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Role of direct microbial electron transfer in corrosion of steels

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Abstract

It has recently been discovered that many microbial species have the capacity to connect their metabolism to solid electrodes, directly exchanging electrons with them through membrane-bound redox compounds, nevertheless such a direct electron transfer pathway has been evoked rarely in the domain of microbial corrosion. Here was evidenced for the first time that the bacterium *Geobacter sulfurreducens* is able to increase the free potential of 304L stainless steel up to 443 mV in only a few hours, which represents a drastic increase in the corrosion risk. In contrast, when the bacterial cells form a locally well-established biofilm, pitting potentials were delayed towards positive values. The microscopy pictures confirmed an intimate correlation between the zones where pitting occurred and the local settlement of cells. *Geobacter* species must now be considered as key players in the mechanisms of corrosion.

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1. Introduction

Microbial corrosion concerns a broad variety of natural and industrial environments, in which microbial biodiversity is extremely wide. Nevertheless, until now, only sulphate reducing bacteria (SRB) have been acknowledged to play an obvious role in corrosion [1]. It is generally agreed that microbial corrosion of iron alloys in anaerobic environments is mainly due to the catalysis of a cathodic reduction of proton/water:

$$2H^+ + 2e- \rightarrow H_2 \text{ or } 2H_2O + 2e- \rightarrow H_2 + 2OH^- \quad (1)$$

SRBs act via the metabolic production of sulphide ions:

$$SO_4^{2-} + 4H_2O + 8e- \rightarrow S^{2-} + 8OH^- \quad (2)$$

which form iron sulphide deposits that catalyses the proton/water cathodic reduction on the material surface [2]. Actually, the mechanisms of anaerobic biocorrosion are more complex than this raw scheme and remain difficult to decipher. The consumption of hydrogen by SRBs cannot have a direct effect on the corrosion rate,, because reaction 2 can be decomposed first into the Volmer reaction:

$$M + H_2O + e- \Leftrightarrow M\text{-}H_{ads} + OH^- \quad (3)$$

(where M represents a metallic site) followed by either Tafel reaction:

$$2M\text{-}H_{ads} \Leftrightarrow 2M + H_2 \quad (4)$$

or Heyrovsky reaction:

$$M\text{-}H_{ads} + H_2O + e^- \Leftrightarrow M + H_2 + OH^- \quad (5)$$

And both Tafel and Heyrovsky reactions are rate-limiting on iron alloy surfaces. Consumption of the hydrogen produced cannot enhance them. Nevertheless, SRBs certainly take advantage of the hydrogen produced by the corrosion process (reaction 1), using it as electron donor, which promotes the production of sulphides. Moreover, the enzyme hydrogenase produced by SRBs can adsorb on steel surfaces and catalyse proton reduction [3,4], and the presence of phosphate buffer in laboratory experiments can introduce a supplementary cathodic reaction [5]. Finally, although SRBs are the predominant subject of academic works, recent studies have demonstrated that biocorrosion can also occur beneath biofilms where SRBs are not predominant [6]. New pathways still need to be deciphered.

In recent years, more and more bacteria have been shown to be able to oxidise organic matter and to transfer the electrons produced directly to solid electrodes [7,8]. Such bacteria can completely oxidise organic electron donors (e.g. acetates, sugars) to carbon dioxide by using a solid electrode as electron acceptor [9]. Bacteria implement different strategies to achieve direct electron transfer: direct contact with the electrode surface established through membrane-bound redox compounds [10] (e.g. c-type cytochromes) or cell-to-cell networking through conductive nanowires [11]. Some bacteria can also produce soluble electron carriers [12]. One of the most studied bacteria, *Geobacter sulfurreducens*, is also able to implement cathodic reactions, extracting the electrons required for its metabolism directly from the surface of a cathode [13,14]. Up to now, the implication of direct electron transfer between material surfaces and microorganisms has been evoked only once in the framework of biocorrosion, with *Desulfobacterium*-like and *Methanobacterium*-like isolates extracted from natural biofilms [15].

