Microbially Influenced Corrosion as a Model System for the Study of Metal Microbe Interactions: a Unifying Electron Transfer Hypothesis

W A HAMILTON*

Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, Scotland, UK

(Received 17 June 2002; in final form 12 August 2002)

The general term biomineralisation refers to biologically induced mineralisation in which an organism modifies its local microenvironment creating conditions such that there is chemical precipitation of mineral phases extracellularly. Most usually this results from an oxidation or reduction carried out by some microbial species, with the formation of a recognised biomineralised product. These reactions play a major role in microbial physiology and ecology, and are of central importance to such engineering consequences as microbial mining and microbially influenced corrosion. This paper will examine metal microbe interactions, both in naturally occurring microbial ecosystems and in two particular cases of biocorrosion, with the objective of putting forward a unifying hypothesis relevant to the understanding of each of these apparently disparate processes.

Keywords: microbially influenced corrosion (MIC); redox; electron transfer; microbial ecosystems; consortia; sulphate-reducing bacteria (SRB); manganese-oxidising bacteria (MOB)

INTRODUCTION

Electrochemical corrosion is characterized by the dissolution of zero valent metal at an anodic site. In terms of the chemistry involved this occurs as an oxidation with the loss of electrons:

$$M^0 \rightarrow M^{2+} + 2e$$

In order for this reaction to proceed, there is a requirement for a complementary reduction at a cathodic site at which some external component is identified as the electron acceptor. This reduction removes electrons from the cathode and so depolarises the metal and allows the anodic oxidation to proceed. There are two elements to such a process; one thermodynamic, the other kinetic.

The tendency for a given material to act as either electron donor (anode) or electron acceptor (cathode) is recorded as its electrochemical potential, measured against either the saturated calomel electrode (SCE) or the standard hydrogen electrode (SHE). Where, for example, two metals of differing electrochemical potential are galvanically coupled, electrons will flow from the more negative to the more positive potential, with the consequent anodic dissolution (corrosion) of the metal with the more negative potential. The propensity of the anodic member of this couple to corrode is dependent on the potential difference between the anode and the cathode. This thermodynamic property allows us to predict the likely direction and extent of electron flow, and therefore of the resulting corrosion. Potential differences are a necessary, but not, however, a sufficient condition for the metals to corrode.

For the corrosion current to reach a sufficient magnitude there must be a kinetic path available to facilitate the flow of electrons between the anode and the cathode, and the rate of that flow determines the rate of corrosion. Within the limits set by the thermodynamics of the system under study, it is the kinetics of the electron transfer from the corroding metal to a suitable oxidant (cathodic reaction) that is often significant in establishing the severity of a particular corrosion mechanism, or the effectiveness of any preventative measure. This is particularly true in microbially stimulated corrosion as whenever biofilms accumulate on cathodic members of galvanic couples, a significant
increase in the reduction current can be expected (Dexter & LaFontaine, 1998). Despite these considerations however, analyses of corrosion processes generally put their primary focus on thermodynamics and place much less emphasis on kinetics.

Biological processes in general, and microbiological processes in particular, are similarly subject to the laws of thermodynamics and dependent on the existence of suitable kinetic mechanisms. Within this formalism, however, metabolic reactions in living (micro)organisms differ fundamentally from the equivalent reactions in metal corrosion. Microbial cells and ecosystems are open systems, dependent for the maintenance of their structural and functional integrity on inputs of energy from external sources such as the oxidation of organic substrates, or in the case of phototrophs, a source of radiant energy. Microbial systems are characterised by the flux of energy, and of organic and inorganic nutrients, into and through the system; at each of the levels, community, cell, enzymatic reaction. The steady state achieved is therefore a dynamic one where individual reactions are integrated into sequentially operating pathways, and neither individual reactions nor pathways are at positions of thermodynamic equilibrium. That is to say, both microbial communities and metals are in unfavourable thermodynamic states, with their energy continuously dissipating. Living organisms, however, have means of regenerating the lost energy by coupling the energy dissipating reactions to energy capturing reactions, while metals do not possess such abilities. Therefore microorganisms are in a state of constant flux of energy while metals can only approach the state of constant dissipation of energy.

Therefore, the best chance of achieving maximal understanding of the special case of microbially influenced corrosion (MIC) comes through examining the kinetics of corrosion. A further prerequisite is identification of the mechanisms whereby particular microbiological and electrochemical corrosion processes can be directly coupled.

The universal mechanism employed by living organisms for the capture and utilisation of energy from environmental sources involves electron flow from negative to more positive potentials. This pattern exactly parallels the electron flow in corrosion, but in biological energy transduction the electron flow is mediated by a series of highly specialised electron (and hydrogen) carriers. These carriers are also universal in nature, being in the form of such intracellular components as ferredoxins, flavoproteins and cytochromes. They have the capability of being either reduced or oxidised, with the reduced form acting as an electron (and/or hydrogen) donor and the oxidised form as an electron acceptor; they are referred to generically as redox couples. Both individual species of microorganisms and multi-species microbial consortia can be characterised by their primary energy source and electron donor, and by the nature of the terminal electron acceptor which acts as the end point for the flow of electrons through the system. Many bacteria, for example, gain their energy from the oxidation of molecular hydrogen. With aerobic species oxygen is the terminal electron acceptor, but for various anaerobic species a number of possible alternative electron acceptors are utilised, e.g. nitrate, ferric iron, sulphate (sulfidogenesis) or CO₂ (methanogenesis). Since several of these oxidation and reduction reactions are also integral to MIC, there is much to be gained from a consideration of the extensive data and related understanding of their role in the balanced functioning of various natural ecosystems.

