS to investigate the effects of microbial communities, designed to simulate anaerobic communities found in gas transmission pipelines, on the corrosion of pipeline steel. A synergistic effect on corrosion was observed when an acetogen, *Eubacterium limosum*, and two sulfate-reducers, a lactate-utilizing *Desulfovibrio* sp. and an acetate-utilizing *Desulfobacter* sp., were included in the consortium. This increase in corrosion rate did not correlate with bulk-phase volatile acid concentration or change in pH, which suggests that enhanced corrosion caused by microbial biofilms is a complex process, and that bacterial production of acid is not solely responsible for the increased corrosion rates.

Dual-cell studies of MIC half reactions

The effect of sulfate-reducing bacteria on the corrosion of stainless steel has been attributed in part to the aggressive nature of sulfur compounds [8••,9••], including sulfide and thiosulfate ions. Newman *et al.* [10••] have used a dual-cell to physically separate the anodic reaction, contained in an anaerobic environment with sulfate-reducing bacteria, from the cathodic reactions, occurring on a large-surface-area steel electrode in an aerobic medium. The authors demonstrated that catalysis of the anodic reaction in both carbon steel and chromium-depleted stain-

less steel proceeds by the adsorption of hydrogen sulfide to the steel. The cathodic reaction for carbon steel in an anaerobic environment can be maintained by hydrogen evolution from the large surface area of the iron sulfide precipitate. In most cases, oxygen reduction on a remote cathode was required to maintain the anodic reaction. In some cases, however, reduction of certain sulfur species, such as polysulfide, could stabilize the anodic reaction on the stainless steel. Pitting corrosion was inhibited when chloride was not the predominant anion in solution.

Localized MIC studies using the scanning vibrating electrode technique

Recently, the scanning vibrating electrode technique (SVET) developed by Hugh Isaacs and associates, has been applied to the study of localized corrosion as a result of MIC [11••,12••]. The SVET uses a vibrating electrode to map the potential fields in solution over local anodic and cathodic sites. The vibrating electrode converts the potential fields into an alternating signal. Frequencies not associated with the alternating signal can be filtered, thereby increasing the signal-to-noise ratio, as compared with a non-vibrating electrode. Franklin *et al.* [11••,12••] have used the SVET to localize corrosion on carbon steel associated with MIC (Fig. 1). They have demonstrated that in sterile, continuously stirred microbiological medium, small pits initiated on the steel, and subsequently became inactive (repassivated). In the presence of microbial biofilms, some of the initiated pits remained active. The active pits in the presence of bacteria propagated and spread until a large area of the sample became anodic. Propagation of pits was shown to be dependent on the presence of bacteria, as spent cell-free microbiological medium did not cause pit propagation. Thus, the presence of the microbial biofilm, rather than changes in the bulk medium, caused pit propagation. The authors have proposed that the microbial biofilms may either inhibit migration of aggressive ions, such as chloride, from inside pits or else inhibit migration of inhibiting ions, such as phosphate and hydroxide ions, from the bulk medium to the pits.

Hydrogen-producing bacteria and hydrogen embrittlement

Certain anaerobic bacteria can produce H_2 as an endproduct of the fermentative metabolism of carbohydrates. As atomic H_2 can cause the embrittlement of certain susceptible metals, Ford *et al.* [13••] and Walsh *et al.* [14••] have studied the effects of microbial H_2 production on the permeation of H_2 through palladium and mild steel. These investigators measured the transport of H_2 across a thin metal membrane by measuring the current associated with the anodically polarized 'output' surface of the membrane. They were able to correlate permeation of H_2 through the membranes with the bacterial growth cycle. In addition, the synergistic or antagonistic effects of certain chemicals in solution were characterized. For example, the authors found that H_2 permeation was enhanced with the production of organic acids in the mild steel. Hydrogen sulfide acted to increase H_2 permeation through palladium. The results of these studies indicated that, in addition to causing enhanced deterioration of metals, bacteria may also be involved in the loss of metal ductility, thus enhancing stress corrosion cracking (SCC). Ford and Mitchell [15••] have studied the effect of bacterial-mediated H_2 embrittlement on SCC of steel. They found that cracks which formed rapidly on stressed samples exposed H_2-producing bacteria.

