milar papers at core.ac.uk

provided by

Open Archive Toulouse Archive Ouverte (OATAO)
OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible.

This is an author -deposited version published in: http://oatao.univ-toulouse.fr/
Eprints ID: 4568

To link to this article: DOI:10.1016/j.elecom.2010.03.017

http://dx.doi.org/10.1016/j.elecom.2010.03.017

To cite this version : Mehanna, Maha and Basséguy, Régine and Délia, Marie-Line and Bergel, Alain (2010) *Geobacter sulfurreducens can protect 304L stainless steel against pitting in conditions of low electron acceptor concentrations.* Electrochemistry Communications, vol. 12 (n°6). pp. 724-728. ISSN 1 388-2481

Any correspondence concerning this service should be sent to the repository administrator: staff-oatao@inp-toulouse.fr

Geobacter sulfurreducens can protect 304L stainless steel against pitting in conditions of low electron acceptor concentrations

Maha Mehanna *, Régine Basséguy, Marie-Line Délia, Alain Bergel

Laboratoire de Génie Chimique, CNRS — Université de Toulouse, 4 allée Emile Monso, 31432 Toulouse, France

Keywords:
Biocorrosion
Microbial corrosion
Geobacter sulfurreducens
304L stainless steel

A B S T R A C T

The effect of *Geobacter sulfurreducens* cells was studied on the electrochemical behaviour of 304L stainless steel, emphasizing the role of the soluble electron acceptor (fumarate). In fumarate-lacking media, the presence of *G. sulfurreducens* induced free potential ennoblement in a few hours. This ennoblement has already been observed in standard media that contained fumarate. Our previous studies have shown that *G. sulfurreducens* shifted the pitting potential toward the positive values. The pits induced by the presence of the bacteria were wider and deeper than in the absence of bacteria. Here, in fumarate-lacking media, similar shift in pitting potential was observed, but the repassivation phase was strongly improved. AFM analysis showed that pits were identical with those observed in the absence of bacteria at lower potential. In contrast with all the previous work where *G. sulfurreducens* enhanced corrosion, here at a low concentration of electron acceptor, the presence of the bacteria protected the steel against pitting.

1. Introduction

Many microbial genera and species have the capacity to connect their metabolism to solid electrodes, directly exchanging electrons with them through different mechanisms [1,2]. One of the most widely studied of these bacteria, *Geobacter sulfurreducens*, has been shown to be able to oxidize organic electron donors (acetate, benzoate, toluene, etc.…) to carbon dioxide using graphite [3,4] or stainless steel [5] anodes as electron acceptor. Conversely, it can also reduce nitrate to nitrite or fumarate to succinate with graphite [6] or stainless steel [7] cathodes as electron donor. Electron transfer was achieved directly between the electrode surface and the membrane cell through periplasmic and outer membrane c-type cytochromes [8].

The implication of direct electron transfer between material surfaces and microorganisms was first evoked in the framework of microbial corrosion with *Desulfobacterium*-like and *Methanobacterium*-like isolates extracted from natural biofilms [9]. Our previous work has shown that *G. sulfurreducens* can exert two different effects on 304L stainless steel [10]: just after inoculating, *G. sulfurreducens* cells create a cathodic reaction on the material, which leads to a fast increase in its open circuit potential (E_{oc}), increasing the corrosion risk; in contrast, after a few days, well-established biofilms shift the pitting potential (E_{pit}) towards positive values, which might be interpreted as a protective effect. Nevertheless, this second effect is correlated with the presence of acetate (electron donor) in the medium and our last study has shown that, in the absence of acetate, the E_{pit} positive shift was no longer observed and the presence of the biofilm actually resulted in deeper and wider pits [10,11].

The present study indicates that the presence of the electron acceptor (fumarate) plays a crucial role in the influence of *G. sulfurreducens* on the corrosion of 304L stainless steel.

2. Experimental section

G. sulfurreducens strain PCA (ATCC 51573) was purchased from DSMZ. The standard growth medium, which was used as described elsewhere [7], contained 25 mM sodium fumarate (electron acceptor) and 10 mM acetate (electron donor). The bacteria were incubated in the growth medium under N_2/CO_2 (80/20) for five days, at 30 °C. Electrochemical experiments were carried out in 0.5 L reactors with continuous N_2/CO_2 (80/20) bubbling, at 30 °C. The electrochemical reactors were inoculated with 5% v/v bacterial culture at 142 000 CFU mL^{-1} at time $t = 24$ h. The reactors contained the same components as the culture medium but with 25 or 0 mM fumarate.

