Recent advances in electron transfer between biofilms and metals

Alain Bergel

Laboratoire de Génie Chimique – CNRS
5 rue Paulin Talabot, BP1301, 31106 Toulouse, France

Alain.Bergel@ensiacet.fr

Keywords: Electron transfer, biofilm, microbial corrosion, biocorrosion, microbial fuel cell.

Abstract. Microbial biofilms produce electrochemical interactions with metal surfaces by following a wide variety of different electron exchange pathways. Reviewing the mechanisms identified in the biocorrosion of steels leads us to distinguish direct and indirect mechanisms for biofilm-catalysed cathodic reactions. Indirect mechanisms are due to the production of metal oxides or hydrogen peroxide (aerobic corrosion) or metal sulphides (anaerobic corrosion), which further react with the metal surface. Direct mechanisms involve adsorbed biocompounds, generally enzymes or their active sites, which catalyse the cathodic reduction of oxygen for aerobic biocorrosion or the proton/water reduction in anaerobic processes. Recent studies dealing with the role of hydrogenases in anaerobic corrosion have shed light on the important role of phosphate species via so-called cathodic deprotonation. Advances in the development of microbial fuel cells have also resulted in new concepts, mainly for oxidation processes. Some microbial cells have been shown to be able to produce their own electron mediators. Others can transfer electrons directly to electrodes through membrane-bound electron shuttles or achieve long-range transfer through conductive pili.

Introduction

Oxidizing nutrients and wasting the electrons to a final electron acceptor is the fundamental process of the life of any organism. The presence of microbial biofilms on solid surfaces is thus unavoidably attended by drastic modification in the local redox conditions close to the material surface. Living organisms have developed a variety of very effective redox catalysts, the oxidoreductases, which are key agents in any microbial redox process. They may be found inside or linked to microbial cells, or released by the cells and entrapped in the biofilm matrix. On metal surfaces, the local modifications induced by the microorganisms organised in biofilms or by the free oxidoreductases retained in the biofilms are sources of several corrosion processes, also called microbial corrosion or biocorrosion. Looking at the mechanisms that have been identified in the biocorrosion of metals is thus a very helpful way to approach the electron transfer pathways between conductive surfaces and microbial cells.

In the great majority of cases, both nutrients and final electron acceptors are dissolved in solution, and electron transfer between the metal surface and biofilms is enhanced *indirectly* by local modifications in pH, oxygen concentration or chemical composition, by the formation of deposits or by the production of aggressive metabolites. In addition, it is now well known that adsorbed enzymes and proteins are able to achieve *direct* electron transfer with electrodes. In the last few years, it has also been demonstrated that bacteria are able to develop unexpected strategies to exchange electrons *directly* with solid surfaces. This early 21st century discovery has produced an abundance of literature, mainly devoted to microbial fuel cells (MFCs). Actually, the capability of biofilms to oxidize organic matter, used as fuel, and to transfer the electrons coming from this oxidation directly to solid anodes has provided unexpected new solutions for designing fuel cells where biofilms play the role of a catalyst. A few works have also described biofilms that are able to catalyse cathodic reductions. Besides biocorrosion, the field of MFCs will also be mentioned here as a source of newly discovered mechanisms of electron transfer between biofilms and metal surfaces.

Aerobic Corrosion

Established enzyme-based mechanisms for oxygen reduction. Marine biofilms have long been known to be remarkable motors of microbial corrosion as they catalyse the reduction of dissolved oxygen on metallic materials such as stainless steels [1,2]. Actually the electrons extracted from the material by the cathodic reaction are provided by the oxidation of the underlying alloy. The first effect is an increase of the free corrosion potential (phenomenon called potential ennoblement), which may lead to the disruption of the oxide layer that naturally protects stainless steel and similar active-passive alloys. The biofilm-driven catalysis of oxygen reduction consequently increases the probability of corrosion onset, and may induce subsequent fast propagation of localised corrosion. The numerous studies that have aimed at deciphering the basic mechanisms of the biofilm-driven oxygen reduction on stainless steels have used different assumptions and different models, which seem pertinent and even complementary. Manganese oxidising bacteria, which consume oxygen to produce manganese oxides, have been demonstrated to be very effective causes of corrosion in waters rich in manganese ions [3]. These bacteria oxidize manganese ions into manganese oxide, which is then reduced on the material surface into manganese hydroxide and back to manganese ions. These successive reactions create a cathodic cycling process that enhances electron transfer from the material to oxygen, the final electron acceptor.

