Electrochemical concepts and techniques in the study of stainless steel Ennoblement

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Abstract

Electrochemical theory and technique used to investigate microbially influenced corrosion is discussed with a focus on methods used to demonstrate the manganic-oxide mechanism of stainless steel Ennoblement. The concept of mixed potential and its relationship to the current-voltage behavior of stainless steel is developed. This concept is used to interpret microbially induced changes in corrosion potential, polarization behavior, surface-oxide abundance, and the redox environment at submerged metal surfaces. Microelectrode, capacitance, and coulometric methods are described that can be used to discriminate electrochemical effects caused by changes in solution properties from those caused by mineral deposition at the metal surface. The variety of electrochemical, wet-chemical, microbiological, and surface analytical techniques used to demonstrate the effect of biomineralized manganese dioxide on the electrochemical behavior of stainless steel are summarized.

Introduction

Microbial colonization of metal surfaces can influence-mediate the corrosion of a variety of metals and metal alloys including aluminum, copper, nickel, iron, and steel. This effect, known as microbially-influenced corrosion (MIC), is widely implicated in the failure of metals exposed to aqueous systems and is the focus of worldwide research efforts. Studies link MIC to the metabolic activities of microorganisms that modify the electrochemical environment near the metal surface; however, the complex chemistry of corrosion in the presence of microorganisms has made identification of exact mechanisms difficult. At present, the true nature of MIC is poorly understood.

One of the most puzzling aspects of MIC is the change in electrochemical properties of stainless steel that occurs as the metal surface is colonized by aerobic microorganisms in natural water. The dominant effects of colonization are a several-hundred millivolt increase in corrosion potential (E_{corr}) to values near +350 mV

versus the saturated calomel electrode (SCE) and a two to three decade increase in cathodic current density at potentials between approximately -300 and +300 mV_{SCE}. These effects, known as Ennoblement, operate in concert to increase the risk of stainless steel corrosion by elevating E_{corr} above the metal pitting potential and by furnishing increased cathodic current density to stabilize and propagate the nucleated corrosion sites. Figures 1 and 2 illustrate the electrochemical characteristics of Ennoblement.

Ennoblement was first observed in the mid-1960's (Crolet 1991) and has remained a controversial issue for the past three decades. Studies have confirmed the effect for a variety of inert or passive metals including stainless steel, titanium, and platinum exposed to marine, estuarine, and fresh waters worldwide (Dexter 1995; Holthe et al. 1989). Recently (Dickinson et al. 1996a), we established a chemical mechanism for Ennoblement in which manganese dioxide naturally deposited on the metal surface acts as a galvanic cathode to elevate cathodic current and shift E_{corr} in the
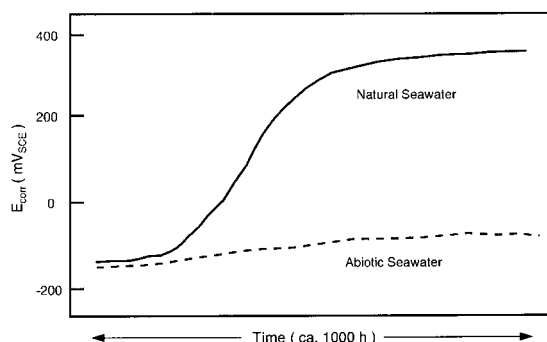
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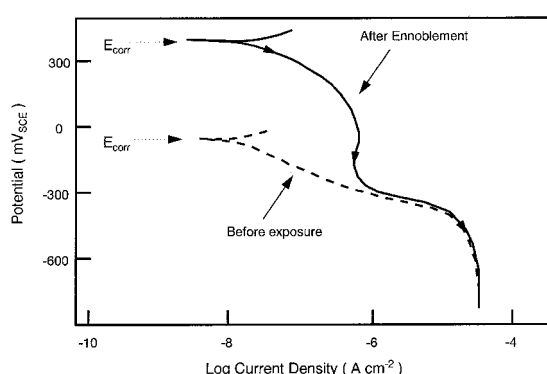Figure 1. Schematic shift in E_{corr} with exposure time in natural and abiotic sea water.

Figure 2. Schematic diagram of cathodic polarization behavior before and after Ennoblement.

noble direction. Evidence for the biological origin of the manganese dioxide was provided by inducing the effect using the manganese-oxidizing bacterium *Leptothrix discophora*.

Defining the electrochemical mechanisms of MIC is crucial to understanding and controlling the biodegradation of metals. The progression of research (Figure 3) used to establish a valid mechanism of Ennoblement, exemplifies the ways in which electrochemical techniques can be used to understand MIC. The present article summarizes the approach taken to investigate the Ennoblement phenomenon with the purpose of reviewing electrochemical theory and techniques used in MIC research.