Working electrodes were 2 cm diameter cylinders made of 304L stainless steel embedded in insulating resin (Resipoly Chrysor). Coupons were polished using SiC papers with P120–P800 grit (Lam Plan) for all coupons and additionally with P120–P4000 for some of them. They were then rinsed thoroughly with distilled water. A platinum grid served as a counter electrode. All potentials were

* Corresponding author. Tel.: +33 5 34 32 36 73; fax: +33 5 34 32 36 97.
E-mail address: mum34@psu.edu (M. Mehanna).

expressed with respect to Ag/AgCl reference. Measurements of open circuit potentials (E_{oc}) were made using a multipotentiostat (VMP-Bio-Logic) and polarization curves were obtained potentiodynamically with a scan rate of 0.5 mV s^{-1}.

Topographic images were recorded with an atomic force microscope (AFM) (AutoProbe M5 with ECAP module from Veeco, Park Scientific Instruments) operated in contact mode using silicon nitride Si_3N_4 cantilevers with a scan rate of 0.5 Hz.

3. Results and discussion

G. sulfurreducens cells were grown in standard bulk conditions, i.e. in media containing 10 mM acetate as electron donor and 25 mM fumarate as electron acceptor. 304L coupons were immersed in 0.5 L medium that was identical to the culture medium (with 25 mM fumarate) or in medium that did not contain fumarate, and the open circuit potential (E_{oc}) was recorded as a function of time. After 24 h, reactors were inoculated with 25 mL (5% v/v) of cell culture that contained 142 000 colony forming units (CFU) per mL.

In each case the addition of bacterial cells led to a sharp increase in E_{oc}, while no such steep E_{oc} increase was observed in the control experiments that were performed by injecting the same volume of fresh medium without bacteria. 3 h after inoculation, the E_{oc} increases ($\Delta E_{oc,3\,h}$) were respectively 0.36 V in the fumarate-containing medium (Fig. 1A) and 0.32 V in fumarate-lacking medium (Fig. 1B). Both experiments were performed with coupons polished up to P800. With finer polishing (P4000), $\Delta E_{oc,3\,h}$ was only 0.18 V (Fig. 1B′) in fumarate-lacking medium. 150 h after inoculation, ΔE_{oc} followed the same descending order: 0.41 V in fumarate-containing medium, 0.35 V and 0.19 V in fumarate-lacking media with standard (P800) and fine polishing respectively. At the end of the 20-day experiments all ΔE_{oc} values were similarly around 0.15 V. The slow ennoblement that was observed in the control experiments performed in sterile conditions was due to the development of the passive layer after polishing. Such ennoblement in sterile conditions was not perceptible in previous experiments made with 1145 mild steel [12].

The number of planktonic cells was fifteen times lower (16 522 CFU mL^{-1} instead of 207 709 CFU mL^{-1}) in the fumarate-lacking medium than in fumarate-containing medium. Actually, in the fumarate-lacking medium, there was a residual amount of fumarate due to the part contained in the inoculum that had not already been consumed during the inoculum preparation. This residual amount was sufficient for the cells to survive but not enough to support significant bacterial development.

Fast ennoblement of free potential just after inoculating *G. sulfurreducens* cells has already been observed on mild steel, ferritic and austenitic steels in standard (fumarate-containing) media [12]. It has been shown that the *G. sulfurreducens* cells that settled on a stainless steel electrode were able to achieve fast electron exchanges with the electrode. The cells can be electrochemically filled with and emptied from electrons, functioning as the so-called "electron sponges" [8,13]. This hypothesis is supported by the abundance of iron in the periplasm and outer membrane of *Geobacter* species in the form of c-type cytochromes [14,15]. The steep E_{oc} increase that is observed as soon as the inoculum is added into solution is provoked by electron exchange between the material and the cells that adhere on the surface. E_{oc} increase was only slightly affected by the absence of fumarate; this phenomenon depends more on the initial redox state of the cells in the inoculum than on their further development during E_{oc} recording. The initial redox state of the cells in the inoculum did not vary since the culture medium was always identical. The significant effect of polishing can be explained by two complementary ways. It has been reported that surface roughness (Ra) close to the size of bacterial cell favour microbial adhesion [16] and we have already observed that the current density provided by *Geobacter* biofilms matched the Ra ratio for values of a few µm [4]. It can be concluded that polishing disfavoured bacterial adhesion here, which diminished the ennoblement kinetics. Besides, it has been shown that small Ra values, lower than 0.9 µm improve the resistance to corrosion of stainless steels [17]. Here both phenomena can occur and contribute to slowing down ennoblement of the smoother surface.

