Biodegradation **8:** 189–200, 1997.

Instrumental analysis of microbiologically influenced corrosion

G. Chen, R.J. Palmer & D.C. White*
Center for Environmental Biotechnology, University of Tennessee/Oak Ridge National Laboratory, 10515 Research Drive, Suite 300, Knoxville, TN 37932, USA (author for correspondence)*

Accepted 20 March 1997

Key words: electrochemical techniques, microbiologically influenced corrosion (MIC), microelectrode, microscopy, piezoelectricity, spectroscopy

Abstract

Appropriate application of techniques for detection and monitoring of microbiologically influenced corrosion is essential for understanding the mechanistic nature of the interactions and for obtaining control methods. This paper reviews techniques and methods applied to microbiologically influenced corrosion in recent years. The techniques presented in this paper include electrochemical noise measurement, concentric electrodes, scanning vibrating electrode mapping, electrochemical impedance spectroscopy, atomic force microscopy, confocal laser microscopy, Fourier transform infrared spectroscopy, x-ray photoelectron spectroscopy, Auger electron spectroscopy, extended x-ray absorption fine structure and utilization of piezoelectric materials. These techniques are reviewed regarding the heterogeneous characteristics of microbial consortia and their possible influences on metal substrata. We hope this review will motivate application and combination of new techniques for practical detection and on-line monitoring of the impact of biofilms on engineering alloys.

Introduction

Microbiologically influenced corrosion (MIC) occurs where the presence or activity of microorganisms change localized conditions at or near the surface of a metal substratum so that corrosion acceleration or inhibition takes place. Formation of a biofilm may lead to gradients of pH, of oxygen and of chloride thereby creating pitting conditions for passive metals. Products released during growth of certain microorganisms may also be corrosive to the metal substratum; enzymatic products may serve as catalysts of corrosion, and biopolymers associated with the bacterial cell surface or with the extracellular matrix of the biofilm can create localized substratum effects. Sulfide production by sulfate-reducing bacteria (SRB) has been known to cause cathodic hydrogen depolarization (Von Wolzogen Kuhr & Van der Vlugt 1934). H_2S resulting from SRB growth may also damage the passivity of stainless steels (SS) by accelerating anodic interactions (Chen et al. 1995a; Newman et al. 1991; Schmidt 1991; Smith & Miller 1975). A copper-tolerant marine bacterium *Oceanospirillum* sp., has been found to increase the extent of copper corrosion (Angel et al. 1995a; Wagner et al. 1991), presumably through a Cu-binding extracellular polymer. Evidence of MIC lies in the conspicuous presence of microorganisms in those areas where the integrity of the material has deteriorated markedly. However, the electrochemistry of MIC has not been clearly defined due to limitations of the techniques applied in detection and monitoring.

Techniques utilized for detection and monitoring of MIC include conventional electrochemical direct current (DC) methods (Dexter et al. 1991; Little et al. 1991; Mansfeld & Little 1991), alternating current (AC) electrochemical impedance spectroscopy (EIS) (Dowling et al. 1988), optical and electron microscopy, atomic force microscopy (AFM) (Bremer et al. 1992; Steele et al. 1994), Fourier transform infrared spectrometry (FTIR) (Geesey et al. 1990), scanning vibrating electrode mapping (SVEM, Franklin et al. 1991a, 1992), concentric electrode technique (Angell et al. 1994, 1995b; Campaignolle et al. 1993), x-ray photoelectron spectroscopy (XPS) and Auger electron spec-

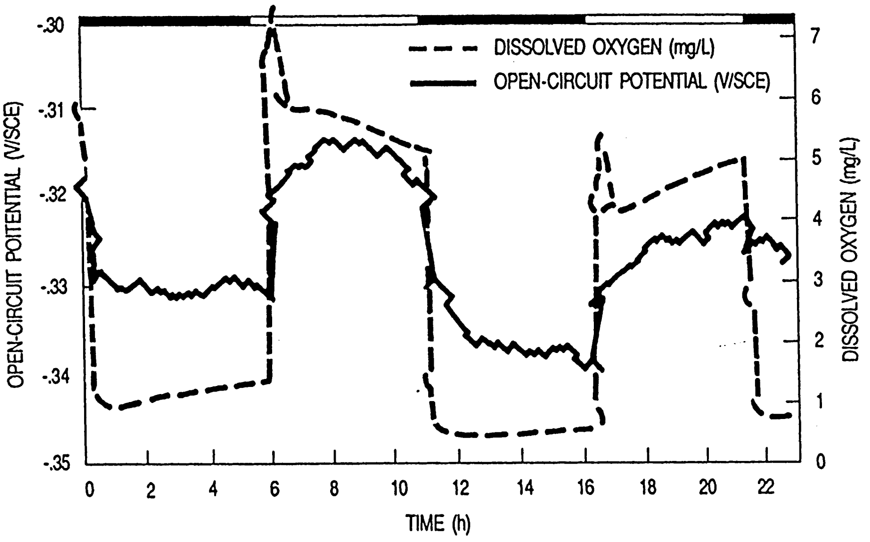

Figure 1. Time dependent changes in open circuit potential of 316L and dissolved oxygen in *Anabena* sp. in dark and light cycle (light period denoted by white slot, dark period by black slot: Dowling et al. (1992), reproduced with permission).

troscopy (AES) (Bremer et al. 1991a; Chen et al. 1995, 1996). These techniques have provided evidence of MIC and mechanistic information about loss of corrosion resistance of engineering alloys. The information on electrochemical interactions provided by DC techniques has been reviewed previously (Dexter et al. 1991; Mansfeld & Little 1991). This review is to evaluate the relatively new techniques applied to MIC. The information obtained by these techniques will be discussed in terms of their electrochemical basis, their efficiency in detecting MIC, and the mechanistic information they provide. Other methods that may have potential application in MIC research will also be introduced.

Electrochemical techniques

Electrochemical noise measurements

Electrochemical noise measurements include measurements of time-dependent changes in open circuit potential and of galvanic current between identical electrodes under identical conditions (Zhang et al. 1989; Johnsen et al. 1985) or galvanic current between identical electrodes in dual biotic and abiotic cells (Dowling et al. 1988; Gerchakov et al. 1986; Little et al. 1986).

Time-dependent changes in open circuit potential of stainless steels and Ti have been interpreted on the basis of the redox potential of the bacterial medium. This greatly assisted estimation of the corrosion tendency of a metal in microbially active environments compared with measurement of open circuit potential only (Zhang et al. 1989). Changes in open circuit potential have also been used to demonstrate oxidation of 316L stainless steel under a photosynthetic biofilm that produced dissolved oxygen upon illumination (Dowling et al. 1992). Periodic fluctuation of dissolved oxygen concentration in the biofilm led to periodic changes in the open circuit potential of 316L SS (Figure 1). This study indicated that surface stability of stainless steels could be altered as the oxygen level changes, and in particular, that an oxygen gradient across a steel surface could be produced if the steel surface is partially illuminated. This result has implications for pit initiation on stainless steels under extended exposure to oxygen-producing bacteria.