Mixed potential theory

The potential of an electrode in solution reflects relative rates of anodic (oxidation) and cathodic (reduction) reactions occurring at the electrode surface. When the net anodic and cathodic reaction rates are equal, the
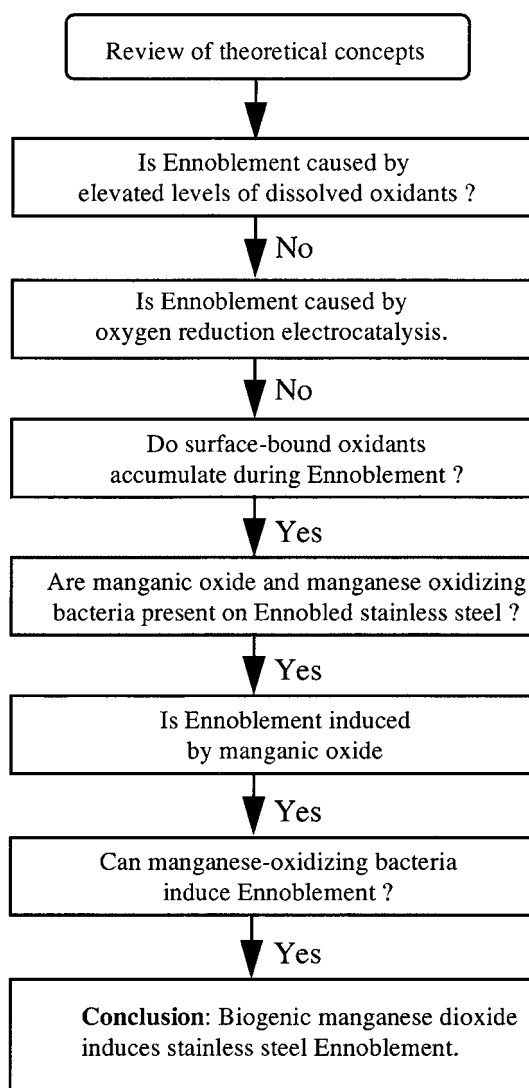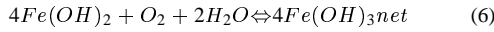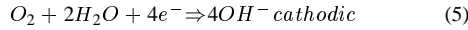

Figure 3. Experimental progression leading to the manganic oxide mechanism of Ennoblement.

electrode potential is constant. For inert or noble metals, such as platinum and gold, the electrode itself does not undergo reaction and serves only to facilitate charge transfer between external redox species. In contrast, electrodes of active metals such as iron do undergo oxidation and this process contributes to the net anodic rate. Metal oxidation is typically the dominant anodic process for freely corroding materials. In aerated, neutral solution, the dominant cathodic reactions are reduction of dissolved oxygen (DO) and reduction of water (Jones 1992). Equations 1–6 show relevant half-reactions and the corresponding net reaction for

the corrosion of iron in aerated aqueous media (Green-wood and Earnshaw 1990; Stumm and Lee 1961)

$$Fe \Rightarrow Fe^{2+} + 2e^- \qquad anodic \qquad (1)$$

$$2H_2O + 2e^- \Rightarrow H_2 + 2OH^- \qquad cathodic \qquad (2)$$

$$2H_2O + Fe \Rightarrow Fe(OH)_2 + H_2 \qquad net \qquad (3)$$

$$4OH^- + 4Fe(OH)_2 \Leftrightarrow 4Fe(OH)_3 + 4e^- \, anodic \qquad (4)$$

$$O_2 + 2H_2O + 4e^- \Rightarrow 4OH^- \, cathodic \qquad (5)$$

$$4Fe(OH)_2 + O_2 + 2H_2O \Leftrightarrow 4Fe(OH)_3 \, net \qquad (6)$$
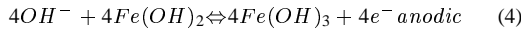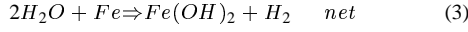
Equations 1–6 indicate that the anodic and cathodic processes occurring on the metal surface correspond to different half-reactions. The term 'mixed potential' is used to describe this condition, and to distinguish it from a reduction potential in which the anodic and cathodic reactions are simply the forward and reverse parts of a single half-reaction. A mixed-potential in which the anodic reaction is metal oxidation is termed the corrosion potential, E_{corr}.

Influence of Cathodic Rate on E_{corr}

The net rate for each half-reaction occurring on the metal surface is given by Butler-Volmer kinetics as (Bard and Faulkner 1980; Jones 1992):

$$i_{net} = \eta F A k^o \left\{ C^*_{red} \exp\left[2.3 \frac{(E-E^{o\prime})}{B_a} \right] \right.$$
$$\left. -C^*_{ox} \exp\left[-2.3 \frac{(E-E^{o\prime})}{B_c} \right] \right\} \qquad (7)$$
$$B_a = \frac{2.3RT}{\alpha_a \eta_a F} \qquad B_c = \frac{2.3RT}{\alpha_c \eta_c F}$$

where anodic and cathodic rates for each half-reaction are described by the first and second exponential terms in Equation 7 respectively, E refers to the electrode potential, and C^*_{ox} and C^*_{red} refer to concentrations of the oxidized and reduced species at the electrode surface respectively. Other symbols are defined in the Notation. Measurable current across the electrode-solution interface is the sum of the net currents for each half-reaction and at steady-state potential, must equal zero. For n separate half-reactions:

$$i_{meas} = \sum_{j=1}^{n} i_{net}^j \qquad (8)$$

Bearing in mind that only a single value of E can exist for a conductive surface, and setting the sum of currents described by Equation 8 to zero, E can be expressed in terms of the reactant concentrations, formal half-cell potentials ($E^{o\prime}$), and kinetic parameters associated with each half-reaction. If as is often the case, the anodic and cathodic rates are dominated by single half-reactions (e.g. reactions 1 and 2), E will acquire a value intermediate between the half-cell potentials for these dominant reactions. Equation 7 shows that in so doing, the cathodic rate for half-reactions possessing a formal potential more positive than E, and the anodic rate for half-reactions possessing a formal potential more negative than E, will increase. At the same time, rates of the reverse reactions decrease. Expressed in another way, for $C^*_{ox} = C^*_{red}$ and $E << E^{o\prime}$, the second exponential term in Equation 7 dominates and the Equation becomes:

$$i_c = -\eta F A k_c^o C_c^* \exp[-2.3 \frac{(E-E_c^{o\prime})}{B_c}] \qquad (9)$$

Where i_{net} has been changed to i_c, to indicate that, at $E = E_{corr}$, the net current for the more positive half-reaction is cathodic. The subscript c has been added to indicate that this Equation describes the current supplied by the cathodic half-reaction.

Taking the logarithm of Equation 9 shows that a plot of E vs. natural log of current density will be a straight line, and that the position of this line with respect to the ordinate axis, is dependent on the intrinsic rate constant and concentration of the cathodic reactant.

$$\ln \frac{i_c}{A} = D + \ln k_c^o + \ln C_c^* - \frac{2.3}{B_c} E$$
$$D = \left(\ln(-\eta F) + \frac{2.3}{B_c} E_c^{o\prime} \right) \qquad (10)$$

Equation 10 pertains for each half-reaction, j, that satisfies the condition $E << (E^{o\prime})_j$. Net cathodic current is then:

$$(i_c)_{net} = \sum_{j=1}^{n} i_c^j \qquad (11)$$

In a similar manner, for $C^*_{ox} = C^*_{red}$ and $E >> E^{o\prime}$, the first exponential term in Equation 7 dominates and the Equation becomes:

$$i_a = \eta F A k_a^o C_a^* \exp\left[2.3 \frac{(E-E_a^{o\prime})}{B_a} \right] \qquad (12)$$

Where i_{net} has been changed to i_a, to indicate that the net current for the more negative half-reaction is anodic.

Measurable current across the electrode-solution interface is the sum of the net anodic and cathodic rates, so that $i_{meas} = i_a + i_c$ and:

$$i_{meas} = \eta FA \left(k_a^o C_a^* \exp\left[2.3\frac{(E-E_a^{o\prime})}{B_a}\right] \right.$$
$$\left. -k_c^o C_c^* \exp\left[-2.3\frac{(E-E_c^{o\prime})}{B_c}\right] \right) \tag{13}$$

For an electrode at steady-state potential, $i_c = -i_a$ and for the special case of a corroding metal, the current at steady-state is termed the corrosion current, i_{corr}. As noted above, under these conditions $E = E_{corr}$. At steady-state, $i_{corr} = i_c = -i_a$, and either Equation 9 or 12 can be used to express i_{corr} as a function of E_{corr}. In terms of the cathodic reaction:

$$i_{corr} = -\eta FA k_c^o C_c^* \exp\left[-2.3\frac{(E_{corr}-E_c^{o\prime})}{B_c}\right] \tag{14}$$

Dividing Equation 9 by Equation 14 yields the expression:

$$i_c = i_{corr} \quad \exp\left[-2.3\frac{(E-E_{corr})}{B_c}\right] \tag{15}$$

Similarly:

$$i_a = -i_{corr} \quad \exp\left[-2.3\frac{(E-E_{corr})}{B_a}\right] \tag{16}$$

Again noting that $i_{meas} = i_a + i_c$:

$$i_{meas} = i_{corr} \left(\exp\left[-2.3\frac{(E-E_{corr})}{B_c}\right] \right.$$
$$\left. -\exp\left[2.3\frac{(E-E_{corr})}{B_a}\right] \right) \tag{17}$$

It can be seen, that for $E << E_{corr}$, or for $E >> E_{corr}$, Equation 17 reduces to Equations 15 or 16 respectively. Equation 17 relates measurable current to the displacement of E from E_{corr} and is an important expression used to experimentally determine i_{corr}. The current-voltage characteristics given by Equations 7 and 17 are shown schematically in Figure 4.