At the end of the 20-day open circuit experiments, voltammetry was performed at 0.5 mV s^{-1} to assess the pitting potential (E_{pit}). E_{pit} was measured when the current reached 0.1 mA (Fig. 2). In each case,

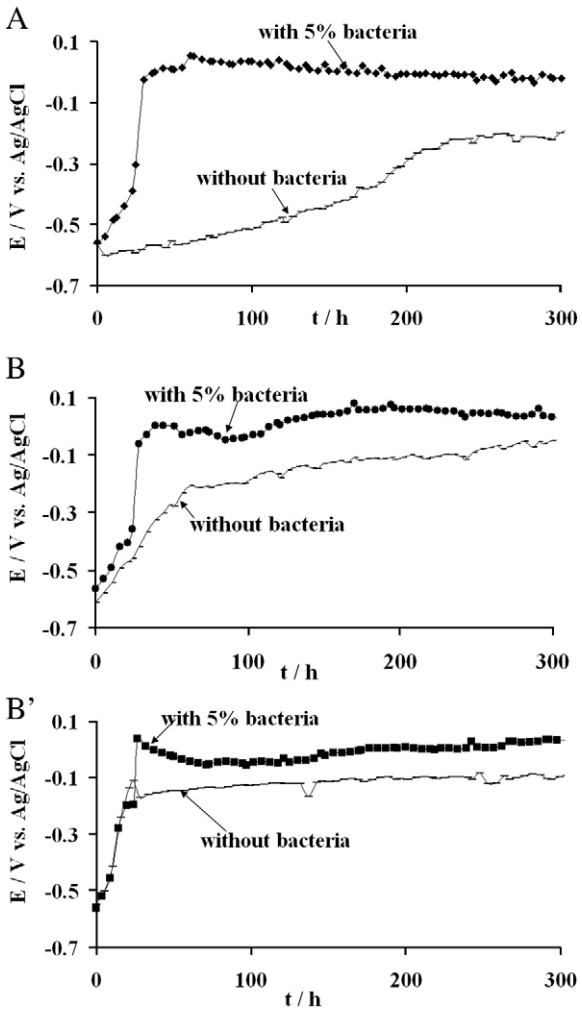

Fig. 1. Variation of E_{oc} of 304L stainless steel with time with 5% v/v *G. sulfurreducens* and without bacteria: (A) medium containing 25 mM fumarate, after polishing at P120–P800 grit; (B) fumarate-lacking medium after polishing with P120–P800 grit; and (B′) fumarate-lacking medium after polishing with P120–P4000 grit.

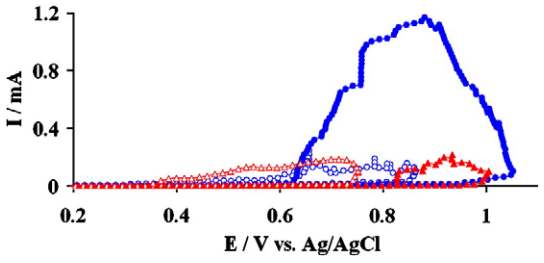

Fig. 2. Pitting curves (scan rate 0.5 mV s^{-1}) of 304L stainless steel after 20 days immersion in a medium containing (○) 25 mM fumarate; (△) 0 mM fumarate. Closed and open symbols represent, respectively the presence of 5% v/v *G. sulfurreducens* and the absence of bacteria.

the presence of *G. sulfurreducens* shifted the pitting potential by 0.2 V or more towards positive values. In the presence of 25 mM fumarate, high pitting currents were observed, which resulted in severe attack of the material. After the pitting curves had been recorded, numerous severely corroded zones appeared (Fig. 3B), while only one or two weakly corroded spots were visible in the control experiments carried out in the absence of bacteria (Fig. 3A). The behaviour was markedly different in fumarate-lacking medium. The pitting current was of the same order of magnitude as the control experiments and repassivation occurred faster: at 0.81 V vs. Ag/AgCl instead of 0.34 V without bacteria. On a macroscopic scale, the material surface exhibited only one weakly corroded spot (Fig. 3D), while two similar spots were observed on the control coupon (Fig. 3C). AFM topography measurements confirmed that only small pits had formed in the presence of bacteria in fumarate-lacking medium (Fig 4B and C). The pit widths (around 20 μm) and the pit depths (a few micrometers), were similar to those observed in the absence of bacteria (Fig 4A). The values extracted from Fig 4 are representative of values obtained from the twenty images taken in different locations of each sample.