The purpose of this work was to check the possible relevance of this newly discovered mechanism in biocorrosion by monitoring the electrochemical behaviour of 304L stainless steel in the presence of *Geobacter sulfurreducens* cells.

2. Experimental

Geobacter sulfurreducens strain PCA (ATCC 51573) purchased from DSMZ was grown in the standard medium [14] that contained 10mM sodium acetate (electron donor) and 25mM sodium fumarate (electron acceptor). The bacteria were incubated for five days at 30°C. Electrochemical experiments were carried out in electrochemical reactors under continuous N_2/CO_2 (80/20) bubbling, at 30°C for optimum bacteria growth. The reactors were filled with 0.5L solution identical to the culture medium but with less acetate (5mM). The bacteria (5% vol/vol. i.e. 142 000 CFU.mL^{-1}) were injected into the reactors after 24 hours. Working electrodes were 2cm diameter cylinders made of 304L stainless steel and embedded in resin. Connections were made through titanium wire protected with resin. Coupons were polished using P120-P800 grit SiC papers and cleaned with ethanol followed by thorough rinsing in distilled water. Electrochemical measurements were performed using a multipotentiostat (VMP-Bio-Logic) with Ag/AgCl reference electrode and a platinum grid as counter electrode. Tafel plots were recorded before inoculation (around 21h), two days after (around 71h) and at the end of the experiment (around 237h) by scanning the potential from E_{oc}-100mV to E_{oc} +200mV, and from E_{oc}-100mV to E_{oc} +350mV for the last one.

At the end of the experiment, electrodes were removed from the reactors and stained with acridine orange (0.03% w/w). Scanning electron microscopy (SEM) pictures were taken with a LEO 435 VP-Carl Zeiss SMT. Epifluorescence microscopy was performed using Carl Zeiss Axiotech 100 microscope equipped with HBO 50/ac mercury lamp and coupled to a

monochrome, digital camera (Evolution VF). Images were treated with Image-Pro Plus 5.0 software.

3. Results and discussion

Cells were first cultured in bulk solution according to the standard procedure, with 10 mM acetate as electron donor and 25 mM fumarate as electron acceptor. The culture was then used to inoculate electrochemical cells which contained the 304L coupons in the culture medium, but with 5mM acetate instead of 10mM. The concentration of the electron donor was lowered in the aim of forcing the microbial cells to search for a supplementary electron source on the steel surface. The experiment, repeated seven times, gave reproducible results (Fig. 1). The open circuit potential (E_{oc}) increased quickly during the three hours following injection of the bacteria, up to ΔE_{oc}=305$\pm$22 mV (average and standard deviation from 7 experiments). It continued to increase slowly for the next twenty hours, up to ΔE_{oc}=443$\pm$51 mV. In control experiments, injection of the sterile culture medium did not cause any E_{oc} increase.

Tafel plots were recorded at various times by scanning the potential around E_{oc}. In the absence of bacteria (Fig. 2A), the E_{corr} values given by the Tafel plots were significantly more negative than the E_{oc} values. In this case, the sole cathodic reaction consisted of the reduction of protons (reaction 2), which had a very low concentration ($6.3 10^{-8}$ M at pH 7.2). H^+ depletion in the diffusion layer that was provoked by the potential scan logically decreased the cathodic current and consequently shifted E_{corr} towards negative potential values. In the absence of bacteria E_{corr} was controlled by the mass transfer limitation of the cathode reaction. In the presence of the bacteria (Fig. 2B-C) there was less than 70mV difference between E_{oc} and E_{corr}. It can be concluded that the cathodic reaction was no longer controlled by the mass-transfer-limited proton reduction and that the presence of bacteria created a new cathodic

reaction that was less sensitive to mass transfer. The anodic part was not significantly modified by the presence of *G.sulfurreducens* during the first few days, confirming that the abrupt increase of E_{oc} observed during the early hours was due to the modification of the cathodic part. The Tafel plot recorded at the end of the experiments showed a clear oxidation wave at potential values above 0.03 V vs. Ag/AgCl, which was not observed on the Tafel plots recorded only 2 days after inoculation. This wave was due to the oxidation of acetate catalysed by the biofilm. As already observed on polarised electrodes, it was confirmed here that acetate oxidation occurred only with well-established biofilms [16].