Equation 17 defines E_{corr} and i_{corr} and correctly relates these quantities for a freely corroding metal, however, anodic current for metals in the passive state is controlled by oxide film dissolution and/or cation transport through the passive film (Heuesler 1990; Irhzo et al. 1986), and is nearly independent of E. Under these conditions, anodic current is equal to the passivation current, i_{pass}, and can be taken as roughly constant (Jones 1992). For a metal in the passive state, i_{corr} then equals the constant value i_{pass}. From Equation 14, it can be seen that an increase in the intrinsic rate
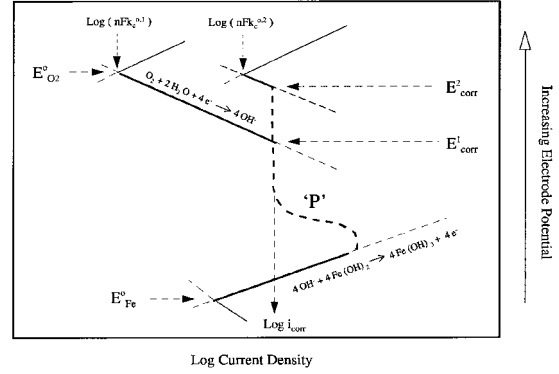

Figure 4. Schematic diagram showing current-voltage relationships according to eq. 7 and 17. Curve 'P' illustrates behavior for a metal in the passive state. E_{corr} shifts from E^1_{corr} to E^2_{corr} as the equilibrium rate for the cathodic reaction increases.

constant or concentration of any cathodic species must shift E_{corr} in the positive direction in order to maintain a constant value of i_{corr}. Equation 9 shows that for a constant value of E, an increase in these same quantities will increase cathodic current. These relationships are shown schematically in Figure 4 for a single cathodic reactant, where curve 'P' is the current-voltage behavior for a metal in the passive state. An increase in the intrinsic rate constant from $k^{o,1}_c$ to $k^{o,2}_c$ shifts E_{corr} from E^1_{corr} to E^2_{corr}. An increase in the concentration of cathodic reactant would qualitatively produce the same effects.

Based on the foregoing, the noble shift in E_{corr} and elevated cathodic current density that define Ennoblement may be related to processes that increase the concentration or the intrinsic rate constant of cathodic reactants. To understand Ennoblement, possible increases in rate constants and reactant concentrations, and their influence on the electrochemical behavior of the metal need to be investigated. The following sections describe several processes by which microbial colonization may alter the electrochemical environment at metal surfaces, and discuss techniques used to study these processes. The order of presentation corresponds roughly to the order in which the techniques were used to elucidate the manganic oxide mechanism of Ennoblement.

Electrochemical studies

A number of electrochemical techniques were applied to investigate Ennoblement. These techniques were used to confirm and to further define the electrochem-

ical characteristics of the phenomenon as well as to assess modifications of redox and surface conditions caused by microbial colonization of the metal.

E_{corr} measurements

The noble shift in E_{corr} towards limiting values of +300 to +400 mV_{SCE} was used throughout our studies to monitor the development of Ennoblement. E_{corr} appears as the voltage difference between a metal specimen and a known reference voltage, and is easily measured using a voltmeter or A/D converter. To avoid polarizing the sample specimen, measurements are made using high impedance ($>10^9\Omega$) equipment. In a typical experiment, metal specimens or coupons are exposed to a test environment and E_{corr} is measured periodically against the SCE.

Potentiodynamic polarization

Elevated cathodic current density is the second defining characteristic of Ennoblement. To ensure that the noble shifts in E_{corr} observed in our experiments were accompanied by this characteristic, potentiodynamic polarization measurements were carried out and an increase in cathodic current density was confirmed. The measurements were made using a conventional three-electrode system by monitoring current between the stainless steel specimen (working electrode) and a graphite or platinum counter electrode as potential difference between the working electrode and an SCE was varied. Potential was scanned beginning at E_{corr} to observe the small current changes associated with biological production of small amounts of oxidants. For a single reaction taking place at potentials where charge transfer is the rate determining step, a plot of E vs. log current density generates a straight line according to Equation 10. As noted, shifts in the slope or position of this line are an indication that changes have occurred in the reaction kinetics or concentration of the reacting specie(s) or that a new electroactive specie(s) has been formed.

Redox environment within biofilms

According to the theoretical discussion outlined above, microbial activity that modifies redox potential near the metal surfaces may induce Ennoblement. Several microbial processes that could influence redox potential are considered below.

Photosynthesis by attached algae and cyanobacteria produce oxygen and may increase the concentration of this cathodic reactant at the metal surface. Extracellular hydrogen peroxide and other active oxygen species produced by microorganisms (Glenn and Gold 1985; Yamashoji et al. 1991) are also strong oxidants and can significantly elevate E_{corr} for stainless steel. Other electroactive species produced during microbial metabolism include organic acids from the citric acid cycle and constituents such as NAD^+, FAD, and quinones that are part of the electron transport system. These species may accumulate within biofilms during cell death and lysis and may be reduced at the metal surface if they possess a reduction potential greater than E_{corr}.

Metallo-porphyrins and other respiratory macro-cycles may elevate E_{corr} by electrocatalyzing oxygen reduction. Analogues of these respiratory compounds have been investigated widely as electrocatalysts (Anson et al. 1985; Nagaoka et al. 1986; Su et al. 1990), and the enzyme laccase has been shown to produce a forty-fold increase in the rate of oxygen reduction on platinum (Srinivasan et al. 1985). Metabolic electron-transfer mediators such as menadione may couple extracellular oxygen reduction to oxidation of membrane bound species of the electron transport system, producing superoxide (Yamashoji et al. 1991). Superoxide formation has been established as the rate determining step in oxygen reduction on iron at neutral pH (Jovancicevic and Bockris 1986), consequently, the rate of cathodic oxygen reduction may increase if superoxide is catalytically produced near the metal surface. Alternatively, extracellular levels of electrocatalytic enzymes may reach sufficiently high levels within biofilms to directly increase oxygen reduction rate.