Biofilm dynamics

In situ evaluation of microbial biofilms

The attachment of bacteria to metal surfaces is widely held as a precursor to MIC. Detection of changes in physiology of sessile bacteria often requires removal and destructive analysis of the sample. New insights have resulted from on-line non-destructive methods. Nivens *et al.* [16••] have demonstrated that attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT/IR) can be used to detect changes in sessile microbial biomass. The ATR-FT/IR studies showed that changes in the physiological properties of the attached bacteria were induced by changes in the bulk phase. They demonstrated that the number of attached caulobacters is directly correlated with the intensity of the infrared amide II asymmetrical stretch band at 1543 cm^{-1}, corresponding to bacterial protein. The technique was sensitive to 10^6 bacteria per cm^2, and changes in the physiological status of the attached bacteria could be measured. For example, production of the intracellular storage lipid, poly-β-hydroxyalkanoate, and production of extracellular polymer, were monitored by absorbance at 1730 cm^{-1} (C=O stretch) and 1084 cm^{-1} (C–O stretch), respectively.

Nivens *et al.* [16••] have also investigated the use of the quartz crystal microbalance (QCM), a very sensitive mass-sensing device, for detecting attached microbial films. Although it provided less chemical information regarding biofilm composition, the QCM was more sensitive to changes in biomass than the ATR-FT/IR, with a detection limit of 10^4 bacteria per cm^2 and a linear range of at least two orders of magnitude. An interesting aspect of both the ATR-FT/IR and the QCM is that the substrata of both techniques can be converted to electrodes for electrochemical analyses. Thus, corrosion information can be obtained while changes in microbial biofilms are monitored.

Geesey *et al.* [17••] have used the ATR-FT/IR not only to detect biofilm formation, but also as a spectroscopic method to monitor metal loss. By sputtercoating a thin film of copper on the germanium internal reflectance element used in ATR-FT/IR, they were able to detect changes in the thickness of the copper films by observing the increase in intensity of the infrared water absorption band at 1640 cm^{-1}. The authors compared copper loss from the thin film in the presence of different bacteria isolated from corroded copper samples. Different rates of metal