**MICROBIAL ECOLOGY AND THE ROLE OF METALS**

A striking characteristic of many microbial ecosystems that has a major influence on their metabolic functioning is the existence of separately identifiable oxic and anoxic zones. This applies equally to soils, deep water columns and aquatic sediments, as it does to biofilms deposited on metal substrata. Within the biosphere as a whole, oxygen is the ultimate and universal electron acceptor. Anoxic regions generally have their origins in the reduction of oxygen by overlying aerobic species at a faster rate than can be compensated for by the diffusion of oxygen into these regions. In functional terms, however, such oxic/anoxic ecosystems operate as a single unified continuum. This arises from the integrated activities of the various redox couples within the ecosystem.

The extracellular role of metals in the maintenance of such microbial ecosystems is one particular area in which there is wide appreciation of the importance of metal–microbe interactions. Key practical issues concern their bioavailability from mineral sources often with low solubility, and their stability under the prevailing environmental conditions of pH and Eh. As with intracellular redox couples, it is the capacity to act as electron donor and/or acceptor that determines their biological utility. Quantitatively the most important redox couples operating in microbial ecosystems are Fe(II)/Fe(III) \([\text{Eo} = 770 \text{mV}_{\text{SHE}}]\) and Mn(II)/Mn(IV) \([\text{Eo} = 402 \text{mV}_{\text{SHE}}]\), due to the relative abundance of iron and manganese in most natural environments, and the compatibility of their midpoint redox potentials with the biological range between the hydrogen \([\text{Eo} = -414 \text{mV}_{\text{SHE}}]\) and oxygen \([\text{Eo} = 820 \text{mV}_{\text{SHE}}]\) half reactions; [all mid point potentials being referred to standard conditions at pH 7, against the hydrogen electrode \(e_{\text{SHE}}\)].

The putative environmental roles of these metals are three-fold, viz. i) the divalent ferrous...
and manganous ions may be oxidised and, at least potentially, serve as the electron donor for cellular energetic and biosynthetic functions; ii) in sediments and other anoxic environments, ferric and manganic ions can serve as terminal electron acceptors, as alternatives to oxygen; iii) in parallel with the redox cycling of electron carriers in cellular membrane energetics, the Fe(II)/Fe(III) and Mn(II)/Mn(IV) redox couples can act as electron carriers transporting reducing equivalents from sulphidogenic and methanogenic regions of sediments to oxygen in the oxic surface zone. In the oxidation and reduction modes a) and b), substrate amounts of the metals are modified in fulfilling their biological function, whereas in c) only catalytic quantities are required.


Environmental Oxidation and Reduction of Iron and Manganese

The oxidation and reduction reactions involving iron and manganese, and the micro-organisms responsible, have been subject to considerable study, particularly over the past ten or fifteen years. What has become clear from such studies is a number of conclusions of general applicability, and of very considerable significance in the context of metal–microbe interactions. Firstly, the oxidation and reduction of iron are best considered as complementary processes, functionally coupled within any given ecosystem. This applies equally to manganese. Secondly, the redox cycles of iron and manganese are often closely interrelated at each of the levels, molecular, cellular and ecological, both through the phylogenetic relatedness and cell physiology of the organisms involved, and through their role in environmental carbon turnover and redox balance. In ecological terms at least, this interrelatedness extends to the sulphidogenic activity of the sulphur- and sulphate-reducing bacteria. Thirdly in part due to the insoluble and extracellular nature of the products of oxidation, Fe(III) and Mn(IV), it is not always clear whether such oxidations are coupled to cell growth, or what might be the mechanisms of their reduction back to Fe(II) and Mn(II). Fourthly, although many microbial species have been identified as having the capability of carrying out one or another of the oxidation or reduction reactions within such complex ecosystems, abiotic reactions can often play a significant role as, for example, with the reoxidation of Fe(II) or sulphide by MnO₂.

Oxidation

Due to the high rate of autoxidation of ferrous iron and the minimal energy available from Fe(II) oxidation, iron-oxidising bacteria employ one of two possible strategies to harness the energy of the reaction for cell growth; they either grow at neutral pH in conditions of low oxygen concentration, or they are obligate acidophiles (Ingleedew et al., 1977; Wood, 1988; Ehrlich, 1999).