The production of hydrogen peroxide in biofilms is at the crossroads of several different hypotheses [4]. The presence of hydrogen peroxide results in the modification of the nature of the surface oxide layers [5], which may be responsible for the catalysis of the cathodic oxygen reduction. This aspect will not be developed here because it seems too specific to stainless steels and other passive alloys. More interesting is the involvement of free oxidoreductases, such as peroxidases, which have been detected in marine biofilms, for instance, and may contribute to the production of hydrogen peroxide in the biofilm. In contrast, catalases, which are produced by most aerobic micro-organisms to protect themselves against oxidative stress, should decrease the concentration of hydrogen peroxide. The balance between the enzymes that produce hydrogen peroxide and the enzymes that consume it should control the corrosive properties of the biofilm [6]. A laboratory model has thus been proposed in which glucose and glucose oxidase (GOx) are added to artificial seawater. The model reproduces the electrochemical action of biofilms on stainless steels that is observed with natural marine biofilms. In the presence of glucose, GOx catalyses the reduction of the dissolved oxygen into hydrogen peroxide:

$$C_6H_{12}O_6 + O_2 + H_2O \rightarrow C_6H_{12}O_7 + H_2O_2 \quad (1)$$

The further reduction of hydrogen peroxide on the material surface:

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \quad (2)$$

is taken to be responsible for the increased risk of corrosion induced by the biofilm. The fine mechanism of this model is certainly more complex because of local acidification due to the side-production of gluconic acid, and also to complex interactions between the adsorbed enzyme and the oxide layers [7].

Both cases commented above deal with *indirect* microbial effects that are induced by some compounds produced by the microbial metabolisms. Other assumptions in biocorrosion require *direct* adsorption of biocompounds on the material surface. Much work in the "Bioelectrochemistry" field has been devoted to the study of direct electron transfers between enzymes and electrodes. In the past, the main target applications were in the design of electrochemical biosensors. For several years, however, studies have also been aimed at devising innovative bio-electronics concepts [8] and the enzymatic catalysis of oxygen reduction has become of interest for designing biofuel cells [9]. In this framework, a few enzymes, such as laccase, have revealed remarkable properties for catalysing oxygen reduction on graphite, carbon and other electrode materials. Catalase, which catalyses the disproportionation of hydrogen peroxide:

$$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2 \quad (3)$$

has also been shown to be able to catalyse oxygen reduction of glassy carbon [10]. It has been proposed that this enzyme is more pertinent for biocorrosion issues because of its usual synthesis by aerobic microorganisms. However, the transfer of this bio-electrocatalysis from the carbon, graphite or gold materials that are commonly used in basic bioelectrochemistry has not proved really convincing yet, as far as we know.

Recent advances in aerobic biocorrosion. The investigations carried out on the direct catalysis of oxygen reduction through adsorbed enzymes led to a promising method focusing on the active site of the enzyme instead of the full protein. A porphyrin compound, also called haemin, constitutes the active centre of a wide variety of proteins active in the respiratory chain (cytochrome-linked compounds) or related to oxygen consumption (catalases, peroxidases, etc.). By simple adsorption on the surface of stainless steel, haemin has been demonstrated to produce a potential ennoblement of the same order of magnitude as that observed in natural seawater, and voltammetry has confirmed its capability to catalyse oxygen reduction on stainless steel [11] and to create locally high oxygen reduction rates.