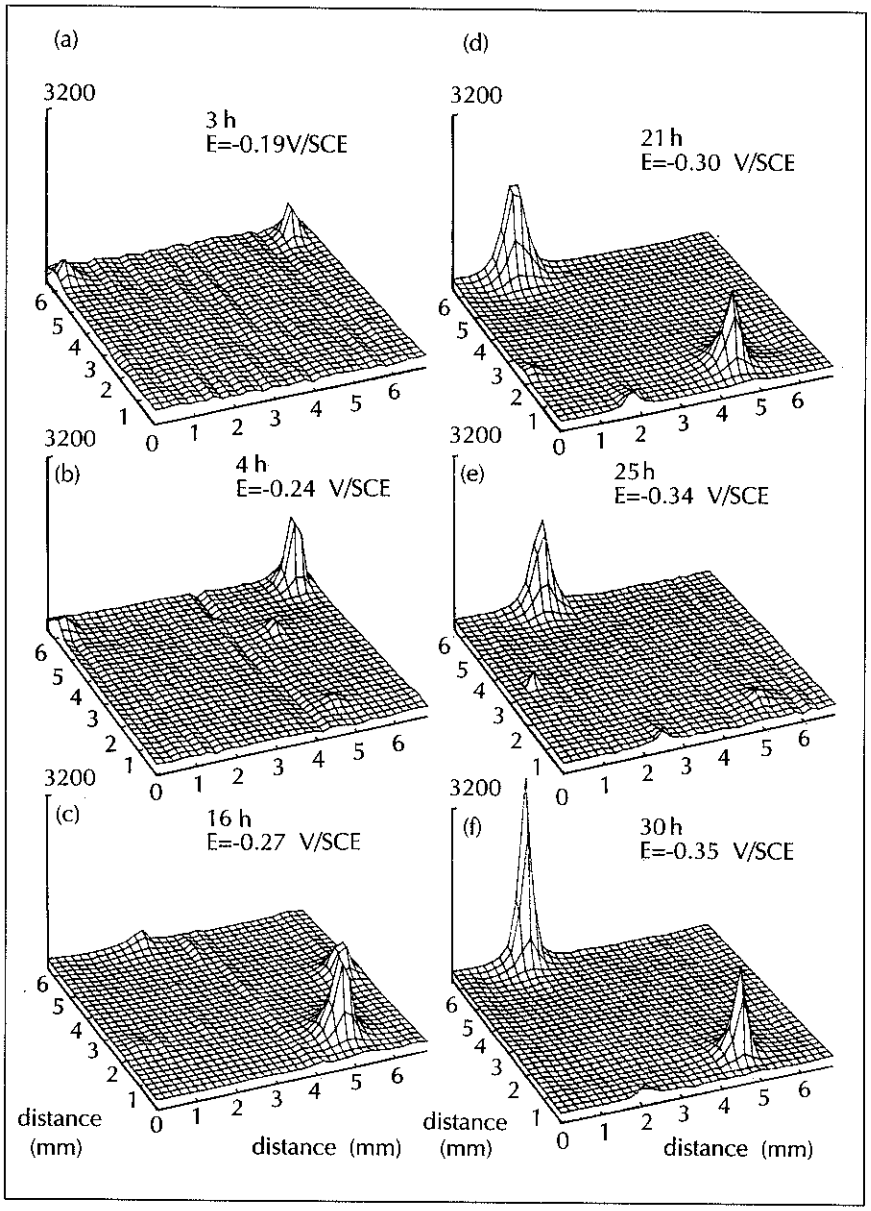

Fig. 1. Current density maps over carbon steel samples obtained with the scanning vibrating electrode technique. The steel was exposed to sterile medium for 17 h (a,b,c), and showed pitting and repassivation. After 17 h, the sterile medium was removed and medium containing a high concentration (10^8 cells ml^{-1}) of fixed bacteria was added. Two of the initiated pits propagated and spread after addition of bacteria (d,e,f). E, open circuit potential; V/SCE, potential versus saturated calomel electrode.

loss were observed in the two cultures. Using this technique, Jolley *et al.* [18••] observed copper loss, caused by the binding by bacterial extracellular polymer, in the absence of bacteria. Additional characterization of these isolates and of the polymer–metal interactions should lead to a better understanding of the nature of bacteria which promote copper corrosion.

Recently, Little *et al.* [19••] have used environmental scanning electron microscopy (ESEM) to study biofilms associated with corroding copper alloys. This technique allowed the investigators to visualize biofilms without the usual dehydration steps required for SEM sample preparation, and to visualize the bacteria within their extracellular polymer matrix (Fig. 2). Use of EDAX in conjunction with ESEM enabled the investigators to analyze the chemical composition of the biofilm-corrosion layers, again with less disruption of the chemistry than when the films were dehydrated. The authors demonstrated that, among other effects of the bacteria, sulfides, produced by sulfate-reducing bacteria, preferentially reacted with the iron and nickel in the copper alloys, resulting in selective loss of these constituents (and loss of the corrosion inhibition provided by these alloying constituents).

Metabolic activities of attached bacteria

Varying the community structure within biofilms leads to changes in corrosion rate that are not dependent on the total microbial biomass [6••]. Thus, the activities of attached bacteria appear to be a more important component in MIC than the attached biomass. Measurement of bacterial activity *in situ* and a comparison of this activity with the corrosion of the metals should provide insight into MIC mechanisms. Roszak and Colwell [20••] have reviewed the techniques commonly used to detect

Fig. 2. Environmental scanning electron micrograph of hydrated biofilm on brass foil. Taken from [19••] with permission.

microbial activities in natural environments. These techniques include transformations of certain radiolabelled metabolic precursors. Phelps *et al.* [21••] and Mittelman *et al.* [22••] have used uptake or transformation of ^{14}C-labelled metabolic precursors to examine the activities of sessile bacteria in natural environments and in laboratory models. Phelps *et al.* used a variety of ^{14}C-labelled compounds to quantify catabolic and anabolic bacterial activities associated with corrosion tubercles in natural gas transmission pipelines. They demonstrated that organic acid was produced from H_2 and CO_2 in natural gas by acetogenic bacteria, and that this acidification could lead to enhanced corrosion of the steel. Mittelman *et al.* [22••] used measurement of lipid biosynthesis from ^{14}C-acetate in conjunction with measurements of microbial biomass and extracellular polymer, to study the effects of differential fluid shear on the physiology and metabolism of *Alteromonas* (formerly *Pseudomonas*) *atlantica*. Increasing the shear force increased the rate of total lipid biosynthesis, but decreased the per cell biosynthesis. Increasing fluid shear also increased the cellular biomass and greatly increased the ratio of extracellular polymer to cellular protein.