There are a number of significant differences between Mn(II) and Fe(II) oxidation which have an impact on their respective roles in natural ecosystems. Mn(II) is chemically stable at neutral values of pH and therefore potentially subject to microbial oxidation. The oxidation product is generally considered to be MnO₂, although it has been suggested from a number of studies that MnOOH may be an intermediate (Tebo et al., 1998). MnO₂ is virtually completely insoluble, and as with Fe(II) oxidation therefore, Mn(II) oxidation gives rise to biomineralisation characterised by the precipitation and extracellular accumulation of manganese oxides (Ehrlich, 1999).

Although iron- and manganese-oxidising bacteria are often considered together (Ghiorse, 1984), only Leptothrix and Crenothrix of the iron-oxidising bacteria have been shown to be capable of manganese oxidation (Tebo et al., 1998). Rather than the restricted range of lithotrophic organisms characteristic of Fe(II) oxidation, Mn(II) oxidation is found to be widespread amongst the α, β and γ Proteobacteria, as well as Gram-positive bacteria and certain fungi. This wider distribution is germane to the question of the utility of Mn(II) oxidation to those organisms with this capability.

Despite biochemical and genetic studies of Mn(II) oxidation in several genera, there is little unequivocal evidence of either lithothrophic or mixotrophic growth driven by oxidation of Mn(II) as electron and energy donor (Nealson et al., 1988; Caspi et al., 1998; de Vrind et al., 1998; Ehrlich, 1999). It now seems likely that the ecological importance of Mn(II) oxidation lies in the chemical reactivity of MnO₂.

Mn(IV) oxides have very high adsorptive and oxidative properties (Laha & Luthy, 1990; Tebo et al., 1998). Striking examples of this come from the demonstration that microbially deposited MnO₂ can serve as the site for adsorption and subsequent oxidation of complex humic and fulvic acids, and of an extensive range of substituted phenols, giving rise to suites of low molecular weight compounds (Stone & Morgan, 1984; Stone, 1987a; 1987b; Sunda & Kieber, 1994). The mechanism of the oxidation is abiotic and the authors argue that such degradative processes, converting highly recalcitrant material to readily assimilable microbial substrates, could constitute a valid and sufficient ecological
and evolutionary role for the microbial oxidation of manganese.

In the context of the present discussion however, by far the most significant chemical oxidations driven by Mn(IV) oxides are those affecting Fe(II) and sulphide (Myers & Nealson, 1988; Nealson & Myers, 1992). The abiotic oxidative activity of microbi ally produced MnO₂ is often found to be critical to redox balance and carbon flux within anoxic ecosystems.

Reduction

The capacity for Fe(III) reduction is distributed widely within both the Archaeal and Bacterial kingdoms (Caccavo et al., 1996; Lovley, 1997; Bridge & Johnson, 1998; Vargas et al., 1998; Kieft et al., 1999).

It is evident from these studies that Fe(III) is only one of several possible electron acceptors capable of being utilised by these organisms, although the identity of the alternatives shows a considerable degree of species-specificity. For example, most sulphur reducing species can also reduce Fe(III) (Lovley et al., 1995; Coates et al., 1995; Caccavo et al., 1996; Lovley, 1997). Many sulphate reducing species, with the exception of Desulfobacter postgatei, have also been shown to be capable of Fe(III) reduction (Coleman et al., 1993; Lovley, 1997). Particularly interesting is the fact that the affinity of D. desulfuricans for H₂ with Fe(III) as electron acceptor is an order of magnitude higher than with sulphate, which suggests that in many natural environments it may be that this so-called sulphate-reducing bacterium behaves rather as an Fe(III)-reducing organism (Coleman et al., 1993).

In contrast to these findings, Shewanella putrefaciens, probably the most studied Fe(III) reducer at the present time, can reduce Mn(IV) and tetrathionate, thiosulphate and sulphite, but not either sulphate or sulphur itself. Another Fe(III) reducer Bacillus infernus, isolated from a deep subsurface environment and the only known anaerobic Bacillus sp., can utilise as electron acceptor Mn(IV), trimethylamine oxide and nitrate, but none of the oxidised forms of sulphur (Boone et al., 1995).

A number of other Fe(III)-reducing species have now also been shown to be capable of reducing Mn(IV) (Lloyd et al., 1998; Francis et al., 2000). Kashefi and Lovley (2000) have concluded from their studies that “after Fe(III), Mn(IV) is the most abundant metal likely to be found as a potential electron acceptor in sedimentary environments, and most Fe(III) reducers have the capacity to reduce Mn(IV)”.

One major factor with the potential to reduce the environmental significance of the microbiological reduction of Fe(III) and Mn(IV) is that they exist as highly insoluble oxides and are not, therefore, readily available to the intracellular enzymes and solution chemistry of the reacting bacteria. Nevin and Lovley (2000) suggest that although Geobacter metallireducens may be able to reduce insoluble Fe(III) by direct cell contact, this activity is stimulated by the addition of the humic acid analogue anthraquinone-2,6-disulfonate (AQDS). In most studies it has been concluded that significant microbial reduction only occurs either with solubilisation, or in the presence of humic acids or AQDS, which latter two are envisaged as operating as electron shuttles between the insoluble oxide and the cellular reductases (Frederickson et al., 1998; Kieft et al., 1999). Lovley and Blunt-Harris (1999) suggest that in microbial Fe(III) reduction the cells first reduce the quinone groups in the humic substances, and that these hydroquinone groups then reduce Fe(III) by an abiotic reaction.