It has already been observed that a biofilm of *G. sulfurreducens* formed in standard medium (containing both fumarate and acetate) shifts the pitting potential towards positive values, but it increases the propagation current by a factor of around 4 [11]. It is known that *G. sulfurreducens* biofilms catalyse the oxidation of acetate, transferring the electrons directly to stainless steel anodes [5]. It has been assumed that this biofilm-catalysed oxidation modified the passive layer in a way that inhibits pit initiation [12]. As a consequence, in voltammetry tests, pitting occurred at more positive potentials logically resulting in higher propagation current and deeper and wider peaks, because higher energy is available at higher potentials. More surprisingly, it has been reported that in the absence of electron donor (acetate), the presence of bacteria did not change the pitting potential value, but pits remained significantly deeper and wider than in the absence of bacteria [11]. It has been concluded that *G. sulfurreducens* cells inhibited pit initiation in the presence of acetate but favoured formation of wider and deeper pits whatever the acetate concentration.

Here, in a fumarate-lacking medium, *G.sufurreducens* cells shifted the pitting potential toward positive values but the propagation currents remained at the same level as in the absence of bacteria. In spite of the higher energy available when pitting occurred, pit formation was similar to that in the absence of bacteria. The gain of more than 450 mV in repassivation potentials confirmed the protective effect of the cells. In a fumarate-lacking medium, the biofilm was not able to develop properly as indicated by the number of planktonic cells; nevertheless, this surviving biofilm was sufficient to have a remarkable protective effect on the material.

4. Conclusion

The fast free potential ennoblement that is induced by *G. sulfurreducens* cells was only slightly dependent on the presence or not of electron acceptor in the medium. In contrast, the long-term effect of biofilm on pitting drastically depended on the composition of the medium. In fumarate-lacking conditions the biofilm revealed a protective effect by shifting the pitting potential to positive values and improving the conditions of repassivation. The effect of *G. sulfurreducens*