Pitting curves recorded at the end of the experiments indicated that the presence of *G.sulfurreducens* shifted the pitting potential (E_{pit}) from 840$\pm$80 mV vs. Ag/AgCl (without bacteria) to 1009$\pm$20 mV vs. Ag/AgCl (Fig. 3). In the presence of bacteria, the abnormally high anodic current, with regard to traditional pitting curves, that was recorded during the scan in the positive direction was due to the biofilm-catalysed oxidation of acetate, as observed on the Tafel plot (Fig. 2C). At high potential values, *G.sulfurreducens* had a clear protective effect, due to the electrons provided to the material by the biofilm-catalysed oxidation of acetate.

SEM micrography showed that the pits were deeper in the presence of bacteria. This is relevant with the higher hysteresis effect observed on the repassivation curves (Fig. 3). In the presence of bacteria, pitting occurred at higher potential values and resulted in higher propagation currents. The epifluorescence microscopy pictures recorded after the pitting curves showed that deep pits formed predominantly in zones where the biofilm was dense (Fig. 4A), while zones free from pits revealed only scattered microbial settlement (Fig. 4B). Two different hypotheses can explain this observation: i) bacteria preferentially colonise the areas that are the most sensitive to further corrosion attacks, for instance because of local surface defaults, or ii) the presence of a locally dense biofilm creates a cathodic area that

promotes corrosion in its vicinity. Whatever are the hypothesis, microbial settlement and pitting zones, showed intimate local correlation.

4. Conclusion

G.sulfurreducens revealed here as a main player in electron transfer between 304L stainless steel and the surrounding medium. Experiments performed at open circuit demonstrated that, in a medium with low electron donor concentration, *G.sulfurreducens* can extract electrons from steel, causing a fast potential increase up to 443 mV, which drastically increases corrosion risk. This reaction was due to the fast electron transfer already observed between electrodes and *G.sulfurreducens* cells as soon as they settle on the electrode surface [16,17]. In contrast, the catalysis of acetate oxidation that occurred at high potential values only with well-developed biofilms delayed the occurrence of pitting. In this case the biofilm revealed a protective effect. The corrosive/protective action of *G.sulfurreducens* on steel surfaces depends strongly on the potential range and the age of the biofilm. From a fundamental point of view, it has been demonstrated here that the mechanism of direct microbial electron transfer can be of crucial importance in anaerobic biocorrosion. Moreover, several *Geobacter* species have been shown to be able to achieve direct electron transfer with solid electrodes and *Geobacter* species are abundant in soils, sediments and other natural environments. Practically, these species should now be considered as possible main contributors to biocorrosion, particularly when buried equipment is concerned.

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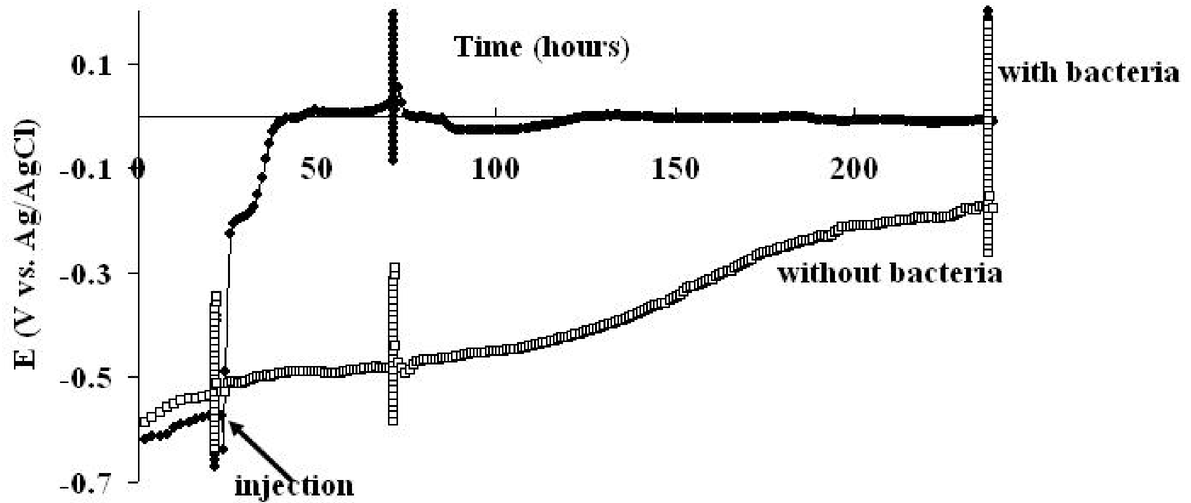

Figure 1 Open circuit potential versus time in the absence and presence of *G. sulfurreducens*. Fluctuations in potential that appeared around 21h, 71h and 237h were due to Tafel plot recording.

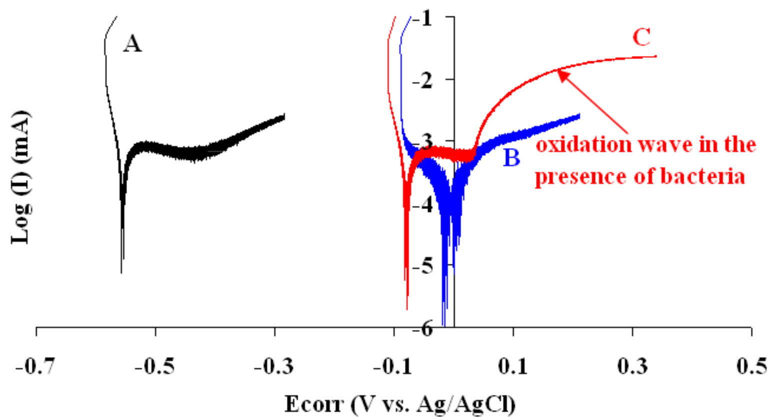

Figure 2 Tafel plots performed from -100mV / E_{oc} to + 200mV / E_{oc} (scan rate $0.5 mVs^{-1}$) A, two days after injection of sterile medium. B, with *G. sulfurreducens* two days after inoculation (142 000 $CFU.mL^{-1}$). C, from -100mV / E_{oc} to + 350mV / E_{oc} at t =237h with *G. sulfurreducens* (142 000 $CFU.mL^{-1}$).

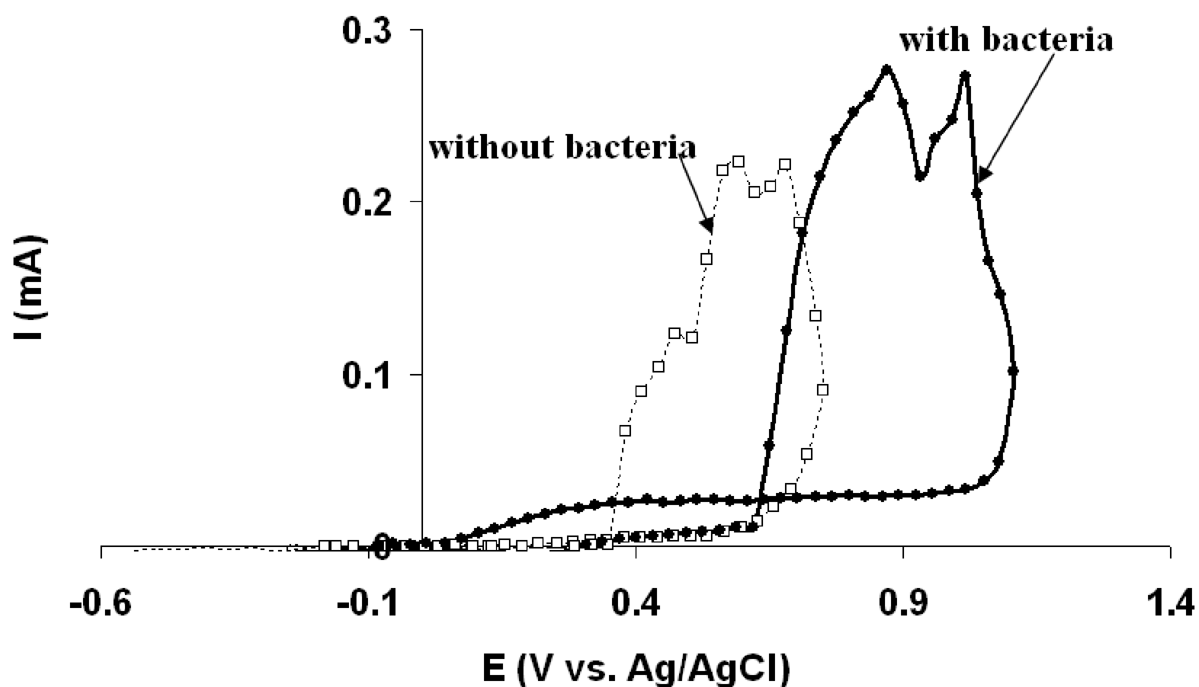

Figure 3 Polarisation curves (scan rate 0.5 mVs^{-1}); scan was reversed when 0.1 mA was reached, performed at t = 240h in the absence and presence of *G. sulfurreducens*.

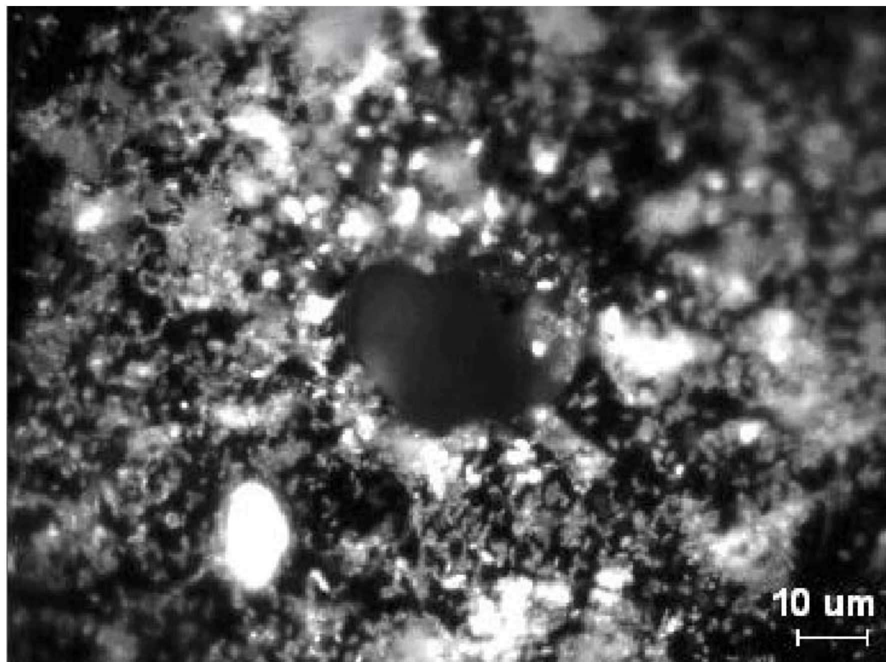

Figure 4 Epifluoresence microscopy of 304L stainless steel in the presence of *G. sulfurreducens:* **A** in the vicinity of a pit, and **B** in a zone free from pits (magnification 100x).