Metal reduction in marine sediments

It has been widely recognised for many years that carbon flux in sediment systems is coupled to the reduction of a series of electron acceptors of increasingly reductive redox potential, and located at increasing depth within the sediment. In marine and estuarine environments, although methanogenesis may be present at some greater depths, the principal terminal electron acceptor is clearly identified as sulphate, along with associated sulphidogenic activity. Particularly in more recent studies, however, the role of iron and manganese reduction has also been stressed (Nealson & Myers, 1992; Canfield et al., 1993; Aller, 1994; Aguilar & Nealson, 1994; Nealson & Saffarini, 1994; Thamdrup et al., 1994; Ramsing et al., 1996; Thamdrup & Canfield, 1996).

This extensive body of work shows that across a wide range of marine habitats there is consistent evidence for the presence of Fe(III) and Mn(IV) reduction linked to organic carbon oxidation. In several instances the data suggest that the major role of iron and manganese takes the form of Fe(II)/Fe(III) and Mn(II)/Mn(IV) redox cycling, lying between and functionally coupling the quantitatively more important aerobic oxygen and anaerobic sulphate reduction zones. Although, as described above, it is known that a broad spectrum of microbial species have the capability of manganese and/or iron reduction, it appears likely that in natural sediment ecosystems much of the iron and virtually all of the manganese reduction is extracellular and occurs abiotically. Thamdrup et al. (1994), for example, have calculated that in their coastal sediment samples 100% of Mn(IV) reduction is coupled to chemical oxidation of Fe(II) and HS⁻, while 63% of Fe(III) reduction is coupled to HS⁻ oxidation. These processes lead to the formation of
elemental sulphur and of pyrite (FeS₂), and show minimal involvement of metal-reducing bacteria. Similar findings were reported by Nealson and Myers (1992).

In summarising this body of data, much of it very recent, concerning the physiology and ecological impact of metal oxidising and reducing bacteria, a number of specific conclusions that are of considerable generic significance can be identified. Firstly, carbon and energy flux in both individual cells and microbial ecosystems require mechanisms of electron transfer, many of which have metal ion oxidation and reduction at their core. Secondly, many natural environments have both oxic and anoxic regions, and the interaction between the principal terminal electron acceptors, oxygen and sulphate respectively, is dependent upon the redox cycling of intermediate electron carriers, such as iron and manganese. Thirdly, the net activity shown by any one component of these complex ecosystems may be influenced by oxygen-lability, solubility and/or chemical reactivity. It seems likely, for example, that manganese redox cycling will involve microbial oxidation but chemical reduction, and that this latter process will have a major impact on iron and sulphide re-oxidation.

**Oxidation of zero valent metals**

Much of the recent impetus to examine inorganic oxidation and reduction processes in greater detail has come from concerns with such practical issues as environmental pollution and microbial remediation. One form of environmental pollution arises from the accumulation in soils and sediments of toxic chemicals derived from various manufacturing processes. Principal amongst these are the halogenated hydrocarbons, both aliphatic and aromatic. Recent studies have shown that these may be biodegraded by bacteria capable of reductive dehalogenation under anoxic conditions, where the halogenated hydrocarbon serves as a terminal electron acceptor during microbial growth (Boyle et al., 1999). Whereas in most such studies, hydrogen, formate or a short chain organic acid such as lactate have been identified as the energy and electron donors driving the reductive dehalogenation, Weathers et al. (1997) demonstrated that finely divided iron filings could serve that same function for the dehalogenation of chloroform in cometabolism with methanogenesis. Earlier in a wider survey, Belay and Daniels (1990) showed that metallic Fe⁰, Al⁰ and Zn⁰ were capable of oxidation and supplying the reducing equivalents for methanogenesis. In these two studies it was established that metal oxidation occurred with the intermediate production of H₂ by what is described as a cathodic reaction, in parallel with the same reaction sequence recognised as a primary event in the corrosion of mild steel structures.

**Metal Corrosion and the Role of Microorganisms**

Before discussing how microbial activities can influence the rate of corrosion of ferrous metals in aqueous environments, it is instructive to consider the nature of the abiotic corrosion that can arise under these conditions.

**Abiotic corrosion of ferrous metals**

When iron or mild steel are exposed to oxygen in an aqueous environment, corrosion, or rusting, takes place with the formation of the characteristic mixed iron oxide/hydroxide as corrosion product. This reaction is not particularly rapid, and more active oxidants such as peroxide or chlorine can stimulate the corrosion. On the other hand, corrosion is effectively zero where oxygen or other suitable oxidants are excluded.