Measurement of galvanic currents produced between abiotic and inoculated test cells demonstrated the effect of biofilms on induction of corrosion. Formation of a biofilm on the electrode in the inoculated test cell resulted in a difference in potential between the inoculated and the sterile cells; a galvanic current was generated. However, it should be mentioned that certain metabolic products were able to migrate into the sterile cells through the membrane and could affect surface conditions of the control electrode.

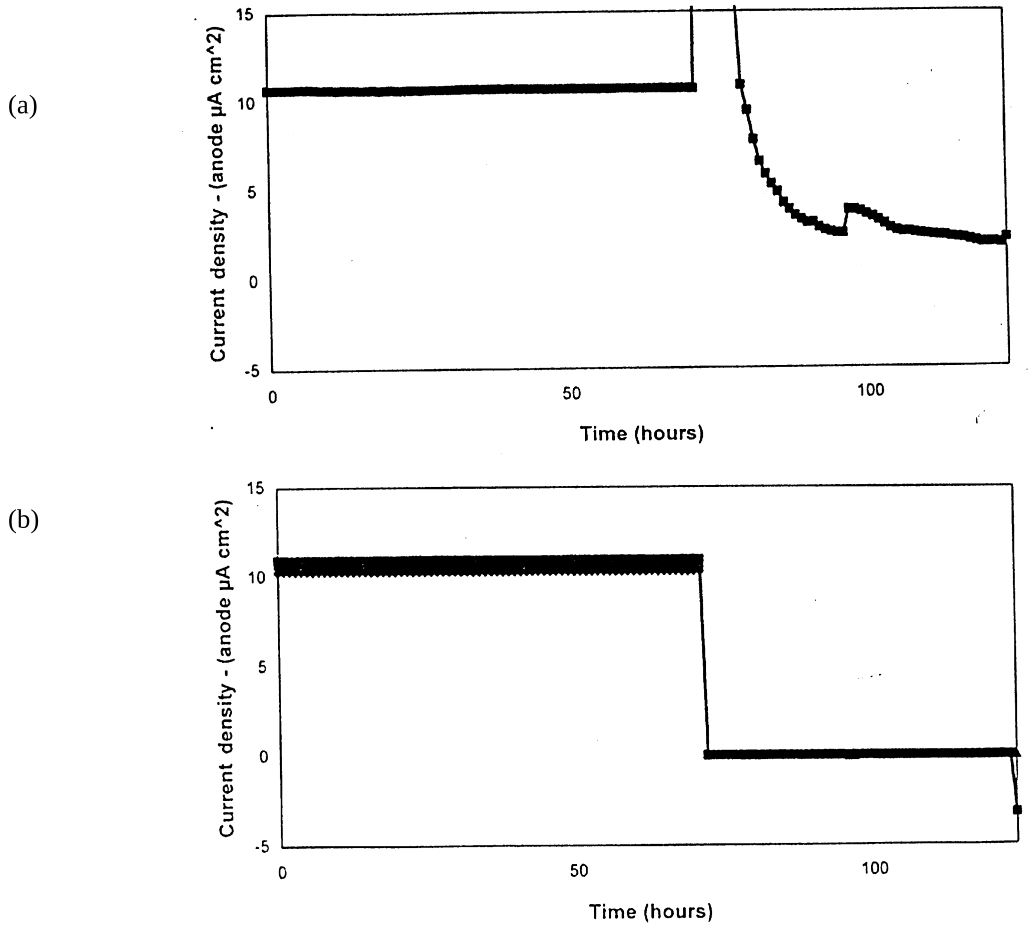

Figure 2. Time-dependent measurement of current flow between anode and cathode for: (a) a mixed culture of *Desulfovibrio vulgaris* sp., (b) sterile medium (Angell et al. (1995b), reproduced with permission).

Concentric electrodes

In this system, a circular small area anode is surrounded by a large area annular cathode. A uniform current profile is produced by applying a small current between the cathode and anode using a galvanostat (Campaignolle et al. 1993; Angell et al. 1995b). Utilization of a large-area cathode reduces the overpotential required to maintain current between the anode and cathode so that a biofilm is less likely to be disturbed by the applied current. Changes in the potential applied to the anode are monitored using a potentiostat, and the galvanic current between the cathode and anode can be measured using a zero resistance ammeter (ZRA) after the applied current is turned off. In SRB-containing anaerobic artificial seawater, this test apparatus indicated that pitting would be initiated due to charge release after applying a 10 μA/cm^2 current for 3 days, and the pits would further develop despite the presence of a biofilm (Figure 2; Angell et al. 1995b). In addition, bacterial response to cathodic and anodic interactions can also be monitored. It was observed that a larger number of bacteria was accumulated on the anode and that a mixed bacterial consortium was able to maintain a longer period of charge release after the applied current was disconnected. Pitting resulted from a longer maintenance of the charge exchange. When applied to the stainless steels, however, this technique has a disadvantage that the over potential to the anode may be too much higher than the redox potential for bacterial growth because the passive film has a blocking effect on anodic dissolution.

Scanning vibrating electrode mapping

Two dimensional (2-D) scanning vibrating electrode mapping (SVEM) measures current density during corrosion (Isaacs 1985, 1989). A highly capacitance-sensitive microelectrode scans over a metal surface exposed to an electrolyte and detects potential differences between anodic and cathodic areas. The potential vector is converted into current by means of an analog/digital board. Current density in pitting areas is recorded, and thus cathodic and anodic areas are mapped. The current density maps can be overlayed with images of the surface obtained through the microscope. This technique finds application in MIC as heterogeneous bacterial colonization on metal substrata often leads to distinct anodic and cathodic areas associated with gradients of pH, of oxygen and of chloride (Mansfeld & Little 1991; Little et al. 1991). Using SVEM, it was seen that *Pseudomonas* sp., isolated from a steel pipe tubercle, caused pitting of carbon steel C1020 (Franklin et al. 1991a). Localized anodic activity of the steel developed as the biofilm was formed and the pits were not able to repassivate. In contrast, the spent medium was unable to induce pitting. Inhibition effects of phosphate on corrosion of C1020 carbon steel in this aerobic growth medium were shown using SVEM (Franklin et al. 1992). The map of current density clearly revealed repassivation of the steel in the presence of phosphate which was added in order to promote bacterial growth (Figure 3). These data were consistent with measurements of open circuit potential and polarization resistance. Anodic areas on a copper surface colonized by *Oceanospirillum* sp. were also observed using SVEM (Angell et al. 1995a).