An innovative approach introduced under the European Project "Electro-Active Biofilms" (6^{th} FP, NEST508866 [12]) resulted in a numerical model that fitted the catalysis of oxygen reduction due to biofilm growth on stainless steel coupons immersed in the sea. This model gave new information on the biofilm-driven kinetics of oxygen reduction, and a clear demonstration that the phenomenon was fully controlled by enzyme kinetics. The same project also resulted in the isolation and identification of numerous microbial species that composed the electrochemically active marine biofilms.

Anaerobic Corrosion

Established mechanisms and role of hydrogenase. It is now commonly agreed that sulphate reducing bacteria (SRB) act on the corrosion of carbon steels through the production of H_2S and the deposition of iron sulphides that enhance the cathodic reduction of the proton. The link between SRB and the hydrogen that is formed cathodically on the material surface is more difficult to ascertain. It has been claimed that the presence of hydrogenase in SRB is a criterion for possible corrosive activity, because only SRB that contain hydrogenase are able to use the hydrogen produced on the material surface as an energy source [13]. Nevertheless, the role in corrosion of this consumption of the cathodically formed hydrogen remains an open issue. Despite the lack of commonly agreed fundamental explanations, several authors have observed that carbon steel corrosion is significantly enhanced in the presence of hydrogenase-positive SRB, while SRB that do not possess hydrogenase have no effect [13-15].

Daumas *et al*. showed that hydrogenase-positive SRB increased the corrosion rates of carbon steels to a similar extent whether they produced H_2S or not [16]. In this case, the presence of hydrogenase was claimed to be more important than the production of H_2S. They also noted that direct contact between the material surface and the cell surface was required. Bryant and Laishley showed that the hydrogenase from *Clostridium pasteurianum* was able to reduce organic electron acceptors by extracting electrons directly from the steel surface [15]. Several studies have demonstrated that hydrogenases adsorbed on the surface of electrodes are able to reduce dissolved compounds using the electrons they accept from platinum [17], cadmium sulphide [18], and stainless steel [19,20]. In all these cases, direct contact between hydrogenase and the material is obviously required.

On the other hand, Rajagopal and Le Gall [21], and Belay and Daniels [22] described experiments in which no contact was possible. Steel coupons were immersed in a bottle that did not contain any bacteria, while SRB were grown in a separate bottle. The two liquid phases were similar but totally separated; the bottles were connected only through the common gas phase. The presence of SRB in the second bottle increased the corrosion rate of the coupon contained in the first bottle by a factor of three to four. Bryant and Laishley reproduced the two-bottle experiment

with free hydrogenase and methyl viologen as the electron acceptor in the second bottle instead of SRB [23]. No contact was possible between the enzyme and the carbon steel but the presence of hydrogenase in the second bottle increased the corrosion rate by 19%.

The experiments where SRB or hydrogenase come into contact with or close to the surface of steel are open to a variety of interpretations: implication of H_2S and of iron sulphides, consumption of adsorbed hydrogen rather than molecular hydrogen, direct electron transfer from the metal to the biological catalyst, etc. In contrast, the experiments where material and catalyst are definitively separated can only be interpreted through the consumption of molecular hydrogen from the gas phase, and consequently through the shift of some still unknown equilibrium. But still, how can consumption of hydrogen in the second bottle affect the irreversible corrosion process occurring in the first bottle?

Recent advances in anaerobic biocorrosion. It has been pointed out that corrosion occurs only when phosphate species are present in the bottle containing the iron coupons [23]. A recent work has given the probably definitive explanation of these results, demonstrating that phosphate species undergo a so-called cathodic deprotonation [24]:

$$H_2PO_4^- + e^- \rightarrow HPO_4^{2-} + \tfrac{1}{2} H_2 \qquad (4)$$

that enhances the production of dihydrogen on the material surfaces. The most recent results suggest that this reaction may be reversible, which could explain why any cause of dihydrogen consumption should shift this cathodic equilibrium towards higher electron extraction rates, and consequently enhance corrosion [25]. Hydrogen consumption does not require the contact of hydrogenases of SRB with the metal surface. As phosphate is a usual buffer solution in most experiments carried out with hydrogenase and SRB, all the past conclusions should be revisited taking into consideration this unexpected cathodic equilibrium. Other weak acids that may be synthesized in natural biofilms may also be responsible for the same kind of cathodic deprotonation.