The open circuit potential (OCP) of a metal electrode in aqueous solution depends on the rates of anodic (oxidation) and cathodic (reduction) reactions at the electrode. Noble metals, such as platinum and gold, do not participate in the anodic reaction and instead serve only to facilitate charge transfer between external redox species. However, electrodes made of less noble more active metals, such as iron, participate in the redox reactions and undergo oxidation, thereby increasing the net anodic rate. In aerated solution, the dominant cathodic reactions are reduction of dissolved oxygen and reduction of water (Jones, 1992).

The following reactions describe corrosion of iron in aerated aqueous solutions:

\[
\text{Fe}^0 \rightarrow \text{Fe}^{2+} + 2e^- \quad \text{anodic reaction}
\]

\[
\text{Fe}^{2+} + 2\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_2 + 2\text{H}^+ \quad \text{iron hydrolysis}
\]

\[
2\text{Fe(OH)}_2 + 1/2 \_2 \rightarrow \text{Fe}_2\text{O}_3 + 2\text{H}_2\text{O} \quad \text{further oxidation}
\]

\[
2e^- + \text{H}_2\text{O} + 1/2 \_2 \rightarrow 2\text{OH}^- \quad \text{cathodic reaction}
\]

The essential point to be recognised in these reactions is that oxygen is acting as the terminal acceptor for electron flow from zero valent metal serving as electron donor. It can be noted here that stainless steels and other passive metals behave differently. The alloying elements, chromium, nickel and molybdenum, react with oxygen to form a stable passive oxide film that protects the underlying metal. While in the case of active metals corrosion current depends directly on the OCP, in case of passive metals corrosion current is nearly independent of the OCP.
and the anodic current is equal to the passivation current. However as OCP of passive metals increases, it may reach the critical pitting potential above which localized corrosion is initiated. Such a scenario leads to a particularly pervasive form of corrosion, localized corrosion, so called because it covers relatively small areas on the corroding surface, limited to the locations where the passive film has been damaged or otherwise altered.

**Indirect MIC from biofilm growth**

The growth of microbial biofilms on metal substrata will clearly affect the chemistry of the near-surface environment. Such effects may be direct where a specific microbial reaction or product of metabolism becomes itself a reactant in the electrochemical corrosion processes. Indirect, or non-specific effects can also be important, however, where the biofilm, or its associated polymer matrix, modifies the physicochemical environment within which these reactions occur.

For example, Roe et al. (1996) demonstrated that the mere deposition of a thin layer of calcium alginate, an extracellular biopolymer excreted by biofilm microorganisms, on mild steel is enough to fix the anodic sites and initiate corrosion. The mechanism of this process was traced to differential aeration cells where the layer of the biopolymer locally impeded oxygen transport to the surface.

On the other hand, biofilm growth is a dynamic process in which initial attachment of individual cells is followed by their increase in numbers, along with proliferation of extracellular polymeric substances (EPS) which form the matrix within which the cells grow. In the early stages of development there is likely to be an uneven distribution of cells and EPS which can lead to areas of the metal substratum being covered by biofilm, with adjoining areas being freely exposed to the surrounding environment. The metabolic activity of cells within the biofilm will lead to reduced concentrations of oxygen at the metal surface, and so form an oxygen concentration or differential aeration cell between those regions under the biofilm (anode), and the uncolonised areas exposed to maximal oxygen concentration (cathode). Again, the fundamental mechanism of corrosion involves the flow of electrons from an anode to a cathodic region where the electron acceptor is oxygen.

This indirect mechanism of MIC has been examined in detail, and its contribution to MIC associated with the sulphate-reducing bacteria (SRB) considered by Lee et al. (1993a; 1993b) and by Lee and de Beer (1995). These authors demonstrated that during the initial stages of SRB biofilm development patchy microbial growth gives rise to a primary phase of oxygen-dependent corrosion with associated tubercle formation and localised regions of low pH. Subsequently, more contiguous growth then shields the substratum as a whole from oxygen and allows the development of sufficient sulphidogenic activity to precipitate iron sulphide corrosion products and establish the sulphide/metal galvanic couple which is responsible for the major, and longer term, SRB-dependent corrosion.

**Anaerobic corrosion of mild steel by SRB**

MIC of mild steel by SRB has recently been the subject of a series of review papers. These have considered the subject within the context of electrochemical reactions in a wide range of biotic and abiotic corrosion processes (Hamilton & Lee, 1995; Lee et al., 1995; Lewandowski et al., 1997; Hamilton, 2000a; Hamilton, 2000b), and against the background of the basic physiology of the SRB (Hamilton, 1998; 1999). The features of primary importance have been identified, and are presented below. By far the most important of these is the central role that oxygen has been shown to play as terminal electron acceptor.