Microelectrode measurements

Microelectrodes were used to directly evaluate the redox environment within biofilms on Ennobled stainless steel. Hydrogen peroxide and dissolved oxygen were measured using amperometric microelectrodes, and E_{corr} for a stainless steel microelectrode was used as a general measure of the oxidizing power within the biofilm. The latter measurement was based on the idea that if unspecified oxidants shift E_{corr} for the metal substratum in the noble direction they should produce a similar shift for a stainless steel microelectrode placed in the same environment.

Results of the microelectrode experiments (Dickinson et al. 1996c) demonstrated that dissolved oxidants did not accumulate in the bulk biofilm during Ennoblement and focused attention on microbial processes that could deposit oxidants or catalysts within a few microns of the electrode surface.

Oxygen reduction rate

According to Equation 9, cathodic current should be directly proportional to oxygen concentration if oxygen is the dominant cathodic reactant. To determine the influence of oxygen on the cathodic reaction, Ennobled coupons were cathodically polarized in the presence and absence of oxygen. The region of elevated cathodic current density between approximately -200 to +400 mV_{SCE} characteristic of Ennoblement was examined to determine if oxygen concentration influenced net current.

Results from this experiment (Dickinson et al. 1996a) showed that cathodic current density over the specified range was independent of oxygen concentration, confirming that dissolved oxygen was not the cause of the increased cathodic current density observed in Ennoblement.

Surface metal oxides

The state of the metal surface can directly influence the rates of chemical and electrochemical reactions occurring at metal electrodess. Metal oxide properties are known to influence intrinsic oxygen reduction rates on iron (Jovancicevic and Bockris 1986; Zecevic et al. 1991), and it has been reported that oxygen reduction proceeds more rapidly on γ-FeOOH than on Fe_3O_4 in the potential range -0.55 to -0.1 V_{SCE} (Vago et al. 1994). Surface films on stainless steel contain varying amounts of reducible iron oxides depending on surface treatment (Ramasubramanian et al. 1990) that can in some instances produce a slight break in the cathodic polarization curve for uncolonized metal samples coincident with the break observed in Ennoblement. This behavior is illustrated by the lower curve in Figure 2. Cathodic curves generated immediately following polarization at -500 mV_{SCE}, or generated by scanning in the positive direction from cathodic potentials do not show the break in slope; however the feature reappears for scans made a few hours after cathodic polarization. This behavior is characteristic of reversible oxide formation as typically observed for

iron (Nagayama and Cohen 1961; Vago et al. 1994). Coincidence of the break in polarization slope for unexposed and Ennobled coupons, with the later break being much more pronounced, suggested that Ennoblement involved enrichment in oxide abundance at the metal surface, and that the redox activity of metal oxides may contribute to Ennoblement.

The complex redox chemistry of iron oxides is reflected in reactions such as:

$$FeOOH + H_2O + e^- \Rightarrow Fe^{2+} + 3OH^- \qquad (18)$$

$$3Fe_2O_3 + H_2O + 2e^- \Leftrightarrow 2Fe_3O_4 + 2OH^- \qquad (19)$$
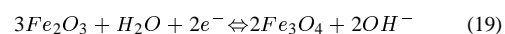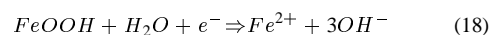
that are implicated in the the reduction of the passive films on stainless steel (Ramasubramanian et al. 1990) and that may participate as cathodic reactions in the atmospheric rusting of iron (Evans and Taylor 1972). Reduction of iron oxides occurs in the same potential region as oxygen reduction (Vago and Calvo 1992), suggesting that reduction of surface oxides could contribute to the increase in cathodic current observed in Ennoblement.

Two approaches were taken to study the relationship between Ennoblement and changes in surface oxide properties of the metal; capacitance was measured during the course of Ennoblement, and the abundance of reducible surface material was assessed before and after Ennoblement.

Capacitance studies

Capacitance is a broad measure of the thickness, area, and dielectric properties of surface films, including the electrical double-layer, present at the metal surface. The passive film on stainless steel is generally considered to have a complex oxide composition, with magnetite (Fe_3O_4), chromium oxides, and ferric oxides present in different proportions depending on conditions of surface treatment (Ferreira and Dawson 1985; Ramasubramanian et al. 1990). The capacitance of these oxides differ, consequently changes in oxide composition should effect metal capacitance. Capacitance measurements were made during the course of Ennoblement and interpreted by assuming that changes reflected modification of the surface (nature of the double-layer, oxide, or other surface films). The absence of change would provide evidence that surface properties were not modified and that new oxide phases did not develop.