New experimental data have demonstrated that hydrogenases can provoke local corrosion of carbon steel in the absence of phosphate species. In this case, only a direct catalysis of proton/water reduction can be suggested as an explanation. In conclusion, the effect of free hydrogenase on the corrosion of carbon steels now seems to have been clearly ascertained and two different mechanisms can be suggested, which involve two new pathways of electron transfer between metals and anaerobic biofilms:

- cathodic deprotonation of weak acids, which leads to the reversible production of hydrogen, and the further consumption of hydrogen in the biofilm,
- direct catalysis of proton/water reduction through adsorbed hydrogenase.

Microbial Fuel Cells (MFCs)

MFCs convert bio-convertible substrates directly into electricity. The concept has been known since the early 20th century but practical applications have proved to be of interest only in the last few years, thanks to the discovery that biofilms can transfer the electrons coming from the substrate they oxidize directly to solid anodes. This discovery was first made on marine microbial fuel cells, in which a graphite anode was embedded in sediment and the cathode was placed above the sediment in aerated seawater. It was observed that the current resulting from the oxidation of the organic matter contained in sediments increased when the biofilm was enriched in electrochemically active microbial species [26]. Several MFCs have since been developed in different natural or industrial environments that contain rich microbial populations, such as waste water treatment plants, or with pure culture of electrochemically active cells. The literature on MFCs has been exhaustively reviewed elsewhere [27-29].

A few biofilm-catalysed cathodes have been proposed [30], sometimes directly derived from the biofilms that are known to catalyse oxygen reduction in biocorrosion [31,32]. The MFC anode processes have been more widely studied and have led to three new mechanisms being

distinguished for the extracellular electron transfer towards anode surface. Some microorganisms have been shown to synthesize their own soluble electron shuttles [33], like *Pseudomonas aeruginosa* known to produce pyocyanin, an electron mediator that can also be used by other species contained in natural biofilms. Other microorganisms achieve electron transfer through membrane-bound electron shuttling compounds. Hydrogenases have been suspected to be involved in such pathways. Genetic studies carried out on *Geobacter sulfurreducens* have demonstrated that many of the key components known to be involved in extracellular electron transfer to soluble oxides, e.g. outer-membrane c-type cytochromes, are also involved in electron transfer to solid electrodes [29,34]. This pathway obviously requires direct contact between the material surface and the cells, but the most recent genetic studies have also identified a long-range electron transfer pathway for microorganism exchange of electrons even far from the electrode surface. Different bacteria, such as *Geobacter sulfurreducens* [35] or *Shewanella oneidensis* [36], develop electrically conductive pili, also called nanowires, that act as an electronic network permeating the biofilm and promoting long-range electrical connection.

In conclusion to this short (and non-exhaustive) review, it can be stated that microbial biofilms enhance electron transfer with metals following a wide variety of pathways that indirectly result from the numerous redox reactions involved in living processes. Besides, it seems that bacteria are also able to develop specific strategies to take advantage of direct electron transfer with conductive surfaces. Recent advances have given a new boost to research in this area, which may even result in new discoveries.