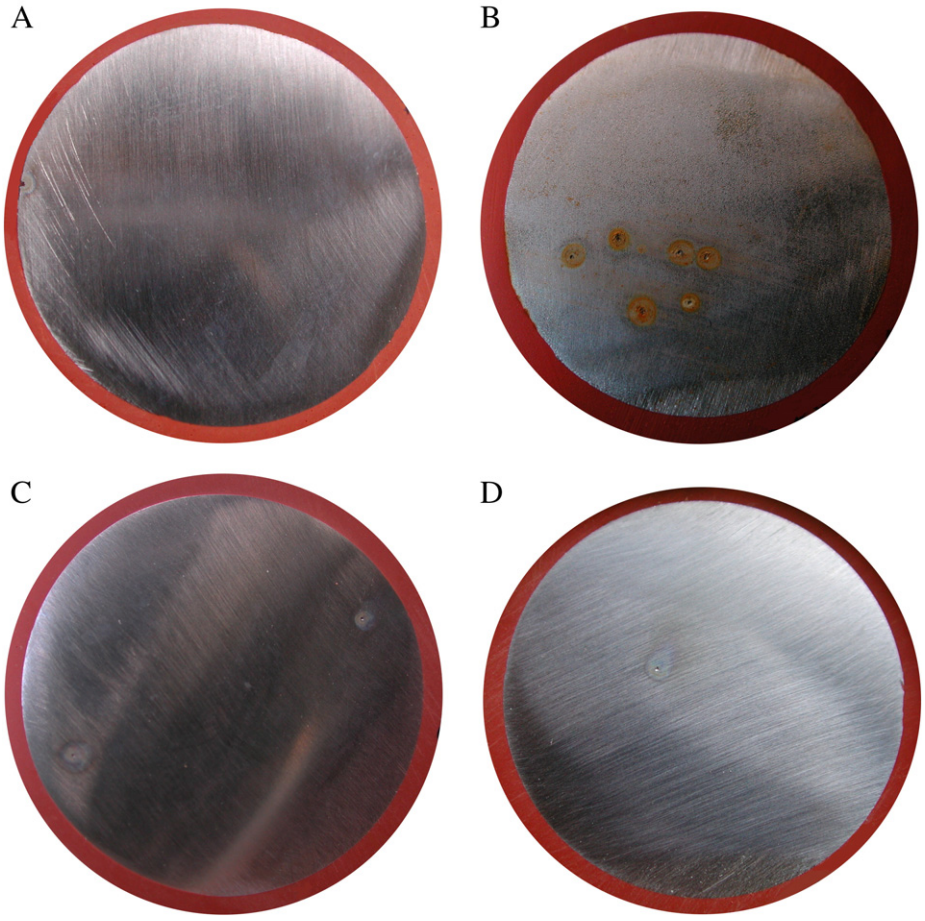

Fig. 3. Photographs of the samples that underwent the pitting polarization reported in Fig. 2 in the absence of bacteria (first column: A, C) or inoculated with 5% v/v *G. sulfurreducens* (second column: B, D). (A, B): Fumarate: 25 mM. (C, D): Fumarate 0 mM.