Capacitance measurements were made using the galvanostatic transient method (Jones 1992). The technique utilizes a constant current to polarize the metal and determines capacitance by fitting polarization vs. time data to the Equation

$$\eta_{act} = I_{app}R_p \left(1 - e^{\frac{t}{R_p C}}\right) \qquad (20)$$

where η_{act} is the polarization or activation overvoltage, R_p is the polarization resistance which reflects the Faradaic reaction rate at the metal surface, and C is the net capacitance. In our studies, capacitance correlated strongly with Ennoblement (Dickinson et al. 1996c) and confirmed that surface properties were modified during the Ennoblement process.

Coulometric titration

The abundance of cathodically active material bound to a metal can be determined by coulometric titration of the metal surface. The technique, also known as galvanostatic reduction, has been used to investigate the nature and abundance of oxide phases on crystalline iron oxides (Stockbridge et al. 1961) and on mild steels and stainless steels (Okada et al. 1970; Ramasubramanian et al. 1990). The measurement is carried out by applying constant cathodic current to a specimen in an oxidant free electrolyte and observing the polarization versus time response. In the absence of dissolved reducible species, the applied current charges the electrode capacitively according to the relationship:

$$\frac{dE}{dt} = \frac{1}{C}I_{app} \qquad (21)$$

As E becomes more negative the cathodic reaction rate for surface-bound species increases exponentially according to Equation 9. If the cathodic rate becomes equal to the applied current, a steady-state is attained and E remains constant. This condition persists as long as surface bound material is available to react and results in a potential lag or plateau. Duration of the plateau corresponds to coulombs of reducible surface material (current × time = coulombs), and the plateau potential is related to the reduction potential of the surface phase. When sufficient charge has been applied to fully reduce the surface phase, the electrode resumes polarization to a more negative potential where other electroactive species can be reduced. Figure 5 shows the coulometric titration curve for Ennobled stainless steel after 9 days exposure to fresh river water. The
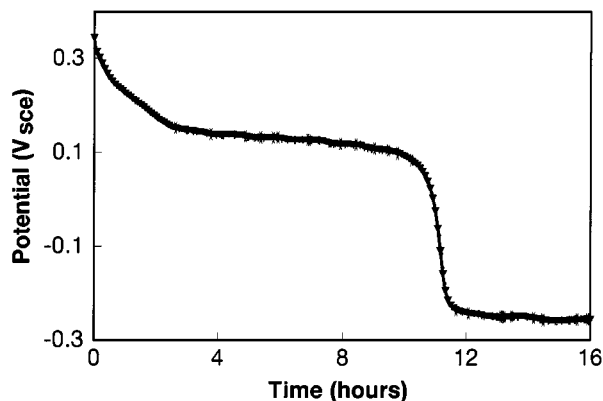
Figure 5. Coulometric titration curve for 316L stainless steel after 9 day exposure to fresh river water. 250 nA cm^{-2} applied cathodic current. The potential plateau near +100 mV$_{SCE}$ corresponds to reduction of surface-bound manganese dioxide. Once the surface material has been reduced, polarization proceeds until oxygen reduction occurs near −250 mV$_{SCE}$.

curve shape is similar to a battery discharge curve obtained at constant discharge current.

In principle, the plateau potential occurs when the electrode reaches the reduction potential for the oxide, however this is dependent on the applied current since excessively high currents will require significant overvoltage to attain steady state (cf. Equation 9). By choosing a suitable value for I_{app}, the plateau and reduction potentials can agree closely. As an example, reaction 19, which has a formal reduction potential of −529 mV$_{SCE}$ at pH 8.5, appears to correspond to the plateau potential near −550 mV$_{SCE}$ frequently observed during reduction of oxide films on iron and stainless steel (Nagayama and Cohen 1961; Ramasubramanian et al. 1990). In the Ennoblement work, potential plateaus were used to determine if new reducible surface phases developed, and to quantify the abundance of these phases.

Coulometric titration confirmed that a new reducible surface phase did develop during Ennoblement (Dickinson et al. 1996c). Furthermore, the plateau potential was much more positive than that observed for iron oxides indicating that the new phase was more strongly oxidizing.

Metal oxide depositing bacteria

Microelectrode, capacitance, and coulometric titration experiments provided strong evidence that Ennoblement was related to the development of a new reducible

surface phase. The nature of this phase was not known, however as noted in the Surface Metal-Oxide Section above, the coincidence of the break in cathodic polarization behavior seen before and after Ennoblement suggested that an enrichment in surface oxide abundance may have occurred. A variety of metal-oxide depositing microorganisms including bacteria, algae, yeast, and fungi exist in nature, and the possibility that microbial metal-oxide deposition produced the new reducible phase and modified the electrochemical parameters observed in Ennoblement became the final focus of the investigation.

Anodic polarization by metal-oxides

Equation 19 exemplifies surface metal-oxide reactivity which, by means of interconversion between surface-bound oxide phases, has the effect of fixing a redox couple at the metal surface. Depending on the uniformity of the lattice structure between the oxidized and reduced phases these reactions can be electrochemically reversible. If both phases are present, and the reaction is the sole significant electrochemical reaction occurring at the surface (i.e an inert electrode), the metal electrode will acquire the reduction potential of the oxide half-cell. In cases where only one phase is present (e.g. only the oxidized phase), the electrode will polarize positively in the manner described for dissolved oxidants, as reduction of the oxide increases cathodic current. This anodic polarization will continue until re-oxidation of the accumulated reduced products, or development of other anodic reactions (e.g. oxidation of water), balance the cathodic current.