References

1- A. Mollica, A. Trevis, Proceedings of the 4th int. congress on marine corrosion and fouling, Antibes, France (1976) p.351
2- V. Scotto, M.E. Lai, Corros. Sci. 40 (1998) 1007
3- B.H. Olesen, R. Avci, Z. Lewandowski, Corros. Sci. 42 (2000) 211
4- H. Amaya, H. Miyuki, Corros. Engin. 44 (1995) 123
5- N. Le Bozec, C. Compère, M. L'Her, A. Laouenan, D. Costa, P. Marcus, Corros. Sci. 43 (2001) 765.
6- I. Dupont, D. Féron, G. Novel, Int. Biodet. and Biodeg. 41 (1998) 13.
7- V. L'Hostis, C. Dagbert, D. Féron, Electrochim. Acta 48 (2003) 1451.
8- I. Willner, B. Willner, Trends in Biotechnol. 19 (2001) 222
9- S.D. Minteer, B.Y. Liaw, M.J. Cooney, Current Opinion in Biotechnol. 18 (2007) 1
10- M.E. Lai, A. Bergel, J. Electroanal. Chem. 494 (2000) 30
11- R. Basséguy, J. Idrac, C. Jacques, A. Bergel, M.-L. Délia, L. Etcheverry, EFC publications 45, Local probe techniques for corrosion research, R. Oltra, V. Maurice, R. Akid, P. Marcus eds. (2007) p.52
12- Information on www.ea-biofilms.org
13- R. Cord-Ruwisch, F. Widdel, Appl. Microbiol. Biot. 25 (1986) 169.
14- R. D. Bryant, E.J. Laishley, Can. J. Microbiol. 36 (1990) 259.
15- R. Cord-Ruwisch, W. Kleinitz, F. Widdel, J. Petrol. Technol. (1987) 97.
16- S. Daumas, Y. Massiani, J. Crousier, Corros. Sci. 28 (1988) 1041.
17- K. Délécouls, P. Saint-Aguet, C. Zaborosch, A. Bergel, J. Electroanal. Chem. 468 (1999) 139.
18- A. Nedoluzhko, I.A. Shumilin, L.E. Mazhorova, V.O. Popov, V.V. Nikandrov, Bioelectrochemistry 53 (2000) 61.
19- S. Da Silva, R. Basséguy, A. Bergel, Bioelectrochemistry 56 (2002) 77.
20- S. Da Silva, R. Basséguy, A. Bergel, in NACE 2002, Denver, USA, 2002.
21- B.S. Rajagopal, J. Le Gall, Appl. Microbiol. Biotechnol. 31 (1989) 406.
22- N. Belay, L. Daniels, Anton. Leeuw. Int. J. G 57 (1990) 1.
23- R.D. Bryant, L. Laishley, Appl. Microbiol. Biot. 38 (1993) 824
24- S. Da Silva, R. Basséguy, A. Bergel, J. Electroanal. Chem. 561 (2004) 93

25- L. DeSilva Munoz, A. Bergel, R. Basséguy, Corros. Sci. (2007) in press
26- D.R. Bond, D.E. Holmes, L.M. Tender, D.R. Lovley, Science 295 (2002) 483
27- B.E. Logan B. Hamelers, R. Rozendal, U. Schröder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, K. Rabaey, Environ. Sci. Technol. 40 (2006) 5181
28- D.R. Lovley, Nature Reviews 4 (2006) 497
29- D.R. Lovley, Current Opinion in Biotechnol., 17 (2006) 1
30- Z. He, L.T. Angenent, Electroanalysis 18 (2006) 2009
31- A. Rhoads, H. Beyenal, Z. Lewandowski, Environ. Sci. Technol. 39 (2005) 4666
32- A. Bergel, D. Feron, A. Mollica, Electrochem. Comm. 7 (2005) 900
33- K. Rabaey, W. Verstraete, Trends in Biotechnol. 23 (2005) 291
34- T. Mehta, M.V. Coopi, S.E. Childers, D.R. Lovley, Appl. Environmen. Microbiol. 71 (2005) 8634
35- G. Reguera, K.D. McCarthy, T. Mehta, J.S. Nicoll, M.T. Tuominen, D.R. Lovley, Nature 435 (2005) 1098
36- Y.A. Gorby, S. Yanina, J.S. McLean, K.M. Rosso, D. Moyles, A.Dohnalkova, T.J. Beveridge, I.S. Chang, B.H. Kim, K.S. Kim, D.E. Culley, S.B. Reed, M.F. Romine, D.A. Saffarini, E.A. Hill, L. Shi, D.A. Elias, D.W. Kennedy, G. Pinchuk, K. Watanabe, S. Ishii, B. Logan, K.H. Nealson, J.K. Fredrickson, PNAS 103 (2006) 11358