Localization of microbial activity

Biofilms are usually spatially heterogeneous, whereas MIC tends to be in the form of localized corrosion. Techniques for analyzing microbial metabolic activity at localized sites are being developed. Franklin *et al.* [23••] incubated microbial biofilms with ^{14}C-metabolic precursors and then autoradiographed the biofilms to localize biosynthetic activity on corroding metal surfaces. The localized uptake of the labelled compounds was related to localized electrochemical activities associated with corrosion reactions, using the SVET. The autoradiographic technique had certain drawbacks with respect to corrosion studies: first, some of the metabolic precursors tend to adsorb to the corrosion products of the steel; and second, the techniques require sample removal and dehydration. It was not possible, therefore, to determine whether localized bacterial activity led to localized corrosion, or whether the bacteria preferentially attached to the corrosion products. In order to better determine the cause and effect of spatial and temporal relationships between the bacterial activity and electrochemical activity, *in situ* techniques for studying both activities must be used. A major breakthrough has been the use of 'reporter' genes that can signal when the activity of specific metabolic pathways are induced. Sayler's research team at the Center for Environmental Biotechnology (CEB) [24••] has engineered the incorporation of a promotorless cassette of *lux* genes into specific operons of *Pseudomonas* so that these operons induce bioluminescence during the degradation of naphthalene. Mittelman *et al.* have used the bioluminescent reporter gene to provide a quantitative measure of the attachment of these engineered organisms onto metal and glass surfaces in a laminar flow system (MW Mittelman, JWM King, GS Sayler and DC White, unpublished data). They found that biofilm light production was directly correlated with biofilm cell numbers in a range of 10^5–10^7 cells per cm^2. In collaboration with CEB, our laboratory is extending this work by resolving, spatially and temporally, the expression of bioluminescent reporter genes in sessile bacteria, to determine the effect of this gene activity on the local electrochemical activity of metal samples.

Perspectives

Although corrosion associated with microorganisms has been recognized for over 50 years, the study of MIC mechanisms is a relatively new field, as the techniques for its study are just now becoming available. Recent stud-

ies have demonstrated that mechanisms of MIC are dependent on the biofilm populations as well as on the metal substrata. They have also shown that MIC represents a local phenomemon in terms of both microbial bioflim distribution and activity, and electrochemical activity. Thus, the combined approaches of electrochemists, material scientists and microbiologists using new on-line methods, will continue to provide elegant new insights into complex interactions between multispecies biofilm consortia and metal surfaces.

Acknowledgements

The authors wish to thank support by contract N00014-87-K-0012 from the Office of Naval Research and RP-3015-1 from the Electric Power Research Institute, the Science Alliance at the University of Tennessee and the Environmental Science Division, Oak Ridge, National Laboratory.

References and recommended reading

Papers of special interest, published within the annual period of review, have been highlighted as:

- • of interest
- •• of outstanding interest

1. •• LITTLE BJ, WAGNER PA, CHARACKLIS WG, LEE W: **Microbial Corrosion.** In *Biofilms* edited by Characklis WG, Marshall KC. New York: John Wiley and Sons Inc. 1990, pp 635–670.

Reviews the electrochemical aspects of microbial corrosion and proposed mechanisms of MIC, including the effects of differential O_2 cells and endproducts of microbial metabolism. In addition, the proposed roles of sulfate-reducing bacteria, metal-reducing and metal-oxidizing bacteria, and extracellular polymers are discussed.

2. •• FORD T, MITCHELL R: **The Ecology of Microbial Corrosion.** *Adv Microb Ecol* 1990, **11**:231–261.

A review which discusses the proposed effects of different physiological types of bacteria on the corrosion of metals. The discussion focuses on the roles of both aerobic and anaerobic processes, including sulfate-reducing bacteria, iron-reducing bacteria and hydrogen-producing bacteria.

3. •• MANSFELD F, LITTLE B: **A Technical Review of Electrochemical Techniques Applied to MIC.** *Corrosion Science* 1991, **32**:247–272.

A review of the electrochemical techniques which have been applied to MIC and the information which was obtained using these techniques. The techniques reviewed include corrosion potential, redox potential, the polarization resistance technique, the dual-cell technique, EIS, electrochemical noise and large-signal polarization techniques.

4. •• LITTLE B, RAY R, WAGNER P, LEWANDOWSKI Z, LEE W, CHARACKLIS WG, MANSFELD F: **Electrochemical Behavior of Stainless Steels in Natural Seawater.** *Corrosion/90, paper no. 150, National Association of Corrosion Engineers*, Houston, Texas, USA 1990.

Microprobes for pH and O_2 and SEM with EDAX, were used to study biofilm–metal interfaces in an attempt to explain the observed increase in corrosion potential of steel exposed to natural seawater. Light was found to influence the composition of microorganisms in the biofilms, and photosynthetic and respiratory activities of the organisms in the biofilms influenced the change in corrosion potential.

5. •• MANSFELD F, TSAI R, SHIH H, LITTLE B, RAY R, WAGNER P: **An Electrochemical and Surface Analytical Study of Stainless Steels and Titanium Exposed to Natural Seawater.** *Corrosion Science* 1991, in press.

EIS, OCP and SEM were used to study biofilm formation and corrosion of steel in flowing seawater from Port Hueneme, California. In contrast to Gulf of Mexico seawater, little change in OCP was observed in illuminated or reduced light conditions, perhaps because of sparse biofilm. EIS indicated no change in sample polarization resistance over the course of the experiments, but the sample capacitance decreased slightly with time.

6. •• JACK RF: **The Effects of Increased Bacterial Metabolic Diversity on the Corrosion of Carbon Steel.** Masters Thesis, University of Tennessee, 1990.

Differences in corrosion rates, measured by EIS, were observed in different consortia of bacteria. In particular, biofilms containing sulfate-reducing bacteria had increased corrosion compared with biofilms without sulfate-reducing bacteria. Biofilm community structures were characterized by cluster analysis of the phospholipid fatty acids.

7. •• DOWLING NJE, BROOKS SA, PHELPS TJ, WHITE DC: **Effect of Selection and Fate of Substrates Supplied to Anaerobic Bacteria Involved in the Corrosion of Pipe-Line Steel.** *Proceedings of the International Congress on Microbially Influenced Corrosion* 1991, Knoxville, Tennessee, USA 1991 in press.

EIS was used to study the effects of an acetogenic bacterium, a hydrogenase-positive sulfate-reducing bacterium, and an acetate-utilizing sulfate-reducing bacterium on the corrosion of carbon steel in an anaerobic marine medium. A synergistic effect on corrosion and less bulk phase acids were observed when the sulfate-reducing bacteria were included in the consortia.

8. •• MORENO DA, DE MELE MFL, IBARS JR, VIDELA HA: **Influence of Microstructure on the Electrochemical Behavior of Type 410 Stainless Steel in Chloride Media with Inorganic and Biogenic Sulfide.** *Corrosion* 1991, **47**:2–9.

A synergistic effect of chloride and inorganic or biogenic sulfide on the corrosion of type 410 stainless steel was observed. Sulfate-reducers lowered the corrosion resistance by forming a non-homogeneous FeS film. Steel samples were prepared with different microstructural conditions to determine the effect of metal microstructure on corrosion resistance.

9. •• DE MELE MFL, MORENO DA, IBARS JR, VIDELA HA: **Effect of Inorganic and Biogenic Sulfide on Localized Corrosion of Heat-Treated Type 304 Stainless Steel.** *Corrosion* 1991, **47**:24–30.

Pitting corrosion of heat-sensitized type 304 stainless steel was studied in the presence of sulfide and chloride. No pitting of the steel was observed in Postgate's C medium, unless chloride was added. Heat-treated samples were more susceptible to corrosion, and a synergistic effect of chloride and sulfide on corrosion was observed.

10. •• NEWMAN RC, WEBSTER BJ, KELLY RG: **The Electrochemistry of SRB Corrosion and Related Inorganic Phenomena.** *J Iron and Steel Institutes of Japan International* 1991, **31**:203–210.

The cathodic and anodic reactions on corroding steel samples mediated by sulfate-reducing bacteria were physically separated and studied, using a dual-cell technique. The anodic reaction could be catalyzed by adsorption of hydrogen sulfide to the carbon or stainless steel. H_2 evolution could act as the cathodic reaction for carbon steel in anaerobic environments, but O_2 reduction was generally required for the cathodic reaction of stainless steel.

11. •• FRANKLIN MJ, WHITE DC, ISAACS HS: **Pitting Corrosion by Bacteria on Carbon Steel, Determined by the Scanning Vibrating Electrode Technique.** *Corrosion Science* 1991, in press.

The SVET was used to map potential fields over carbon steel samples exposed to sterile medium, cell-free spent medium, and bacteria. In sterile-stirred medium, small pits initiated on the carbon steel and subsequently become inactive. In the presence of bacteria, some of the pits became irreversibly active.

12. •• FRANKLIN MJ, WHITE DC, ISAACS HS: **Effect of Bacterial Biofilms on Carbon Steel Pit Propagation in Phosphate-Containing Medium.** *Proceeding of the International Congress on Microbial Influenced Corrosion* 1991, in press.

The effect of biofilms containing viable or formaldehyde-fixed bacteria on the pitting corrosion of carbon steel were studied using the SVET. Irreversible pit propagation occurred in both viable and fixed biofilms, suggesting that the biofilm may help to maintain the aggressive environment in pits that is required for propagation. The time required for

irreversible pitting was dependent on the viability and on the concentration of bacteria.

13. •• FORD TE, SEARSON PC, HARRIS T, MITCHELL R: **Investigation of Microbiologically Produced Hydrogen Permeation Through Palladium.** *J Electrochem Soc* 1990, 137:1175–1179.

H_2 permeation through commercially pure palladium by H_2-producing bacteria was demonstrated. Reducing agents included in the growth-medium affected the rate of H_2 permeation through the metal.

14. •• WALSH M, FORD TE, MITCHELL R: **Influence of Hydrogen-Producing Bacteria on Hydrogen Uptake by Steel.** *Corrosion* 1989, 45:705–709.

The ability of H_2-producing bacteria to mediate H_2 embrittlement of carbon steel was demonstrated. H_2 permeation across a carbon steel foil was correlated with the growth phase of the bacteria, and a synergistic effect of H_2 permeation and bacterial organic acid production was demonstrated.

15. •• FORD T, MITCHELL R: **Microbiological Involvement in Environmental Cracking of High Strength Steels.** *Proceedings of the International Congress on Microbial Influenced Corrosion,* Knoxville, Tennessee, USA 1991, in press.

Stressed wires were exposed to continuous culture of H_2-producing bacteria. Longitudinal cracks, containing bacterial biofilms, appeared on the stressed surface of the wire after only 6 weeks of exposure, indicating that bacterial-mediated H_2 embrittlement can contribute to SCC.

16. •• NIVENS DE, CHAMBERS JQ, WHITE DC: **Non-Destructive Monitoring of Microbial Biofilms at Solid–Liquid Interface Using On-Line Devices.** *Proceedings from the International Congress on Microbial Influenced Corrosion,* Knoxville, Tennessee, USA 1991, in press.

ATR-FT/IR and QCM were used to monitor biofilm formation. ATR-FT/IR provided chemical information about changes in the biofilm that occurred as a result of changes in the bulk medium. QCM was used to detect on-line as few as 10^4 bacteria cm^{-2}.

17. •• GEESEY GG, BREMER PJ: **Application of Fourier Transform Infrared Spectrometry to Studies of Copper Corrosion Under Bacterial Biofilms.** *Mar Technol Soc J* 1990, 24:36–43.

Differences in the rates of copper thin film removal from germanium crystals by different bacterial isolates were observed using ATR-FT/IR. ATR-FT/IR was also used to obtain spectra of surface-bound *Alteromonas atlantica*, and *A. atlantica* exopolymer.

18. •• JOLLEY JG, GEESEY GG, HANKINS MR, WRIGHT RB, WICHLACZ PL: ***In Situ*, Real-Time FT-IR/CIR/ATR Study of the Biocorrosion of Copper by Gum Arabic, Alginic Acid, Bacterial Culture Supernatant and *Pseudomonas atlantica* Exopolymer.** *J Appl Spec* 1989, 43:1062–1067.

FT-IR was used to study the removal of thin films of copper by monitoring the increase in the infrared water absorbance. The different polymers showed different degrees of removal of the copper thin films. Some of the removed copper was incorporated into the polymer matrix, indicating that extracellular polymer can facilitate metal loss.

19. •• LITTLE B, WAGNER P, RAY R, JONES JM: **Microbiologically Influenced Corrosion of Copper Alloys in Saline Waters Containing Sulfate-Reducing Bacteria.** *Corrosion/91 paper no. 101. National Association of Corrosion Engineers.* Houston, Texas, USA.

ESEM showed hydrated biofilm including extracellular polymer matrix associated with copper alloys. Energy dispersive X-ray analysis indicated selective reaction of the alloying agents with bacterial-produced sulfide.

20. •• ROSZAK DB, COLWELL RR: **Survival Strategies of Bacteria in the Natural Environment.** *Microbiological Reviews* 1987, 51:365–379.

Included in this review of bacterial survival strategies are the methods to detect bacterial activities, including the activities of viable but non-culturable bacteria.

21. •• PHELPS TJ, SCHRAM RM, RINGELBERG D, DOWLING NJ, WHITE DC: **Hydrogen Mediated Acetogenesis as a Possible Mechanism for Microbially Influenced Corrosion of Natural Gas Transmission Lines.** *Biofouling* 1991, in press.

Radiolabelled metabolic precursors were used to quantitate microbial activities, including acetogenesis, fermentative metabolism and lipid biosynthesis, in biofilms associated with gas transmission pipelines.

22. •• MITTELMAN MW, NIVENS DE, LOW C, WHITE DC: **Differential Adhesion, Activity, and Carbohydrate: Protein Ratios of *Pseudomonas atlantica* Attaching to Stainless Steel in a Linear Shear Gradient.** *Microbial Ecology* 1990, 19:269–278.

^{14}C-acetate-incorporation into lipids and diffuse reflectance FT/IR were used to study *P. atlantica* biofilms and bacterial activity in a differential fluid shear apparatus. The results indicate that microbial biomass and exopolymer increase with increasing fluid shear, and per cell acetate incorporation decreases with increasing fluid shear.

23. •• FRANKLIN MJ, GUCKERT JB, WHITE DC, ISAACS HS: **Spatial and Temporal Relationships Between Localized Microbial Metabolic Activity and Electrochemical Activity of Steel.** *Corrosion/91 paper no. 115. National Association of Corrosion Engineers.* Houston, Texas, USA 1991.

The local microbial biosynthetic activity of steel was analyzed using autoradiography after incubation of biofilms with ^{14}C-acetate. The SVET was used to study the relationship between this biosynthetic activity and the local electrochemical activity of corroding steel.

24. •• KING JMH, DIGRAZIA PM, APPLEGATE B, BURLAGE R, SANSEVERINO J, DUNBAR P, LARIMER F, SAYLER GS: **Rapid, Sensitive Bioluminescent Reporter Technology for Naphthalene Exposure and Biodegradation.** *Science* 1990, 249:778–781.

A bioluminescent reporter plasmid was developed by insertion of the *lux* gene cassette from *Vibrio fischeri* into a naphthalene catabolic plasmid of *Pseudomonas fluorescens.* Induction of the catabolic plasmid as a result of addition of naphthalene or salicylate to the bioreactor was monitored by light production.

MJ Franklin, Institute for Applied Microbiology, University of Tennessee, 10515 Research Dr. Suite 300, Knoxville, Tennessee 37932-2567, USA.

DC White, Institute for Applied Microbiology (and Department of Microbiology), University of Tennessee, 10515 Research Dr. Suite 300, Knoxville, Tennessee 37932-2567, USA.