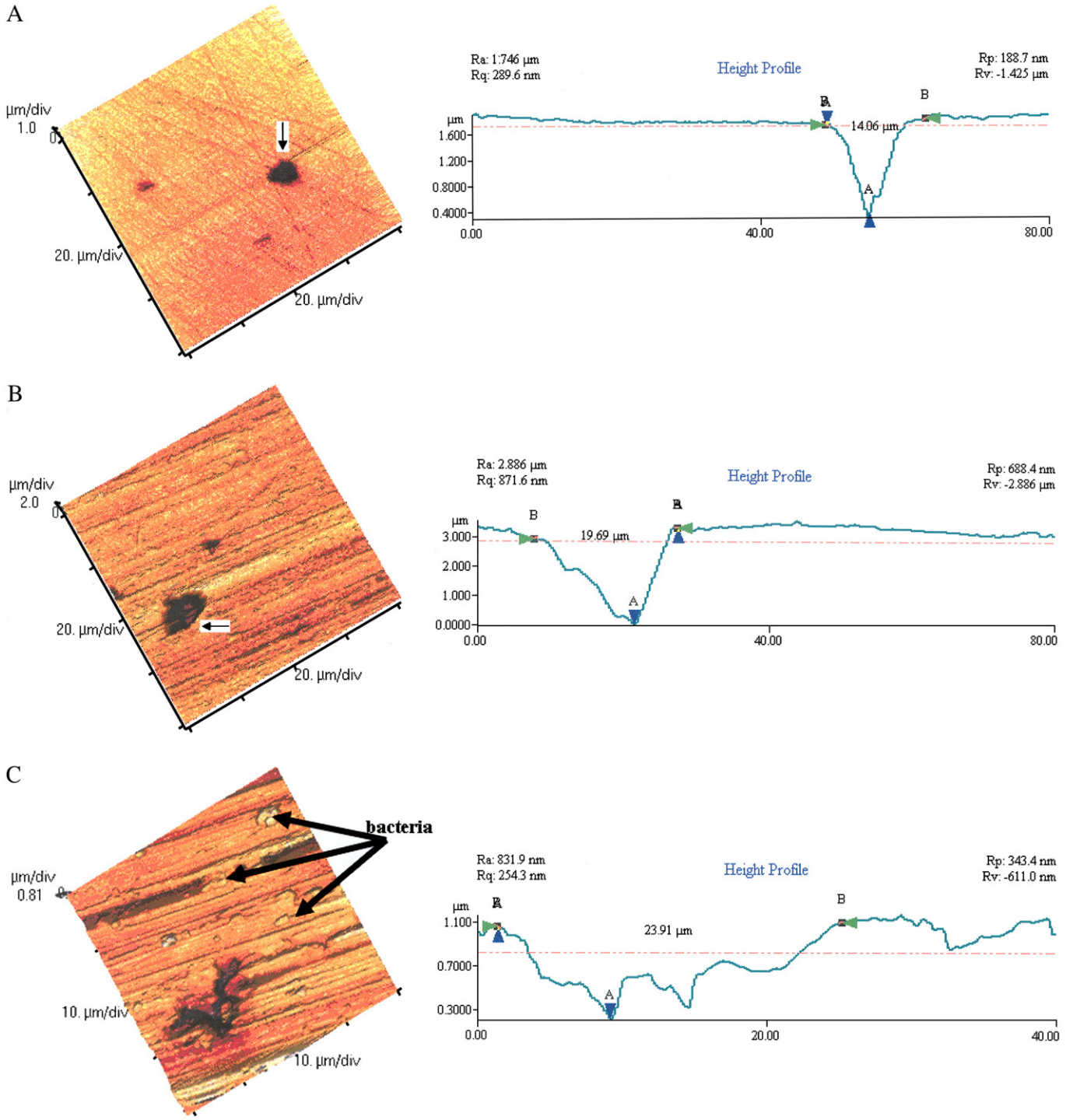

Fig. 4. AFM image and topographic representation of 304L surfaces from the experiments reported in Fig. 2B with 0 mM fumarate: A in the absence of bacteria (pit width ≈ 14 µm, depth ≈ 1.6 µm); B and C in the presence of 5% v/v *G. sulfurreducens* (pit width ≈ 20 µm, depth ≈ 3 µm in B; pit width ≈ 24 µm, depth ≈ 1 µm in C); some adherent bacterial cells are visible in picture C.

cells on the corrosion behaviour of 304L steel can be drastically changed depending on the presence or absence of soluble electron acceptor.

Acknowledgements

This work was supported by a grant from CNRS-DRI (department ST2I). It was part of CNRS work for the European network "Surfaces of materials in living environments (SMILE)". We are grateful to Dr. Damien Féron from CEA/DEN/SCCME Saclay (France) for helpful discussions. We thank Luc Etcheverry and Dr. Benjamin Erable from the Laboratoire de Génie Chimique, Toulouse (France), for their technical and scientific support and Dr. Rolf Gubner and Namurata Sathirachinda from the Swedish Corrosion Institute — Swerea KIMAB, Stockholm (Sweden) for AFM facilities.

References

[1] D.R. Lovley, Nat. Rev. Microbiol. 7 (2006) 497.
[2] K. Rabaey, W. Verstraete, Trends Biotechnol. 23 (2005) 291.
[3] D.R. Bond, D.R. Lovley, Appl. Environ. Microbiol. 69 (2003) 1548.
[4] C. Dumas, R. Basseguy, A. Bergel, Electrochim. Acta 53 (2008) 3200.

[5] C. Dumas, R. Basseguy, A. Bergel, Electrochim. Acta 53 (2008) 5235.
[6] K.B. Gregory, D.R. Bond, D.R. Lovley, Environ. Microbiol. 6 (2004) 596.
[7] C. Dumas, R. Basseguy, A. Bergel, Electrochim. Acta 53 (2008) 2494.
[8] D.E. Holmes, S.K. Chaudhuri, K.P. Nevin, T. Mehta, B.A. Mehté, A. Liu, J.E. Ward, T.L. Woodard, J. Webster, D.R. Lovley, Environ. Microbiol. 8 (2006) 1805.
[9] H.T. Dinh, J. Kuever, M. Mussmann, A.W. Hassel, M. Stratmann, F. Widdel, Nature 427 (2004) 829.
[10] M. Mehanna, R. Basseguy, M.-L. Delia, A. Bergel. Electrochem. Commun. 11 (2009) 568.
[11] M. Mehanna, R. Basseguy, M.-L. Delia, A. Bergel. Electrochem. Commun. 11 (2009) 1476.
[12] M. Mehanna, R. Basseguy, M.-L. Delia, A. Bergel. Corr. Sci. 51 (2009) 2596.
[13] S. Parot, M.-L. Delia, A. Bergel. Electrochim. Acta 53 (2008) 2737.
[14] A. Esteve-Nunez, J. Sosnik, P. Visconti, D.R. Lovley, Environ. Microbiol. 10 (2008) 497.
[15] D.R. Lovley, Geobiology 6 (2008) 225.
[16] S.H. Flint, J.D. Brooks, P.J. Bremer, J. Food Eng. 43 (2000) 235.
[17] L.R. Hilbert, D. Bagge-Ravn, J. Kold, L. Gram, Int. Biodeterior. Biodegradation 52 (2003) 175.