Firstly, SRB stimulate corrosion as a consequence of their activities within mixed species microbial consortia in the form of biofilms adherent to the mild steel substratum. Maximal corrosion activity is demonstrated where there is access to oxygen and the biofilm develops a characteristic oxic/anoxic (O₂/AnO₃) interface. Secondly, the primary role of the SRB is generic and comprises the production of sulphide. At least a proportion of this sulphide arises directly from the oxidation of hydrogen formed at the cathode of the electrochemical corrosion cell. Thirdly, iron dissolution takes place at the electrochemical anode and combines with the biogenic sulphide to give precipitated iron sulphides as corrosion products. The chemical nature and physical form of the iron sulphides are dependent upon a number of factors, viz. the relative concentrations of soluble iron and sulphide, the access of oxygen; the presence of other bacteria within the biofilm, e.g. iron and/or sulphur oxidising species; the time course of development of the biofilm and associated corrosion processes. Fourthly, iron sulphide corrosion products may be either protective or corrosive. Where they are in the form of a tightly adherent thin film or tarnish, they protect the underlying unreacted steel in a manner directly analogous to the oxide film that constitutes the corrosion resistance of stainless steel. In the case of iron sulphides, however, such films are inherently unstable and their rupture gives rise to extremely active corrosion cells between the iron sulphide (cathode) and the exposed-steel surface (anode). Also, whereas oxide films on stainless steel have the capacity to repassivate after any perturbation,
In order for iron sulphide corrosion products to function by this mechanism, they must be in direct electrical contact with the underlying steel substratum. Sixthly, a major feature of corroding biofilms is the nature and extent of the heterogeneities they demonstrate, viz. consortia of mixed microbial species, almost certainly located at separate sites within the biofilm; O₂/AnO₂ interface; a range of iron sulphide corrosion products overlaid, in the presence of oxygen, with ferric oxide/hydroxide; separate foci of electrochemical activity with iron dissolution and pit formation at anodic sites.

Despite the extent of present knowledge of MIC caused by the sulphate reducers, there remain areas where there are significant uncertainties, however. The quantitative importance of the oxidation of cathodic hydrogen has not yet been established. It is still not possible to relate unambiguously protective and corrosive effects to the nature of the corrosion product(s) FeₓSy deposited. Perhaps most importantly, it remains unclear what is the nature of the relationships, direct and indirect, between the heterogeneity of the electrochemical corrosion reactions and the heterogeneities, microbial, chemical and physical, within the biofilm itself.

**Model for SRB corrosion**

The most full and convincing exposition of the current state of understanding of the mechanism of so-called anaerobic corrosion of mild steel caused by the SRB is given in the papers by Lee et al. (1993a; 1993b) and Nielsen et al. (1993), which follow on from earlier work by Hardy and Bown (1984) and McKenzie and Hamilton (1992). Issues of central importance are the establishment of electrical contact between the metal substratum and the biomineralised iron sulphides as corrosion product, and the direct involvement of oxygen.

The dissolution of ferrous iron is an anodic process involving an oxidative reaction with the loss of electrons.

\[
\text{Fe}^+ \rightarrow \text{Fe}^{2+} + 2e^-
\]

In order for this reaction to proceed it must be coupled to a complementary reductive reaction. This occurs at a cathodic region on the metal surface where, in the absence of oxygen, protons or hydrogen sulphide (Costello, 1974) may act as electron acceptor.

\[
2\text{H}^+ + 2e^- \rightarrow 2\text{H} \Rightarrow \text{H}_2
\]

or

\[
2\text{H}_2\text{S} + 2e^- \rightarrow 2\text{HS}^- + \text{H}_2
\]

These anodic and cathodic reactions, and their location at separate sites on the metal surface, constitute an electrochemical corrosion cell. Each partial reaction is thermodynamically characterised by its redox potential, and they are coupled at a potential value midway between the more negative anodic oxidation and the more positive cathodic reduction. For mild steel at neutral pH values this corrosion potential (Ecorr) is around −700 mV, as measured against the saturated calomel electrode (SCE); or −459 mV SHE. The actual value of Ecorr however, and the rate of any subsequent corrosion in a given experimental situation is determined by environmental and kinetic factors affecting each of the individual partial reactions.

The molecular hydrogen resulting from these coupled reactions is subject to biological oxidation by the SRB. This removal of hydrogen from its site of production is termed cathodic depolarisation as it facilitates the electrochemical cathodic reaction which is generally considered to be the controlling step in the overall corrosion process. It should be mentioned at this point, however, that a number of authors have taken a contrary view in which it is proposed that SRB stimulate corrosion by a mechanism of anodic depolarisation (Hamilton & Lee, 1995). A key element of this hypothesis is the localized acidification at the anode which results from the formation of iron sulphide corrosion products (Crolet, 1992; Daumas et al., 1993).

\[
\text{Fe}^{2+} + \text{HS}^- \Rightarrow \text{FeS} + \text{H}^+
\]

Common to both hypotheses, however, is the production of sulphide which results in the precipitation of ferrous sulphide corrosion products within the anoxic regions of the biofilm. Across the O₂/AnO₂ interface however, both reduced ferrous and sulphide ions are subject to oxidation, abiotic and/or biotic, with oxygen as terminal electron acceptor. This, in turn, generates as further corrosion products the ferric oxide/hydroxide and elemental sulphur which are recognised as being diagnostic for active SRB corrosion and, in the case of sulphur, may itself play a direct role in the corrosion mechanism as a cathodic reactant (Lee et al., 1993a; 1993b; Nielsen et al., 1993).

\[
\text{S}^{0} + \text{H}_2\text{O} + 2e^- \Rightarrow \text{HS}^- + \text{OH}^- 
\]

In this regard, it is important to note a) that the biomineralised product, S₀, is only effective as a non-soluble form in galvanic contact with the underlying metal, and b) that the precipitated iron sulphides are highly electron conductive and can therefore serve as a mediator for the electron
transfer from the metal to the elemental sulphur (Schmitt, 1991).