Use of a confocal laser microscope (CLM) to acquire biofilm images while recording current density with the SVEM shall allow elucidation of the role of biofilm in corrosion: overlapping the current maps with the biofilm image obtained by CLM. Application of SVEM to MIC can be extended to measurement of gradients of protons, of oxygen and of chloride over biofilms using ion-specific microelectrodes (De Beer et al. 1994; Lewandowski et al. 1993). By overlapping the map of current density with distributions of these pit-inducing ions, understanding of the mechanism of MIC will be improved.

Electrochemical impedance spectroscopy

In electrochemical impedance spectroscopic analysis, a reasonably small magnitude of sinusoidal potential around open circuit potential (usually 5 to 10 mV rms) does not markedly affect bacterial growth and activity (Franklin et al. 1991b). This is one reason for increased interest in EIS for MIC research. Secondly, decreases in the phase angle and total impedance at low frequencies promptly indicate initiation of pits that may be too small to observe using microscopic methods. Exposure of metallic substratum in local areas causes decrease in the low-frequency imaginary impedance which is a function of the double layer capacitance and diffusional property of the passive film (Macdonald 1991; Mansfeld & Lorenz 1991). EIS measurement of the anode used in the concentric electrode test showed a marked decrease in the polarization resistance, presumably as a result of bacterial colonization and application of anodic current (Angell et al. 1995b). EIS analyses of stainless steels and of Ti metal exposed to natural seawater for different periods were conducted using seawater and NaCl solutions having different pH as test electrolytes (Little et al. 1991; Mansfeld et al. 1992). Double layer capacitance was not markedly altered by extended exposure period. Thus, passive films formed on these alloys were not considerably deteriorated.

EIS data have been interpreted through equivalent circuit diagrams representing structures of passive films and coatings in simple aqueous solutions. The contribution of biofilm to the impedance measurement has not been clarified. Heterogeneous biofilms on metal substrata may contribute to resistance and capacitance values by: (1) modification of double layer capacitance and diffusional properties through incorporation of certain species into the passive film and through interfacial interactions with the passive film, (2) convolution of the EIS spectrum through electron, charge and composition exchange activities at the biofilm-bulk liquid interface during bacterial growth and, (3) changes in the bulk medium composition due to bacterial growth that may not be shown timely at low frequencies. It is difficult to evaluate these contributions because EIS data alone provides a summation of the electrochemical properties of a system regardless of spacial heterogeneity. In this regard, measurement of EIS properties of natural seawater-exposed metals in a sterilized seawater or salt solution eliminated some of the variables that could not be suitably evaluated. Certain metallic compounds in the biofilm can be incorporated into the passive film and thereby modify its electrochemical properties through diffusion, formation of clusters, and other physical and chemical interactions. As a result, the electrical and diffusional properties of the passive film will be changed. For

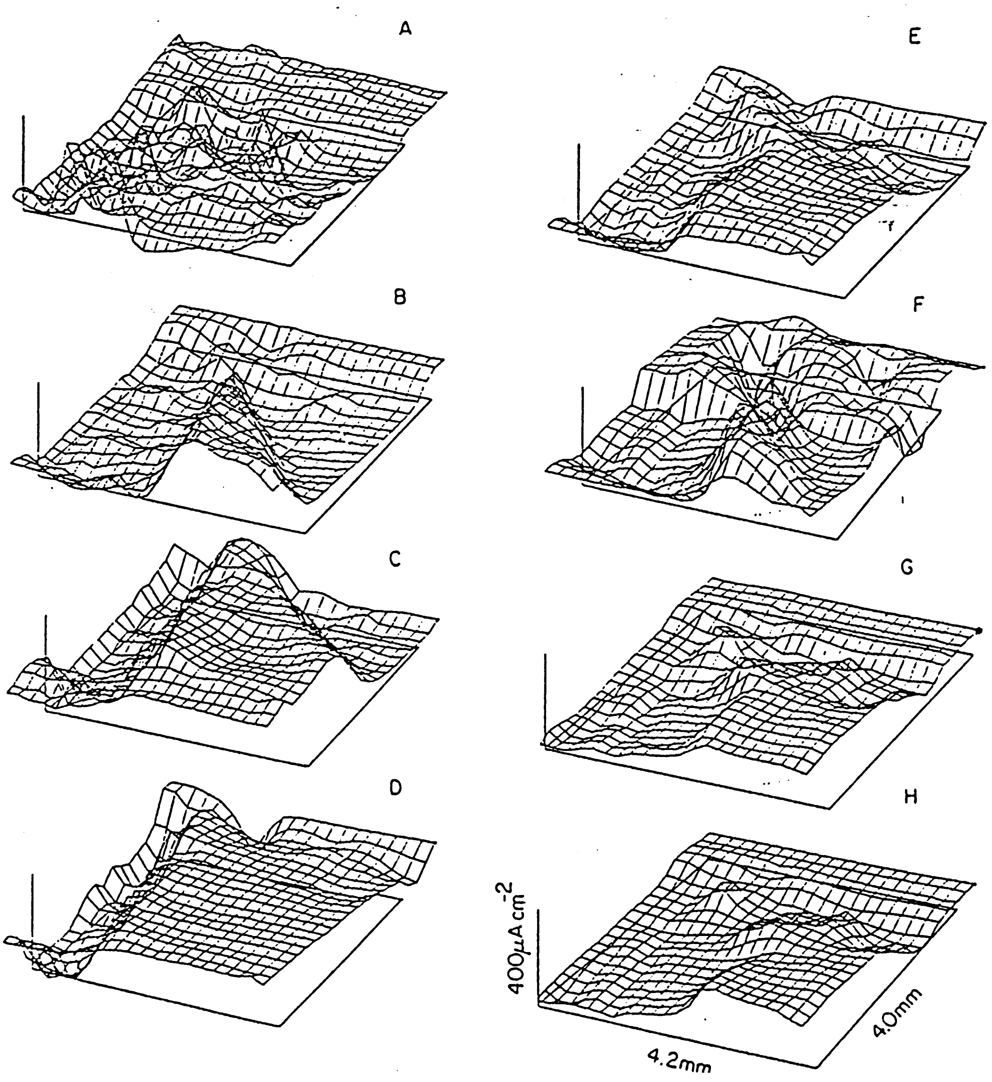

Figure 3. 2-D current density map over carbon steel in an aerobic medium for growth of *Pseudomonas* sp. Left column: (A) 6 minutes after immersion; (B) 60 minutes after immersion; (C) 130 minutes after immersion; (D) 260 minutes after immersion, then 0.2 mM phosphate added to the medium. Right column (with 0.2 mM phosphate): (E) 270 minutes after immersion; (F) 320 minutes after immersion; (G) 460 minutes after immersion; (H) 600 minutes after immersion and phosphate concentration had been increased to 0.8 mM before this measurement (Franklin et al. (1992), reproduced with permission).

example, ennoblement and increases in polarization resistance of stainless steel took place after exposure to aerobic river water (Dickinson & Lewandowski 1995). The interactions at the biofilm/metal interface change the interfacial energies and consequently electrochemical dynamics and kinetics will be altered. This has not been properly demonstrated by EIS measurement. Furthermore, electron exchange activity during bacterial growth in bulk liquid contributes additional variables to the resistance of the liquid, and a consortium of bacteria will result in differences in electron exchange activities between local volumes.

Difficulties involved in EIS measurement in MIC can be reduced through careful experimental design. As mentioned, EIS measurement of seawater-exposed metal samples in a sterile salt solution eliminated some variables. Interpretation of EIS data in the presence of biofilm will be greatly assisted by running control tests in sterile medium representing the situation without microbial interaction and in the presence of the bacteria but using an inert metal representing all of the interactions except those at the interface between the biofilm and an reactive metal substratum. With modern microscopic and surface analysis techniques, such as confocal laser microscopy to characterize the structure of biofilm, XPS and AES to analyze the resultant surface chemistry and surface structure, EIS will play an important role in study of the electrochemical nature of MIC.

Microscopic techniques

Scanning electron microscopy

The existence of bacteria in pitted areas has been demonstrated by several groups using SEM (Jack et al. 1996; Lee & Charaklis 1992; Little et al. 1991; Nivens et al. 1986). SEM has been employed to reveal the presence of microorganisms at the cross sections of pitted areas of CuNi alloy and mild steels. Energy dispersive x-ray spectroscopy (EDX) is routinely applied to analyze pit composition. However, few SEM photographs have shown microbially induced pitting on stainless steel. This is probably because the degree of interaction is only limited to a few surface monolayers and the information is overshadowed by characteristic x-rays from deeper regions in EDX analysis.

Atomic force microscopy

Atomic force microscopy was applied to MIC soon after its development (Bremer et al. 1992; Steele et al. 1994). AFM utilizes a microprobe mounted on a flexible cantilever to detect surface topography by scanning at a subnanometer scale. The repulsion by electrons overlapping at the tip of the microprobe causes deflection of the cantilever which is subsequently detected by a laser beam. The signal is read by a feedback loop to maintain a constant tip displacement by varying voltage to a vertical piezoelectric control. The variations in the volgate mimic the topography of the sample and, together with the movement of the microprobe in horizontal plane, are converted into an image. Corrosion of 316L SS under the biofilm of a consortium of SRB (*Desulfovibrio gigas*), aerobes (*Pseudomonas aeruginosa*) and bacteria isolated from a corroded pipework was studied using AFM (Steele et al. 1994). Extracellular polymeric substance was seen to be associated with different morphological cells and pits were formed in the areas having surface deposits. Profiles, depth and size of pits were analyzed. AFM has the advantage of enabling analysis of sample topography in ‘as received’ states, unlike electron imaging that requires a conductive sample.

Confocal laser microscopy

Confocal laser microscopy (Lawrence et al. 1994) is another new imaging technology that may be used to monitor *in situ* biofilm formation in MIC. This microscope utilizes confocal apertures to create a plane of focus and thus the out of focus light will be eliminated. A laser light source is employed to excite fluorophores and the resultant fluorescence is detected by photomultiplier tubes forming a digital image. Confocal laser microscopy has the advantage in that it resolves 3 dimensional images and thus allows study of the structure of living biofilm using suitable stains to visualize the bacteria without damaging their viability. By optically sectioning a biofilm, spatial arrangement of microorganisms may be visualized and thus the information regarding their interactions with the substratum may be obtained. Confocal laser microscopy has been utilized to determine the diffusion coefficients in biofilms combined with fluorescein and size-fractionated fluoro-conjugated dextrans. This has the potential of becoming an important method in study of kinetics of MIC. If EIS is performed under confocal laser microscope, EIS data may be more properly evaluated. By combining with concentric electrode techniques and scanning vibrating electrode mapping, confocal laser microscopy has the potential of *in situ* mapping the responses of microorganisms to cathodic and anodic interactions by overlapping the biofilm images with the maps of current density.

Spectroscopic analysis

Fourier transform infrared spectroscopy

Advantages of Fourier transform infrared spectroscopy over dispersive instruments have been well recognized for studies of living biofilm (Nivens et al. 1995). Attenuated total reflection (ATR) FTIR is designed for study of biofilms in aqueous environments using an IR-transparent internal reflection element (IRE) as a substratum, such as Ge (Nivens et al. 1993a). With total internal reflection, a standard wave of radiation penetrates into the solution from the IRE. The molecules near the outer surface of the IRE absorb the radiation wave and their absorption composes an spectrum.

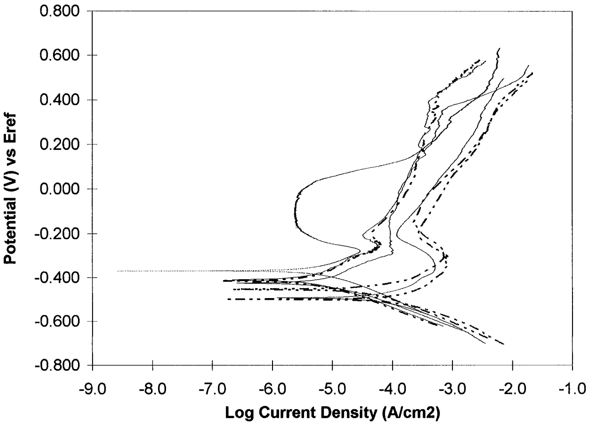

Figure 4A. Potentiodynamic polarization diagrams of SRB-exposed 304 SS coupons in deaerated 0.1 M HCl (E_{ref} vs. saturated calomel electrode, ..., 'As polished'; —— In SRB for 5 days, unrinsed; – – – – In SRB for 5 days and then rinsed: Chen (1996b)).

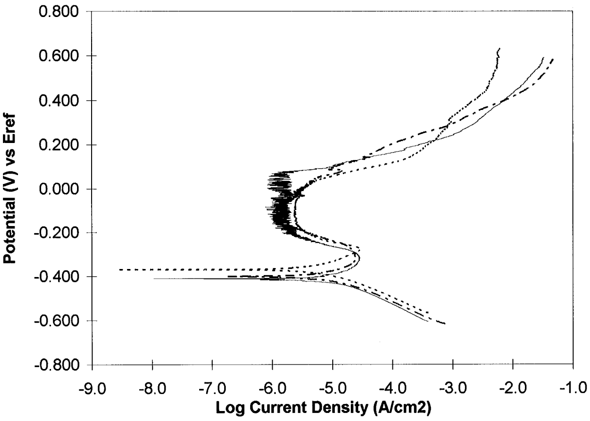

Figure 4B. Potentiodynamic polarization diagrams of control samples of 304 SS in deaerated 0.1 M HCl following immersion in uninoculated medium for the same period (E_{ref} vs. saturated calomel electrode, ..., 'As polished'; —— In uninoculated medium for 5 days, unrinsed; – – – – In uninoculated medium for 5 days and then rinsed: Chen (1996b)).

Copper corrosion under the biofilm has been monitored using FTIR by coating a thin Cu film on Ge (Bremer et al. 1991b; Geesey et al. 1990, 1988; Jolley et al. 1989). Dissolution of Cu was revealed by an increased absorption of the water band at 1640 cm^{-1}. Using flow cells, FTIR detected that the bonding mechanism of biofilm of *Catenaria anguillulae* was contributed by extracellular proteins (Tunlid et al. 1991). A major disadvantage of FTIR is its inability to differentiate dead cells from living ones. In addition, application of this technique to MIC is also limited by strong absorption of IR by water. If properly combined with electrochemical techniques, FTIR has the potential of *in situ* monitoring interfacial interactions between a biofilm and a metal substratum. For example, a controlled electrochemical interaction such as potentiostatic, galvanostatic or polargraphic experiment may be conducted using an appropriately designed reactor that fits a FTIR spectrometer.

X-ray photoelectron spectroscopy and Auger electron spectroscopy

Techniques for analysis of solid surfaces, such as XPS and AES have been applied to study MIC in recent years (Bremer et al. 1991a; Chen et al. 1995, 1996). Unlike SEM and EDX, the kinetic energies of Auger electrons and x-ray-excited photoelectrons are far lower than those of fluorescence x-rays generated by electron beam in SEM so that meaningful information can be obtained only when they are not considerably attenuated by surface layers. Namely, only photoelectrons and Auger electrons from a few monolayers of the top surface are characteristic of the elements and molecules in these monolayers.

As the binding energies of the photoelectrons and Auger electrons are specific for an atom and electron distribution around the atom brings about changes in the binding energies of the electrons, the XPS spectrum and fine structure of Auger electron spectrum provide information about the surface composition as well as chemical states through which the electron exchange process may be understood. In addition, compositional depth profile may be determined using Ar^+ bombardment to remove the top layers during XPS and AES analysis, and by varying take-off angles of photoelectrons with respect to sample surface (Hofmann 1983; Briggs & Riviere 1983).

In an investigation of the loss of passivity of stainless steel exposed to SRB (*Desulfovibrio desulfuricans*, ATCC 7757), XPS was employed to analyze the resultant surface changes, combined with DC polarization tests in deaerated 0.1 M HCl (Chen et al. 1995, Chen 1996b). This method was successful in studying the mechanisms of SRB-induced pitting formation, because pits are usually formed in local areas having a low pH and high chloride content. Therefore, any surface changes arising from the exposure to SRB would be revealed and easily understood, as 0.1 M HCl is a commonly known testing electrolyte. The potentiodynamic polarization diagrams of the SRB-exposed 304 SS and the control samples are given in Figure 4. The XPS spectra of $Fe2p_{3/2}$ obtained at different stages of

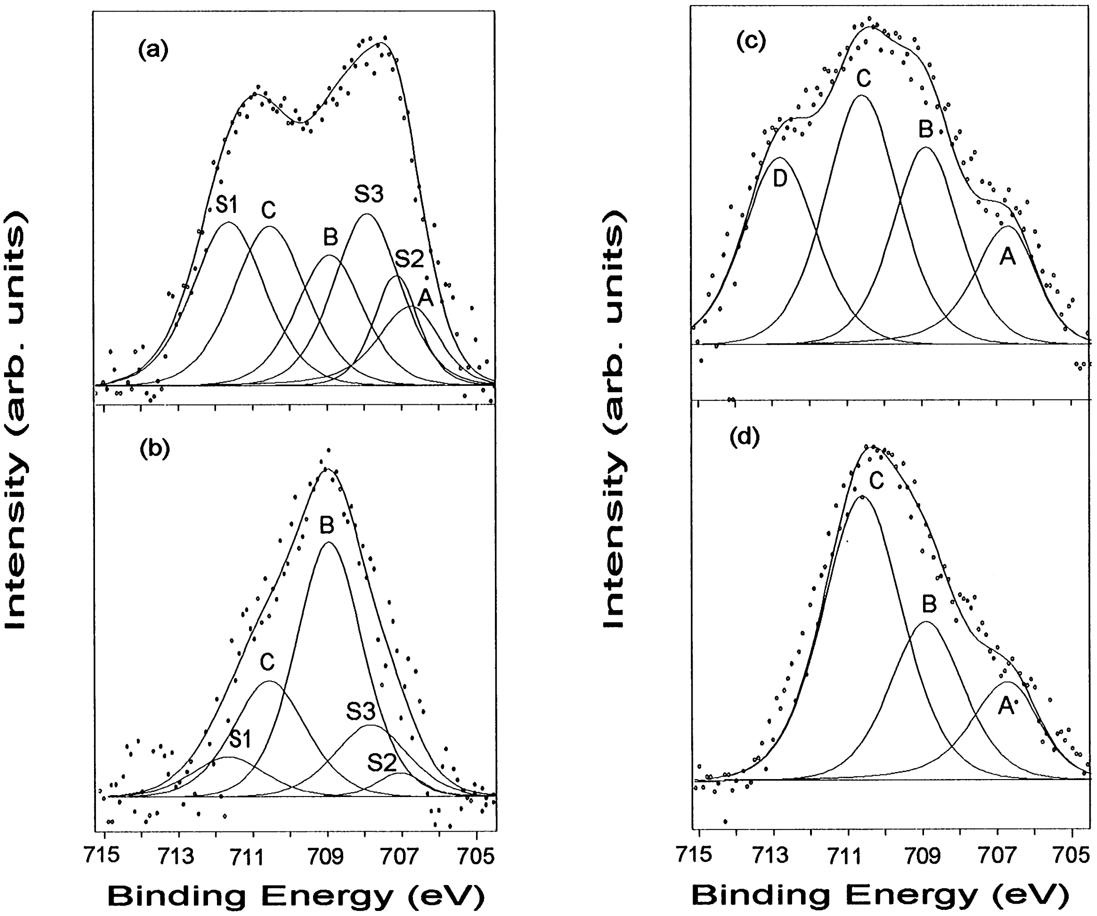

Figure 5. XPS spectra of $Fe2p_{3/2}$ obtained from the SRB-exposed and control samples, take-off angle 20°: (a) immediately after exposure to SRB; (b) Exposed to SRB for 5 days rinsed and then anodically polarized at – 160 mV_{SCE} in deaerated 0.1 M HCl for 5 minutes; (c) control sample corresponding to (a); (d) control sample corresponding to (b) [A: Fe; B: FeO; C: Fe_2O_3; D: FeOOH; S1: FeS; S2: FeS_2; S3: Fe_xS: Chen (1996b)].

the polarization test are given in Figure 5 to demonstrate the process of the passivity loss. In this study, it was known that exposure to SRB resulted in formation of various types of sulfides including FeS, FeS_2, Fe_xS, NiS and a small amount of Cr_2S_3. Uneven depth distribution of the sulfides resulted in undercutting of the passive film. Unlike ferric oxide that normally exists in the passive film, ferrous oxide, an indicator of active dissolution (Pourbaix & De Zoubov 1966), was present on the SRB-exposed surface. This explained the rapid anodic dissolution during polarization in 0.1 M HCl even though a small portion of the surface showed a tendency to passivate. In addition to sulfides, hexavalent chromium species were also detected from the SRB-exposed sample, probably due to production of dissolved oxygen during sulfidation of iron oxides and to the activity of extracellular proteins in providing electrons for SRB growth.

XPS provides information about composition and valence states of surface elements over a relatively large area. In addition to detection of MIC, the mechanistic nature of the interaction can also be explored. But as a new technique applied in MIC, it will be helpful to provide a confirmative analysis using techniques familiar to both corrosion scientists and microbiologists when XPS is utilized to analyze metastable products (Chen 1996b). In contrast, Auger electron spectroscopy may be used to analyze smaller areas (0.1 μm^2) by adjusting the size of incident electron beams. AES is able to obtain chemical information of pits and to provide a secondary electron image. Scanning Auger elemental mapping provides information about elemental distribution over an area. However, chemical shifts in AES spectrum are more difficult to interpret and electron bombardment may damage the surface compounds by causing reduction of metal oxide and evaporation of unstable species, because the density of energy is much higher than x-ray beams used in XPS. Because both XPS and AES are operated under ultrahigh vacuum ($\leq 10^{-9}$ torr), appropriate cooling devices should be used when analyzing bioproducts in order to prevent evaporation and decomposition. In particular, it is important to confirm the chemical states derived from these analyses using alternative methods suitable for liquid analysis because dehydration by ultrahigh vacuum may cause changes.

Extended x-ray absorption fine structure

Another technique utilized to analyze corrosion products of MIC is extended x-ray absorption fine structure (EXAFS). Extended x-ray absorption fine structure refers to the oscillations of x-ray absorption spectrum in the range of 1 keV above the K absorption edge (Feldman & Mayer 1986). These oscillations are due to the scattering of the outgoing electron by nearby atoms and thus contain information on both the type of the absorbing atom as well as the number of atoms around it. EXAFS is able to probe about 6 Å immediately around the absorbing atom. As a bacterial consortium creates complex corrosion products, the ability of EXAFS to determine local structure and bond strength of a specific atom may be employed to characterize enzymatic products on metal surfaces. EXAFS has become a popular technique in study of catalysts and of abiotic corrosion (Davenport et al. 1995; Davenport & Sansone 1995).

Piezoelectric technology

Piezoelectricity of a material is its property of deformation upon exposure to an electric field. The quartz crystal microbalance is an instrument that utilizes piezoelectric materials (Nivens et al. 1995; Lu 1984). In an alternating electrical field, a piezoelectric electrode oscillates with a certain frequency, and mass changes will bring about changes in the oscillation frequency (Sauerbrey 1959):

$$\triangle f = -[2f_0^2/A(\mu_q \rho_q)^{1/2}]\triangle m \qquad (1)$$

where $\triangle$f is the change in frequency, f_0 is the resonant frequency, A is the piezoelectric area, μ_q is the quartz shear modulus, ρ_q is the quartz density, and Δm is the change in mass.

By exposing the electrode to an aqueous solution, piezoelectric microbalance may be utilized to study electrochemical interactions and to monitor biofilm formation and biomass deposition (Buttry & Ward 1992; Deakin & Buttry 1989; Nivens et al. 1993b). By depositing a thin metal film on a piezoelectric microelectrode, the dissolution rate of the metal can be monitored. A disadvantage is that the biofilm on the electrode may add variables to the measurement such as mass increases due to the biofilm formation and insoluble products on the metal film.

Summary

Table 1 gives the advantages and limitations of the instrumental methods introduced in this review. Research in MIC benefits from the development of modern analytical techniques. As MIC increasingly draws attention from corrosion engineers, environmental scientists and applied microbiologists, the variety of techniques applied for detection and monitoring also increases. Unfortunately, separation among the disciplines of electrochemistry, microbiology, solid state physics, physical chemistry, and materials engineering impedes a comprehensive understanding of MIC. As mentioned above, EIS data from MIC will be better understood if combined with *in situ* monitoring of biofilms using confocal laser microscopy and FTIR followed by characterization of resultant surface changes in the metal using XPS and AES. Difficulties in the study of MIC are also opportunities for future exploration. In addition to the techniques mentioned above, there are also many other techniques that may find application in this field such as nuclear magnetic resonance (NMR) and electron spin resonance (ESR) to characterize the interaction products, secondary ion mass spectroscopy (SIMS) to analyze the corrosion products on metal surfaces, high performance liquid chromatography (HPLC) to analyze metabolic acids and optical electrochemical spectrometry to monitor corrosion processes *in situ*. By combining and modifying techniques and instruments, laboratory detection and monitoring of MIC will provide more pertinent mechanistic information, and on-line/on-site MIC monitoring devices may be made more practical for corrosion control.

Table 1. A summary of advantages and limitations of instrumental techniques for MIC research

	Techniques	Advantages	Limitations
1.	Open circuit potential (OCP) measurement.	a. easy experimental setup; b. provides information about changes in the passive film; c. reproducibly indicates the trend of an interaction.	a. may be time-consuming; b. often insufficient to indicate pitting initiation when the data is used alone; c. values may vary in a large range because of structural and compositional differences between samples.
2.	Redox potential measurement	a. easy experimental setup; b. assist understanding of changes in OCP.	an index of oxygen activity but not an index of corrosive power of a system.
3.	Galvanic current between dual cells	straight forward theoretical basis.	metabolic products may diffuse into the sterile cell.
4.	Concentric electrode	a. creates distinct anodic and cathodic areas; b. allows accelerated tests.	potential to the anode may be too high for normal bacterial growth.
5.	Scanning vibrating electrode mapping (SVEM)	able to identify anodic and cathodic areas.	performance of a SVE may be affected by biofilm formation on the tip.
6.	Electrochemical impedance spectroscopy (EIS)	able to detect pitting initiation promptly.	biofilm may complicate the system and convolute the measurement.
7.	Scanning electron microscopy (SEM) and energy dispersive x-ray (EDX)	a. commonly available and straight forward; b. good technique for failure analysis of corroded metals.	a. requires an abundant amount of corrosion products; b. samples need cleaning and insulating samples also need conductive coating.
8.	Atomic force microscopy (AFM)	visualizes the topography of corroded sample in ‘as received’ states.	no compositional information.
9.	Confocal laser microscopy (CLM)	*in situ* monitors biofilm structure and visualizes pitting formation.	a. staining methods for different bacteria may alter normal growth conditions; b. focal length limits the medium volume and adds difficulties to mimic a real microbial consortium.
10.	Fourier transform infrared spectrometry (FTIR)	*in situ* analyzes changes in the composition of a biofilm.	a. water strongly absorbs infrared light; b. dissolution rate of a thin metal film may differ from that of the bulk metal; c. unable to tell the living cells from the dead ones.
11.	X-ray photoelectron spectroscopy (XPS)	a. analyzes changes in the surface chemical states; b. does not need a considerable amount of products.	a. dehydration may change the chemical states of interaction products; b. some chemical states determined by XPS may need confirmation by another technique.

Table 1. Continued

	Techniques	Advantages	Limitations
12.	Auger electron spectroscopy (AES)	analyzes far smaller areas that XPS.	a. high energy density of electron beam more easily causes radiation damage than XPS; b. for chemical information, spectra are more difficult to interpret.
12.	Extended x-ray absorption fine structure (EXAFS)	provides information about molecular structure.	x-ray energy may be too high for *in situ* monitoring.
13.	Quartz crystal microbalance	reveals the tendency of an interaction by detecting weight changes.	biofilm formation may overshadow dissolution of a metal film.

References

Angell P, Luo JS & White DC (1994) Use of 2-D vibrating electrode technique in MIC studies. CORROSION/94, paper 266, National Association of Corrosion Engineers, Houston, Texas, USA

Angell P, Sonnerson A, Wagner PA, White DC & Little BJ (1995a) Role of *Oceanospirillum* exopolymer in marine copper corrosion, paper 347, Symposium of Microbiologically Influenced Corrosion, National Association of Corrosion Engineers, Houston, Texas, USA

Angell P, Luo J-S & White DC (1995b) Microbially sustained pitting corrosion of 304 stainless steel in anaerobic seawater. Corrosion Sci. 37 (7): 1085–1096

Bremer PJ, Geesey GG, Drake B, Jolley JG & Hankins MR (1991a) Characterization of a thin copper film to investigate microbial biofilm formation. Surface and Interface Anal. 17: 767–772

Bremer PJ & Geesey GG (1991b) Laboratory-based model of microbiologically induced corrosion of copper. Appl. Environ. Microbiol. 57: 1956–1962

Bremer PJ, Geesey GG & Drake B (1992) Atomic force microscopy examination of the topography of a hydrated bacterial biofilm on a copper surface. Current Microbiology 24: 223–230

Briggs D & Riviere JC (1983) Spectral interpretation. In: Briggs D & Seah MP (Eds) Practical Surface Analysis by Auger and X-ray Photoelectron Spectroscopy (pp 87–139). John Wiley & Sons Inc., New York

Buttry DA & Ward MD (1992) Measurement of interfacial processes at electrode surfaces with the electrochemical quartz microbalance. Chem. Rev. 92: 1355–1379

Campaignolle X, Luo JS, Bullen J, White DC, Guezennec J & Crolet J-L (1993) Stabilization of localized corrosion of carbon steel by surfate-reducing bacteria. CORROSION/93, paper 302, National Association of Corrosion Engineers, Houston, Texas, USA

Chen G, Sadowski RA & Clayton CR (1995) Influence of sulfate-reducing bacteria on passive film formed on austenitic stainless steel AISI 304. CORROSION/95, paper 217, National Association of Corrosion Engineers, Houston, Texas, USA

Chen G, Kagwade S, French GE, Clayton CR, Ford TE & Mitchell R (1996a) Metal ion and exopolymer interaction: a surface analytical study. Corrosion 52 (12): 891–899

Chen G (1996b) Ph. D Dissertation. Department of Materials Science and Engineering, State University of New York at Stony Brook, New York

Davenport AJ, Bardwell JA & Vitus CM (1995) *In situ* XANES study of galvanostatic reduction of the passive film on iron. J. Electrochem. Soc. 142 (3): 721–724

Davenport AJ & Sansone M (1995) High resolution *in situ* XANES investigation of the nature of the passive film on iron in a pH 8.4 borate buffer. J. Electrochem. Soc. 142 (3): 725–730

Deakin MR & Buttry DA (1989) Electrochemical applications of the quartz crystal microbalance. Anal. Chem. 61: 1147A–1154A

De Beer D, Srinivasan R & Stewart PS (1994) Direct measurement of chloride penetration into biofilms during disinfection. Appl. Environ. Microbiol. 60 (12): 4339–4344

Dexter SC, Duquette DJ, Siebert OW & Videla HA (1991) Use and limitations of electrochemical techniques for investigating microbiological corrosion. Corrosion 47 (4): 308

Dickinson W & Lewandowski Z (1995) Electrochemical and microelectrode studies of stainless steel ennoblement. CORROSION/95, paper 223, National Association of Corrosion Engineers, Houston, Texas, USA

Dowling NJE, Guezennec J, Lemoine ML, Tunlid A & White DC (1988) Analysis of carbon steels affected by bacteria using electrochemical impedance and direct current techniques. Corrosion 44 (12): 869–874

Dowling NJE, Guezennec J, Bullen J, Little BJ & White DC (1992) Effect of photosynthetic biofilms on the open circuit potential of stainless steel. Biofouling 5: 315–322

Feldman LC & Mayer JW (1986) Extended x-ray absorption fine structure. In: Fundamentals of Surface and Thin Film Analysis (pp 201–204). North-Holland Inc., New York

Franklin MJ, White DC & Isaacs HS (1991a) Pitting corrosion by bacteria on carbon steel determined by the scanning vibrating electrode technique. Corrosion Sci. 32 (9): 945–952

Franklin MJ, Nivens DE, Guckert JB & White DC (1991b) Effect of electrochemical impedance spectroscopy on microbial cell numbers, viability and activity. Corrosion 47: 519–522

Franklin MJ, White DC & Isaacs HS (1992) A study of corrosion inhibition by phosphate ions and by organic buffer using a scanning vibrating electrode. Corrosion Sci. 33 (2): 251–260

Geesey GG, Iwaoka T & Griffths P (1987) Characterization of interfacial phenomena occurring during exposure of a thin copper film to an aqueous suspension of an acidic polysaccharide. J. Coll. Interface Sci. 120: 370–375

Geesey GG & Bremer PJ (1990) Applications of Fourier transform infrared spectrometry to studies of copper corrosion under bacterial biofilms. Mar. Technol. Soc. J. 24 (3): 36–43

Gerchakov SM, Little BJ & Wagner P (1986) Corrosion 42: 689

Hofmann S (1983) Depth profiling. In: Briggs D & Seah MP (Eds) Practical Surface Analysis by Auger and X-ray Photoelectron Spectroscopy (pp 141–179). John Wiley & Sons Inc., New York

Isaacs HS (1985) Application of current measurement over corroding metal surfaces. In: Nuccitelli R (Ed) Progress in Clinical and Biological Research: Ionic Currents in Development (pp 37–44). Alan R. Liss Inc., New York

Isaacs HS (1989) Applications and limitations of *in situ* current density mapping. CORROSION/89, paper 83. National Association of Corrosion Engineers, Houston, Texas, USA

Jack TR, Van Boven G & Wilmott M (1996) Evaluation of performance of coatings to biologically active soils. Materials Performance 35 (3): 39–45

Jolley JG, Geesey GG, Hankins MR, Wright RB & Wichlacz PL (1989) *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. Appl. Spectrosc. 43: 1062–1067

Lawrence JR, Wolfaardt GM & Korber DR (1994) Determination of diffusion coefficients in biofilm by confocal laser microscopy. Appl. Environ. Microbiol. 60 (4): 1166–1173

Lee W & Characklis WG (1992) Corrosion of mild steel under anaerobic biofilm. Corrosion 49 (3): 186–199

Lewandowski Z, Lee WC, Characklis WG & Little BJ (1993) Dissolved oxygen and pH microelectrode measurements at water immersed metal surfaces. CORROSION/93, paper 93. National Association of Corrosion Engineers, Houston, Texas, USA

Little BJ, Wagner P, Gerchakov SM, Walch M & Mitchell R (1986) The involvement of a thermophilic bacterium in corrosion processes. Corrosion 42: 533

Little BJ & Mansfeld FB (1991) The corrosion behavior of stainless steels and copper alloys exposed to natural seawater. Werkstoffe und Korrosion 42: 331–340

Little BJ, Wagner P & Mansfeld F (1991) An overview of microbiologically influenced corrosion. Electrochemica Acta 37 (12): 2185–2194

Lu C (1984) Theory and practice of the quartz crystal microbalance. In: Lea C & Czanderna (Eds) Application of Piezoelectric Quartz Crystal Microbalances (pp 19–61). Elsevier, New York

Macdonald DD (1991) Application of electrochemical impedance spectroscopy in electrochemistry and corrosion science. In: Varma R & Selman JR (Eds) Techniques for Characterization of Electrodes and Electrochemical Processes (pp 515–580). John Wiley & Sons Inc., New York

Mansfeld F & Lorenz WJ (1991) Electrochemical impedance spectroscopy (EIS): Application in corrosion science and technology. In: Varma R & Selman JR (Eds) Techniques for Characterization of Electrodes and Electrochemical Processes (pp 581–647). John Wiley & Sons Inc., New York

Mansfeld FB & Little BJ (1991) A technical review of electrochemical techniques applied to microbiologically influenced corrosion. Corrosion Sci. 32 (3): 247–272

Mansfeld FB, Tsai R, Shih H, Little BJ, Ray R & Wagner P (1992) An electrochemical and surface analytical study of stainless steels and titanium exposed to natural seawater. Corrosion Sci. 33 (3): 445–456

Mueller E, Sikes CS & Little BJ (1993) Peptide interactions with steel surfaces: inhibition of corrosion and calcium carbonate precipitation. Corrosion 49 (10): 829–835

Newman RC, Webster BJ & Kelly RG (1991) The electrochemistry of SRB corrosion and related inorganic phenomena. Iron and Steel Institute of Japan 31 (2): 201

Nivens DE, Nichols PD, Henson JM, Geesey GG & White DC (1986) Reversible acceleration of the corrosion of AISI 304 stainless steel exposed to seawater induced by growth and secretions of the marine bacterium *Vibrio Natriegens*. Corrosion 42 (4): 204–210

Nivens DE, Schmitt J, Sniatecki J, Anderson TR, Chambers JQ & White DC (1993a) Multichannel ATR/FT-IR spectrometer for on-line examination of microbial biofilms. Appl. Spectrosc. 47: 669–671

Nivens DE, Chambers JQ, Anderson TR & White DC (1993b) Long-term on-line monitoring of microbial biofilms using a quartz crystal microbalance. Anal. Chem. 65: 65–69

Nivens DE, Palmer RJ & White DC (1995) Continuous nondestructive monitoring of microbial biofilms: a review of analytical techniques. J. Industrial Microbiol. 15: 263–276

Pourbaix M & De Zoubov N (1966) In: Pourbaix M (Ed) Atlas of Electrochemical Equilibria in Aqueous Solutions (pp 307–321). Pergamon Press, New York

Sauerbrey G (1959) Z. Phys. 155: 206

Schmidt G (1991) Effect of elemental sulfur on corrosion in sour gas system. Corrosion 47 (4): 285–308

Smith JS & Miller JDA (1975) Nature of sulphides and their corrosive effect on ferrous metals: A review. Br. Corros. J. 10 (3): 136–143

Steele A, Goddard DT & Beech IB (1994) An atomic force microscopy study of the biodeterioration of stainless steel in the presence of bacterial biofilms. In: International Biodeterioration & Biodegradation (pp 35–46). Elsevier Science Limited, UK

Tunlid A, Nivens DE, Jansson H & White DC (1991) Infrared monitoring of adhesion of *Catenaria anguillulae* zoospores to solid surfaces. Exp. Mycol. 15: 206–214

Von Wolzogen Kuhr CAH & Van der Vlugt LS (1934) The graphitization of iron as an electrochemical process in anaerobic soils. Water 18: 147

Wagner PA, Little BJ & Stiffey AV (1991) An electrochemical evaluation of copper colonized by a copper-tolerant marine bacterium. CORROSION/91, paper 109, National Association of Corrosion Engineers, Houston, Texas, USA

Zhang X, Buchanan RA, Stansbury EE & Dowling NJE (1989) Electrochemical responses of structural materials to microbiologically induced corrosion. CORROSION/89, paper 512, National Association of Corrosion Engineers, Houston, Texas, USA