The values of E_{corr} and the metal-oxide reduction potential determines whether a specific oxidant will be cathodically active at the metal surface. Oxides with reduction potentials more negative than E_{corr} will be stable with respect to reduction and will not generate cathodic current. In contrast, oxides with reduction potentials more positive than E_{corr} will behave as galvanic cathodes when coupled to the metal, with oxidation of the metal serving as the anodic reaction. Galvanically coupled manganic oxides have been used in this fashion to inhibit corrosion in strongly acidic media by anodically polarizing stainless steel into the passive region (Tomashov and Chernova 1967) and the possible influence of these compounds on E_{corr} for microbially colonized stainless steel has been suggested (Duquette and Ricker 1986).

E_{corr} for stainless steel in neutral aerated waters lies in the range -50 to -200 mV$_{SCE}$. Based on the above

discussion, ferric oxide ($E^{o\prime}_{pH8} = -529$ mV$_{SCE}$, cf. Equation 19) should not anodically polarize the metal under these conditions. In the Ennoblement studies, this point was confirmed when synthetic ferric oxide paste applied to the surface of stainless steel caused no shift in E_{corr}. As a consequence, attention was focused on manganic rather than ferric-oxides in assessing the role of biodeposited metal-oxides in Ennoblement.

Manganese-oxidizing bacteria

Manganese-oxidizing bacteria (MOB) are widely distributed in both fresh and marine waters and are considered to be 'directly or indirectly, the major catalyst of manganese cycling in the natural environment' (Gounot 1994). These bacteria catalyze oxidation of dissolved divalent manganese to form insoluble manganic oxides. The oxides are formed extracellularly and encrust the polymeric material (bacterial capsules) that surround individual cells or cell aggregates. The genera *Leptothrix* and *Siderocapsa* are particularly associated with formation of highly enriched manganic oxide deposits (Emerson and Ghiorse 1992; Hanert 1981). *Leptothrix* are typified by filamentous growth in tube-like polymeric sheaths (Mulder 1989), while sessile species of *Siderocapsa* grow as aggregates of a few to several dozen cells surrounded by a common ring-shaped polymeric capsule (Tuovinen et al. 1989). Manganese deposits that form on the extracellular polymers of these genera can frequently be visualized without magnification. Such deposits are expected to be electroactive when formed in immediate proximity to metal surfaces and present a plausible mechanism by which bacteria could alter the electrochemical behavior of metals leading to Ennoblement. To test whether biofouling by manganic oxides could account for Ennoblement, the following threefold approach was developed.

MOB and manganese deposition

Microbiological and surface analytical techniques were used to test for the accumulation of manganese and MOB within biofilms on Ennobled stainless steel (Dickinson et al. 1996a). Reflected-light microscopy was used to determine the presence of mineral encrustations. Colorimetric spot tests and surface analysis by scanning electron microscopy with energy dispersive x-ray analysis (SEM/EDS) were used to confirm the presence of manganese in the deposits. Leuco-crystal violet, the reduced, colorless form of the dye crystal

violet, was chosen as the reagent for the spot test. On contact with manganic oxide, the reagent is rapidly oxidized to produce an intense violet color discernible by eye. In natural waters the reagent is highly specific for manganic oxides and is used both as a spot test and as a quantitative measure of MnO_2 abundance (Kessick et al. 1972; Spratt et al. 1994).

Epifluorescence microscopy at $400 - 1000 \times$ magnification was used to visualize the microbial composition of intact biofilms on stainless steel. Staining was carried out using the nucleic acid fluorochrome acridine-orange which is widely used to visualize and enumerate microorganisms (Bitton et al. 1993; Hobbie et al. 1977). Characteristic cell morphology, colonial habit, and the presence of distinctive structures such as bacterial sheaths were noted. The presence of MOB in the biofilms was assessed by streak plating inoculums from natural biofilms onto a manganese(II) containing growth medium and staining isolated colonies with leuco-crystal violet. Formation of the violet color confirmed that the colonies produced manganic oxides.

Reductive dissolution of MnO2
To confirm that Ennoblement was dependent on the presence of manganic oxides, biofilms on Ennobled coupons were treated with sodium sulfite to reductively dissolve MnO_2. Sulfite and SO_2 quantitatively reduce MnO_2 to Mn(II) (Herring & Ravitz 1965) and are used under acidic conditions to leach manganese from low-grade ores. In the Ennoblement work, sulfite solution was used at pH 8.4 to retain the pH value of the natural water exposure. E_{corr} was monitored as Ennobled coupons were exposed to sulfite and at the conclusion of the experiment, the sulfite electrolyte was analyzed for the presence of Mn(II) either by atomic absorption or colorimetrically using the formaldoxime method (Brewer & Spencer 1971). A decrease in E_{corr} to pre-Ennobled values and a corresponding release of Mn(II) into solution demonstrated a causal link between manganic oxides and Ennoblement (Dickinson et al. 1996a).

Electrochemical changes induced by MnO2 paste
The sulfite experiment tested whether Ennoblement could be eliminated by removing MnO_2. To demonstrate the reverse, i.e. that MnO_2 could produce the electrochemical characteristics of Ennoblement, E_{corr} and cathodic polarization measurements were made on stainless steel coated with chemically prepared MnO_2 paste. The results were compared with the electro-chemical characteristics of Ennobled coupons. The MnO_2 paste, prepared by addition of permanganate ion to an alkaline solution of manganous ion (Lovley & Phillips 1988), was also used to calibrate the potential of the plateau observed during coulometric titration of Ennobled coupons. Agreement between Ennobled and MnO_2 paste coated coupons with respect to E_{corr}, the potential of the coulometric titration plateau, general cathodic curve shape, and current density at specified potentials confirmed that MnO_2 could induce Ennoblement.

Ennoblement by Leptothrix discophora

The relationship between manganic oxides and Ennoblement was firmly established by the foregoing experiments, but the relationship between MOB and Ennoblement remained circumstantial, i.e. both MOB and manganese were identified in the biofilms, but no strict causal relationship could be inferred. To demonstrate that Ennoblement could be induced by MOB, controlled experiments were carried out with pure cultures of the manganese-oxidizing bacterium *Leptothrix discophora*. Masses of sheathed cells characteristic of this species were among the bacteria present in biofilms on Ennobled coupons. A strain of *L. discophora* identified as SP-6 (Emerson & Ghiorse 1993) was obtained from the American Type Culture Collection (Rockville, MD) and grown in a mineral salts-vitamin-pyruvate medium. In the presence of Mn(II), this organism formed copious manganic oxide deposits on all submerged surfaces. Experiments were performed by exposing stainless steel coupons in three identical reactors containing the bacteria and dissolved Mn(II). For each reactor, measurements of E_{corr}, concentration of Mn(II) in solution, surface cell density on the coupons, and surface-bound Mn on the coupons were made. Solution Mn(II) was measured colorimetrically by the formaldoxime technique, surface cell density was determined by acridine-orange direct count, and surface-bound Mn was determined using both the sulfite dissolution and the coulometric titration techniques. Two control experiments were performed, one with bacteria but no added Mn(II), the other with Mn(II) but no bacteria. Ennoblement of E_{corr} in the first experiment and the absence of Ennoblement in both control experiments established that MOB can induce the phenomenon and validated the biological basis of Ennoblement (Dickinson et al. 1996b).

Concluding remarks

The experimental approach outlined in this paper demonstrates the use of electrochemical techniques in the study of MIC. Broad measures of electrochemical properties such as redox potential and capacitance are useful in discriminating between solution and surface effects while polarization measurements provide information on the identity and abundance of electroactive species produced by microbial processes. Used in conjunction with basic microscopy technique and simple chemical spot tests, electrochemical methods can provide signatures for specific forms of MIC.

Results from the Ennoblement project point out the impact of biogenic surface deposits on the electrochemistry of metals. These deposits, acting alone or in concert with physical effects such as differential aeration, increase the risk of localized corrosion of stainless steel and may play a role in the corrosion of other metals.

Acknowledgments

Notation

i_{meas}	=	measurable current, the sum of anodic and cathodic currents for all half-reactions
i_{net}	=	the sum of anodic and cathodic currents for a single half-reaction
i_a	=	anodic current
i_c	=	cathodic current
i_{corr}	=	corrosion current
i_o	=	exchange current
i_{pass}	=	passivation current
k^o	=	standard rate constant for electrode reactions; (cm sec^{-1})
k^o_c	=	standard rate constant for cathodic electrode reaction
k^o_a	=	standard rate constant for anodic electrode reaction
n_a	=	number of electrons transferred in rate determining step
n	=	number of electrons transferred in net reaction
A	=	electrode area; (cm^2)
C	=	capacitance; (coul volt^{-1})
C^*_{ox}	=	concentration of oxidized specie at the electrode surface; (moles cm^{-3})
C^*_{red}	=	concentration of reduced specie at the electrode surface
C^*_c	=	concentration of cathodic reactant at the electrode surface
C^*_a	=	concentration of anodic reactant at the electrode surface
DO	=	dissolved oxygen

E	=	electrode potential
$E^{o\prime}$	=	formal half-cell potential
$E^{o\prime}_c$	=	formal half-cell potential for cathodic reactant
$E^{o\prime}_a$	=	formal half-cell potential for anodic reactant
E_{corr}	=	steady-state electrode mixed potential
F	=	Faraday constant, 96485 coulombs equivalent^{-1}
I_{app}	=	externally applied current
MOB	=	manganese-oxidizing bacteria
R	=	gas constant; (8.31414 volt-coulombs mole^{-1} -deg^{-1} (K))
R_p	=	polarization resistance; (ohm cm^2)
SS	=	stainless steel
SCE	=	saturated calomel electrode
SRB	=	sulfate-reducing bacteria
T	=	temperature; (degrees, K)
α_a	=	anodic transfer coefficient
α_c	=	cathodic transfer coefficient
β_a	=	anodic Tafel slope; (Volts per decade of current)
β_c	=	cathodic Tafel slope; (Volts per decade of current)

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