That is to say, the apparent paradox of the key involvement of oxygen in so-called anaerobic SRB corrosion is solved, and a proper theoretical framework is established for the underlying mechanisms. This reaction scheme is illustrated in Figure 1.

SRB corrosion of mild steel occurs by a process of electron transfer from the base metal to oxygen as the ultimate electron acceptor, through a series of coupled redox reactions of, respectively, electrochemical, biotic and abiotic character. The microbial activity at the metal surface has resulted in a kinetically favoured pathway of electron flow giving rise to increased oxidation (corrosion weight loss) of the zero valent iron.

**Corrosion of copper and copper alloys**

Although nor strictly within the province of this review, it is instructive to consider some comparable studies of MIC with copper and copper alloys.

Syrett et al. (1979) have examined the effects of polluted deaerated sea water on the corrosion of 90:10 and 70:30 Cu:Ni alloys. In aerated sea water in the absence of any sulphide pollution, there is minimal corrosion with oxygen serving as cathodic reactant and electron acceptor. The Cu⁺ ions hydrolyse at the surface to form a protective Cu₂O film ensuring that the corrosion becomes self-limiting. In deaerated sea water without oxygen as terminal electron acceptor, even this limited corrosion will be absent. In this latter case, however, the presence of sulphide, sulphur or polysulphides can have a major impact. Corrosion potentials shift to more active values and hydrogen evolution becomes a possible cathodic process. In this instance, Cu⁺ ions formed at the anode react with the HS⁻ ions in solution to form a cuprous sulphide film. Although, again, corrosion is slight under these conditions, it is significantly increased on subsequent introduction of oxygen, since the porous cuprous sulphide film now inhibits the formation of the normal protective cuprous oxide.

The authors also noted the presence of colloidal sulphur in their experimental system and hypothesised that S⁰ might also serve as a further cathodic reactant, with any sulphide formed then being able to be re-oxidised to sulphur by dissolved oxygen. They pointed out that, in this instance, the HS⁻ ⇌ S⁰ reaction can be regarded as an indirect oxygen reduction process. Clearly, both these experimental findings, and their interpretation in terms of pathways of electron flow from the metal to oxygen as terminal electron acceptor, closely parallel the analysis of SRB MIC of carbon steel described above.

In another study of MIC of copper in the presence of a number of non-SRB bacteria, Gerchakov et al. (1985) quite explicitly, and perhaps for the first time, put forward the idea that the organisms could play a direct role in the corrosion process through their own inherent capability to both oxidise and reduce copper as part of the normal functioning of their electron transport chain.
Corrosion of stainless steel with manganese oxidising bacteria

As has been outlined earlier in this paper, pitting corrosion of metals such as stainless steel requires that some condition modifies the passive oxide film such that the resting potential, in the region of $-150 \text{ mV}_{\text{SCE}}$, is increased to a value more positive than the pitting potential, around $300 \text{ mV}_{\text{SCE}}$. Ennoblement is the term used to describe this shift of potential, which is associated also with an increase in cathodic current density. Ennoblement is considered a necessary first step in the initiation of corrosion in all passive metals and alloys. The mechanism of the actual pitting corrosion, however, is dependent rather on the presence of chloride ions. Ennoblement causes stainless steel to be susceptible to pitting corrosion at low concentrations of chloride to which non-ennobled steel would be resistant. Ennoblement has only been demonstrated in non-brakish waters, presumably due to the subsequent pitting with loss of ennoblement in the presence of high chloride. These features have been discussed by Dexter (1995), with particular regard to the possible cause-and-effect nature of ennoblement and pitting corrosion.

In the past a number of studies have implicated an involvement of microbial biofilms in ennoblement, but it has only been with the more recent work of the groups of Linhardt, Dexter and Lewandowski that details of the mechanism of the phenomenon have begun to emerge. Dickinson et al. (1996) identified the deposition of MnO$_2$ as being the likely causative agent. These authors noted ennoblement of 316L stainless steel coupons after exposure for periods up to 35 d in a natural fresh water stream. Potentials increased from $-150 \text{ mV}_{\text{SCE}}$ and held steady at values close to $350 \text{ mV}_{\text{SCE}}$, with an associated increase in cathodic current density. The biofilm formed during the exposure included characteristic annular deposits which were shown to be rich in precipitated MnO$_2$. Similar findings have been reported by Linhardt (1994) and Ruppel et al. (2001).

In an extensive study involving both field exposure in a range of natural environments and detailed laboratory experimentation under controlled conditions, the Bozeman group have further characterised the manganese effect and proposed its likely mechanism. Ennoblement has been achieved with exposure of stainless steel coupons to pure cultures of the manganese oxidising bacterium (MOB) Leptothrix discophora (Dickinson et al., 1997). Evidence has been obtained that the oxidation of Mn(II) to MnO$_2$ proceeds by two one-electron steps with MnOOH as an intermediate (Shi et al., 2002). Field studies have demonstrated the parallel between the amount of MnO$_2$ deposited at different exposure sites and the consequent rate and extent of ennoblement (Braughton et al., 2001). It is hypothesised that the manganese oxides, deposited as insoluble biomineralised products on the steel surface, act as cathodic reactants and are recycled to Mn(II) in two one-electron reductions (Dickinson & Lewandowski, 1996; Lewandowski et al., 1997; Olesen et al., 2000; Lewandowski & Hamilton, 2002). Further, it has now been shown that when stainless steel coupons, which have first been ennobled by either microbiological or chemical deposition of MnO$_2$, are subsequently exposed to 0.35% NaCl, there is extensive pitting corrosion which is absent from control coupons without ennoblement (Olesen et al., 2001).

The essential features are that the insoluble manganese oxides are in direct electrical contact with the steel surface, and that the metal exhibits an equilibrium potential compatible with the two reactions:

$$\text{MnO}_2 + H^+ + e^- \Rightarrow \text{MnOOH} \quad \text{Eo'}383 \text{ mV}_{\text{SCE}}$$

$$\text{MnOOH} + 3H^+ + e^- \Rightarrow \text{Mn}^{2+} + 2H_2O \quad \text{Eo'}336 \text{ mV}_{\text{SCE}}$$

where the potentials are calculated for pH 7.2 and $[\text{Mn}^{2+}] = 10^{-6} \text{ M}$.

A model of the proposed mechanism is shown in Figure 2. Electron transfer from the metal to oxygen as the terminal acceptor is mediated by a manganese redox cycle comprising microbial oxidation and electrochemical reduction; this latter exactly parallels the chemical reduction of MnO$_2$ which contributes to the redox balance in natural sediment ecosystems.

It should be stressed, however, that specific details of this proposed mechanism remain to be confirmed at this time. For example, Dexter and his colleagues (Ruppel et al., 2001) have performed a similar set of experimental studies to those of the Bozeman group and, while agreeing in general terms with their conclusions, have hypothesised that MnOOH, and its disproportionation may have a central role in the manganese redox cycle.

CONCLUSION

The principal focus of this review has been the corrosion of ferrous metals as the direct result of microbial action; microbivably influenced corrosion (MIC). The mechanisms that have been elucidated, however, are of much broader significance within the general field of metal microbe interactions. They apply equally to reactions involving zero valent metals as they do to ionic species, and they embrace the metallic element as either electron donor or electron acceptor. In terms of practical relevance, they underpin reactions in such apparently disparate
processes as MIC, bioremediation and mineral mining. The term biomineralisation is often used with reference to this range of basic mechanisms, along with their engineering, economic and ecological impact.

The comparison between SRB-induced corrosion of mild steel and the role of MOB in the corrosion of stainless steel illustrates how apparently quite different processes are in fact dependent on the same fundamental mechanism. In the case of the SRB, the microbial oxidation of molecular hydrogen both depolarises the cathodic region of the steel, and gives rise to biomineralised iron sulphides as corrosion products. A galvanic corrosion cell is thus established, and a kinetic mechanism generated for the flow of electrons from the zero valent metal to oxygen as the ultimate electron acceptor. On the other hand, manganese oxide deposited on, and galvanically coupled to stainless steel substrata, is the ideal cathodic reactant to create a corrosion cell, which is then maintained by the recycling action of the MOB.

In both cases, microbial action has supplied products which are thermodynamically compatible with the potentials of the putative corrosion reactions of the relevant substratum and, crucially, has generated appropriate kinetically favoured pathways of electron flow from the metal anode to the universal electron acceptor, oxygen.

Although this unifying hypothesis represents a novel approach to the study of MIC, it stems directly from knowledge of microbial physiology and from understanding of how that determines the controlled functioning of microbial ecosystems.

Acknowledgements

Much of the development of the ideas put forward in this paper took place during short sabbatical visits by the author to the Center for Biofilm Engineering at Montana State University, Bozeman. The support of a Leverhulme Emeritus Fellowship and a travel grant from the Carnegie Trust for the Universities of Scotland is gratefully acknowledged. Many collaborative discussions with Zbigniew Lewandowski and his research colleagues over the years, and direct contributions to the preparation of sections of this manuscript, were invaluable and are acknowledged with thanks.

References

Belay N, Daniels L (1990) Elemental metals as electron sources for biological methane formation from CO₂. Antonie Leeuwenhoek 57: 1–7
Boyle A W, Phelps C D, Young L J (1999) Isolation from estuarine sediments of a Desulfovibrio strain which can grow on lactate.
coupled to the reductive dehalogenation of 2,4,6-tribromo-mophenol. *Appl Environ Microbiol* 65: 1133–1140


Streamlined:


