Effect of *Geobacter sulfurreducens* on the microbial corrosion of mild steel, ferritic and austenitic stainless steels

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A B S T R A C T

The influence of *Geobacter sulfurreducens* was tested on the anaerobic corrosion of four different steels: mild steel 1145, ferritic steel 403 and austenitic steels 304L and 316L. Within a few hours, the presence of cells induced a free potential (E_{oc}) ennoblement around +0.3 V on 1145 mild steel, 403 ferritic steel and 304L austenitic steels and slightly less on 316L. The kinetics of E_{oc} ennoblement depended on the amount of bacteria in the inoculum, but the final potential value depended essentially on the nature of the material. This effect was due to the capacity of *G. sulfurreducens* to create a direct cathodic reaction on steel surfaces, extracting the electrons directly from material. The presence of bacterial cells modified the corrosion features of mild steel and ferritic steel, so that corrosion attacks were gathered in determined zones of the surface. Local corrosion was significantly enhanced on ferritic steel. Potential ennoblement was not sufficient to induce corrosion on austenitic steels. In contrast *G. sulfurreducens* delayed the occurrence of pitting on 304L steel because of its capability to oxidize acetate at high potential values. The electrochemical behaviour of 304L steel was not affected by the concentration of soluble electron donor (acetate, 1–10 mM) or the amount of planktonic cells; it was directly linked to the biofilm coverage. After polarization pitting curves had been recorded, microscopic observations showed that pits propagated only in the surface zones where cell settlement was the densest. The study evidenced that *Geobacter sulfurreducens* can control the electrochemical behaviour of steels in complex ways that can lead to severe corrosion. As *Geobacteraceae* are ubiquitous species in sediments and soils they should now be considered as possible crucial actors in the microbial corrosion of buried equipment.

1. Introduction

Corrosion costs 4% of the GDP of industrialised countries and 20% of this cost is estimated to be due to the action of microorganisms [1,2]. While the implication of sulphate-reducing bacteria (SRB) in anaerobic corrosion is now well accepted [3–9], recent studies have demonstrated that SRB are not the only cause of anaerobic corrosion and that biocorrosion can occur beneath biofilms even when SRB are absent [10]. The electron transfer pathways that lead to microbial corrosion are far from being fully deciphered.

In addition, some bacteria have recently been shown to be able to switch from natural soluble electron acceptors such as oxygen or nitrite to solid anodes. The so-called anodophilic bacteria naturally adhere to the anode surface and catalyse the oxidation of organic compounds, transferring the electrons produced directly to the anode [11–13]. One of the most widely studied anodophilic species, *Geobacter sulfurreducens*, has been shown able to completely oxidize organic electron donors, generally acetate, to carbon dioxide by using only an electrode as electron acceptor [14–16]. Direct electron transfer to solid electrodes, without the need for soluble electron mediator, is achieved through periplasmic and outer membrane *c*-type cytochromes. The genome of *G. sulfurreducens* encodes 111 *c*-type cytochromes, which implies a significantly higher number of cytochrome genes than reported in any other organism whose sequence is available [17]. OmcS (Outer membrane cytochrome S) and, to a lesser extent, OmcE have been shown to be important in electron transfer to electrodes. OmcB, which is required for optimal electron transfer to Fe(III) oxide and Fe(III) citrate particles, was not required for electron transfer to a solid electrode [18]. Outer membrane proteins such as OmpJ and even some kind of conductive pili that serve as biological nanowires are also involved in the electron transfer chains, mainly to Fe(III) and Mn(IV) oxides [19]. Conductive pili do not seem to be absolutely required for electron transfer to solid anodes but may contribute to cell to cell electron transfer in thick biofilms [20,21]. This demonstrates the complexity of the electron transfer pathways, but also the variety of tools that such bacteria possess to implement direct electron transfer with particles and solid electrodes.

On the other hand, *G. sulfurreducens* has also been demonstrated to catalyse the reduction of nitrate to nitrite or fumarate to

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succinate with a graphite electrode serving as sole electron donor [22]. Recently, both the direct oxidation of acetate [16] and the direct reduction of fumarate [23] have been performed with *G. sulfurreducens* biofilm-coated stainless steel electrodes under constant polarization. *G. sulfurreducens* was thus demonstrated to be able to achieve efficient direct electron transfer with stainless steel material under constant polarization. Moreover, our last work has demonstrated that these reactions could drastically affect the electrochemical behaviour of 304L stainless steel coupons at open circuit [24]. A cathodic reaction occurred as soon as the bacterial cells came into contact with the material surface and provoked the ennoblement of the free potential by more than 300 mV in a few hours only and up to 443 mV after a few days. In contrast, well-established biofilms were shown to delay pitting of the material. Pitting curves recorded in the presence of mature *G. sulfurreducens* biofilms showed pitting potentials more than 200 mV higher than in the absence of bacteria. This effect has been attributed to the oxidation of acetate that occurs at anodic potential during the potential scan, shielding the material from pitting. To the best of our knowledge, this was the first time that such a mechanism of direct electron transfer between material surface and bacterial cells was clearly demonstrated in the field of corrosion. Direct electron transfer between material surfaces and microorganisms had been suggested only once previously in the framework of corrosion, with *Desulfobacterium*-like and *Methanobacterium*-like isolates extracted from natural biofilms [25].

Geobacter sulfurreducens, previously classified as a strict anaerobe, tolerates exposure to atmospheric oxygen for at least 24 h and can grow with oxygen as the electron acceptor. Growth on oxygen required that the cells be pregrown on fumarate and then inoculated in a medium low in fumarate (5 mM instead of 20 mM) with multiple additions of 5% oxygen [26]. *Geobacter* species may thus survive in oxic subsurface environments, being poised to rapidly take advantage of the development of anoxic conditions. These findings are important for corrosion which is known to be favoured by the presence of both oxic zones and anoxic zones. *Geobacter* being ubiquitous species in sediments and soils, the electrochemical effect they can induce on materials may be of crucial concern for the corrosion of buried industrial equipment such as off-shore and harbour structures, oil and gas pipes and buried storage tanks.

The purpose of this study is to assess the possible influence of *G. sulfurreducens* on the corrosion of different kind of steels. Mild steel 1145, ferritic steel 403 and stainless steels 304L and 316L were tested in the presence of *G. sulfurreducens* using electrochemical techniques and microscopy. Cells were first cultured in standard medium with a soluble electron donor (acetate) and soluble electron acceptor (fumarate). This culture was used to inoculate the electrochemical reactors, which contained the metallic coupons immerged in a solution with a lower concentration of electron donor (acetate). It was expected that lowering the concentration of soluble electron donor should unbalance the redox state of the bacterial cells and thus favour electron extraction from the material. Epifluorescent microscopy of the coupon surfaces helped in ascertaining the role of the bacterial biofilm.

2. Experimental

2.1. Metal sample preparation

Working electrodes were 2-cm-diameter cylinders made of either mild steel 1145, ferritic steel 403, or austenitic steels 304L or 316L (elemental composition by weight percentage given in Table 1) embedded in insulating resin (Resipoly Chrysor). The electrical connection was made through titanium wire protected with resin. Coupons were successively abraded using SiC papers of P120, P180, P400, and P800 grit (Lam Plan) and rinsed thoroughly with distilled water.

2.2. Microbiological culture and inoculation

Geobacter sulfurreducens strain PCA (American Type Culture Collection ATCC 51573) was purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen). The growth medium contained 28 mM NH_4Cl, 5 mM NaH_2PO_4, 1.3 mM KCl, 29.7 mM $NaHCO_3$, 10 mM sodium acetate (electron donor), 25 mM sodium fumarate (electron acceptor), 10 mL L^{-1} vitamin mix (ATCC MD-VS) and 10 mL L^{-1} trace mineral mix (ATCC MD-TMS) (pH 7.2). The bacteria were incubated in the growth medium under anaerobic conditions for 5 days at 30 °C. The number of planktonic cells was evaluated by measuring the absorbance or optical density (OD) at 620 nm. The absorbance was correlated to cell forming units per millilitre (CFU mL^{-1}) by calibration formula [11]:

$$[\text{CFU mL}^{-1}] = \text{OD}_{620\text{nm}} \times 472,067$$

The reactor medium contained the same components as the culture medium but with lower concentrations of acetate. Experiments were carried out in 0.5 L electrochemical reactors, with continuous N_2/CO_2 (80/20) bubbling, at 30 °C for optimum bacterial growth. The electrochemical reactors were inoculated at time t = 24 h with 5% v/v or 0.5% v/v bacterial culture containing 142,000 CFU mL^{-1} or 14,200 CFU mL^{-1}. At the end of the experiments, the amount of acetate was measured using an enzymatic kit (Essigsaure (Acetate), R-Biopharm).

2.3. Electrochemical measurements

All electrochemical measurements were carried out in 0.5 L electrochemical reactors with a three-electrode system. Working electrode potentials were referred to an Ag/AgCl reference electrode. The total chloride concentration in the culture medium was 29.3 mM. In the conditions of the study, the potential of the reference was E = 0.31 V vs. SHE. A platinum grid served as the counter electrode. Measurements of open circuit potentials (E_{oc}) were made and pitting curves were plotted using a multipotentiostat (VMP-Bio-Logic). Pitting curves were recorded at 0.5 mV s^{-1}. The potential scan was reversed when the current reached the value of 0.1 mA, which was considered to correspond to the formation of stable pits. Pitting potential (E_{pit}) was taken to be equal to this upper potential value. The repassivation potential (E_{rep}) was determined when the current returned to zero during the reverse potential scan.

2.4. Microscopy methods

At the end of the experiment, the electrodes were removed from the reactors and stained with a solution (0.03% w/w) of acridine orange (A6014, Sigma).

2.4.1. Scanning electron microscopy

Scanning electron microscopy (SEM) pictures were taken using a LEO 435 VP-Carl Zeiss SMT at 1000× and 1000 K× magnification

Table 1
Chemical composition of the steels (wt.%).

Alloy	Ni	C	Mn	Cu	Si	S	P	Mo	Cr
1145	0.1	0.46	0.65	0.11	0.31	0.032	0.01	0.02	0.1
403	–	⩽0.08	⩽1	–	⩽1	0.03	0.04	–	11.5/13.5
304L	9.68	0.02	1.43	0.35	0.35	0.03	0.03	0.40	18.26
316L	10.69	0.03	1.41	0.33	0.33	0.02	0.04	2.10	17.09

working at 10 kV acceleration voltage. The depth of the pits was evaluated by the displacement of the lens required to focus the image on the periphery of the pit and then inside at its bottom.

2.4.2. Confocal laser microscopy

Epifluorescence images were taken with a confocal Leica laser SP2 microscope using an argon laser ($\lambda_{emission}$ = 488 nm) (magnification 400×). Images were treated with the LCS Light Leica software.

2.4.3. Light microscopy

A Carl Zeiss Axiotech 100 microscope was also used to analyse the electrode surfaces (magnification 100×) with a HAL 100 lamp. Images were acquired with a monochrome digital camera (Evolution VF) and processed with the Image-Pro Plus 5.0 software.

When indicated, the coupons were cleaned in order to remove the corrosion products before being imaged. The electrodes were cleaned in a solution containing 50% v/v HCl (36%) and 5 g L^{-1} corrosion inhibitor, hexamethylentetramine $C_6H_{12}N_4$, in an ultrasonic cleaning machine (Ultrasonic T950/H Prolabo) at ambient temperature for 30 s, followed by thorough rinsing with distilled water.

3. Results and discussion

*3.1. Influence of **Geobacter sulfurreducens** on different steels*

Geobacter sulfurreducens cells were cultured for five days following the standard procedure in ATCC culture medium that contained 25 mM fumarate as electron acceptor and 10 mM acetate as electron donor. The culture was used to inoculate 0.5 L electrochemical reactors, generally at 5% v/v, which corresponded to 7100 CFU mL^{-1} into the reactors. The metallic coupons were set up in the reactors 24 h before inoculation and kept under continuous N_2/CO_2 (80/20) flow. The electrochemical reactors contained the culture medium but with lower concentration of acetate, 1 mM instead of 10 mM, with the objective of unbalancing the redox state of the bacterial cells, forcing them to search for a new source of electrons on the material surface. The concentration of electron acceptor (fumarate) was kept the same as in the culture medium (25 mM). Four different materials were tested: mild steel, ferritic and austenitic 304L and 316L stainless steels.

3.1.1. Mild steel

Fig. 1 shows the variation of the open circuit potential (E_{oc}) as a function of time for eight separate experiments performed with mild steel. In this case only, the medium contained 1 mM of chloride instead of the usual amount (30 mM) in order to limit uniform corrosion and the experiments were stopped after only 48 h. Addition of 5% v/v *G. sulfurreducens* cells after 24 h induced an increase of the open circuit potential of around 0.32 V in less than 3 h. When only 0.5% v/v *G. sulfurreducens* cells were injected, E_{oc} increased more slowly, rising by around 0.22 V to stabilize after 4 h. In each case, the potential increased gradually over a few hours, indicating that it was not related to electrostatic changes in the double layer capacity. Electrostatic phenomena due to the injection of microbial cells near steel surfaces have already been identified, but the time scale of such phenomena is seconds [27]. The kinetics of E_{oc} increase observed here are fully consistent with bacterial adhesion on a solid surface, which requires a few hours to stabilize. Moreover, the rate of potential ennoblement is directly related to the amount of the bacteria injected. Control experiments were performed by injecting only the fresh culture medium or the bacterial inoculum after filtration through a 0.2 μm filter. This filtrate contained all the components of the bacterial inoculum except the bacterial cells. The control experiments did not show any change in E_{oc}. Potential ennoblement was consequently due to the contact of bacterial cells with the surface and not to a component released in the medium. The coupons removed from the reactors after 48 h presented significant uniform corrosion, both in the presence of bacteria and for control experiments. Coupons were cleaned to remove the corrosion products and imaged by microscopy (Fig. 2). Coupons coming from control experiments exhibited uniform corrosion features, and the polishing stripes had almost completely disappeared. In the presence of bacteria, the images showed more contrasted patterns, with large clear zones that seemed not to be significantly affected by corrosion, as the polishing stripes remained perfectly visible on them, and large dark zones which indicated marked corrosion attack. A similar corrosion pattern has already been observed in the case of microbial corrosion induced by SRB [5]. It has been attributed, in this case, to the microbial production of iron sulphide which catalyses the cathodic reaction of proton reduction. The corrosion occurred preferentially in the vicinity of the zones where iron sulphide deposited, that is to say where the bacterial cells developed. Similarly, here the presence of bacteria changed the corrosion pattern by grouping corrosion attack into large zones while, in contrast, the zones where the cells grew and set up cathodic electron transfer were protected against corrosion. Besides, other studies have showed that the presence of adhered microbial cells changed the corrosion patterns. The anaerobic bacterium, *Citrobacter freundii*, and the sulphate-reducing bacterium, *Desulfovibrio gigas*, have been shown to colonize non-randomly 316L stainless steel surfaces, leading to changes in alloy elemental composition in the surface film. Selective colonization by *C. freundii* resulted in significant depletion of chromium relative to nickel at grain boundaries. Significant depletion of iron relative to nickel in near-surface regions of the oxide film at grain boundaries was observed when colonized by a co-culture of *C. freundii* and *D. gigas*. These chemical changes may weaken the oxide film at specific locations allowing halides such as chloride ions greater access to the underlying bulk alloy thereby facilitating localized attack and pit formation and propagation [28]. Indeed, it was shown that SRB induced pitting in the form of large radial growth patterns on carbon steel [29] and triggered the breakdown of passive films on stainless steels in the form of micro-pitting corrosion [30,31].

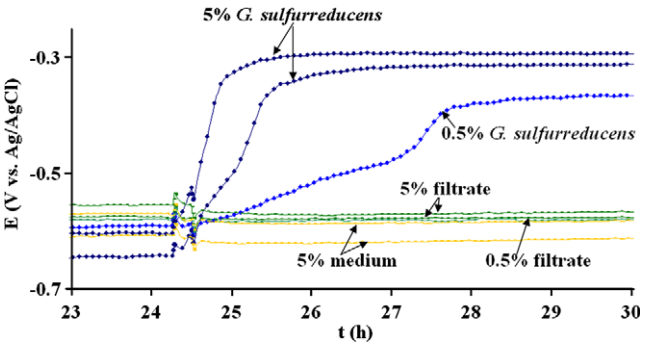

Fig. 1. Variation of the open circuit potential with time (first 30 h) for 1145 mild steel in the presence of *G. sulfurreducens* (5% and 0.5% v/v), 1 mM acetate; control experiments were carried out by injecting the culture medium only or the inoculum after filtration at 0.2 μm.

3.1.2. Ferritic stainless steel

Two independent experiments performed with ferritic steel 403 in the presence of 5% v/v *G. sulfurreducens* gave identical potential ennoblement values of around 0.35 V (Fig. 3). No E_{oc} increase was observed in the control experiment with 5% filtrate or 5% medium injected.

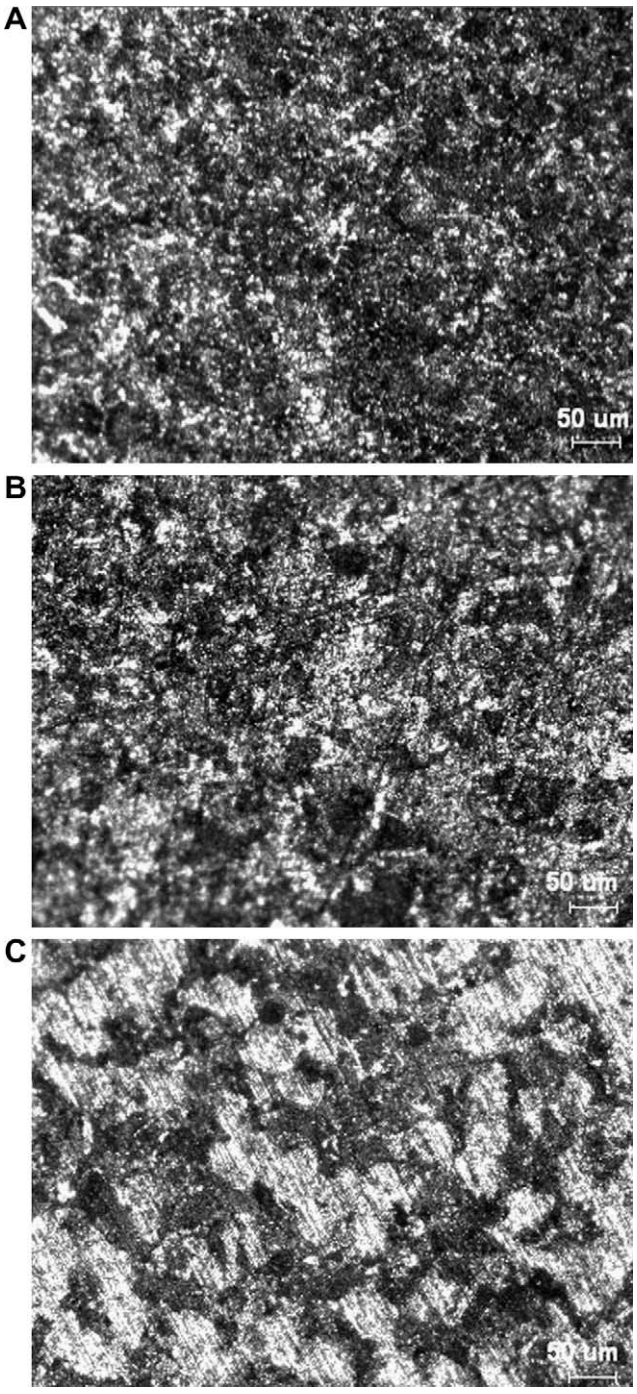

Fig. 2. Microscopy picture of 1145 mild steel coupons at the end of 48 h immersion, with injection at 24 h of 5% v/v of (A) fresh culture medium, (B) culture solution after filtration, (C) *G. sulfurreducens* culture solution (magnification 100×).

At the end of the experiment (350 h) the number of corroded zones is globally the same in the presence of the bacteria or with the filtrate injected, but the size and the distribution of these zones were different. The presence of *G. sulfurreducens* induces larger and deeper corroded areas that were grouped together whereas in the absence of the bacteria, small corroded areas were randomly distributed (Fig. 4). In the presence of 5% *G. sulfurreducens*, SEM micrographs shows large and deep corroded areas of 5.6 μm in diameter and 4.7 μm depth on average. Only smaller corroded areas appears on the control electrode (5% of filtrate), 1.8 μm in diameter and 2.3 μm depth on average.

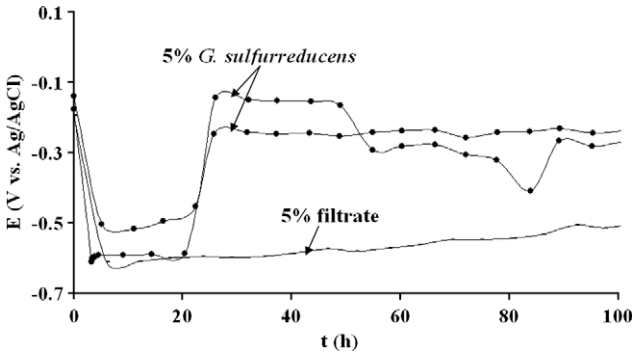

Fig. 3. Variation of the open circuit potential with time (first 100 h) for ferritic steel 403 in the absence and presence (5% v/v) of *G. sulfurreducens*; 1 mM acetate.

3.1.3. Stainless steels

Similar experiments were performed with 316L stainless steel. 5% v/v *G. sulfurreducens* provoked a fast E_{oc} ennoblement around ΔE_{oc} = 0.21 V, from −0.20 V to +0.01 V vs. Ag/AgCl, then E_{oc}

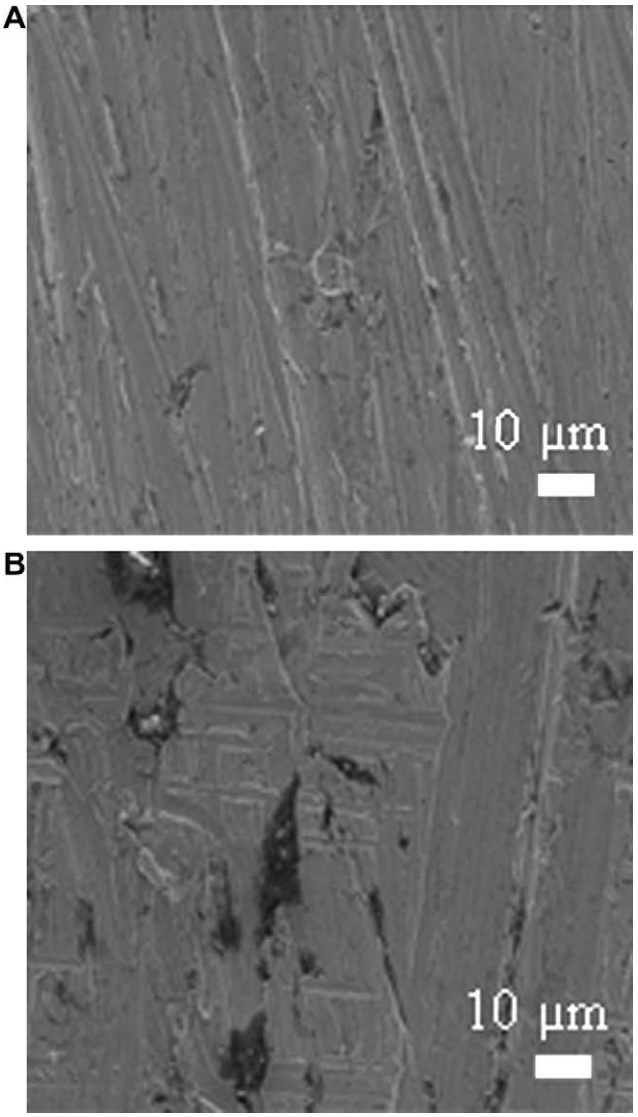

Fig. 4. SEM micrographs showing corrosion on ferritic steel after exposure in a medium containing 5% v/v of (A) culture solution after filtration, (B) *G. sulfurreducens*.

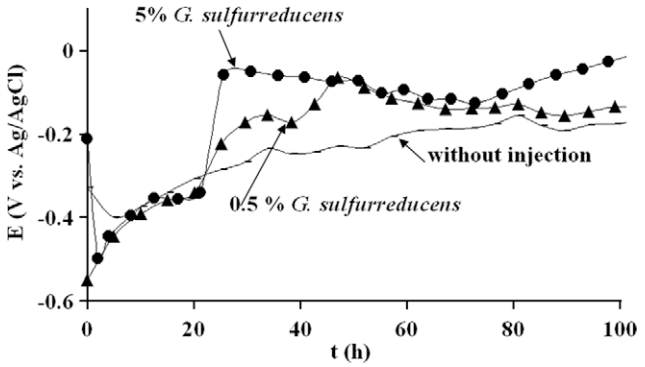

Fig. 5. Variation of the open circuit potential with time for 304L stainless steel in the absence and presence of *G. sulfurreducens* (5% and 0.5% v/v); 1 mM acetate.

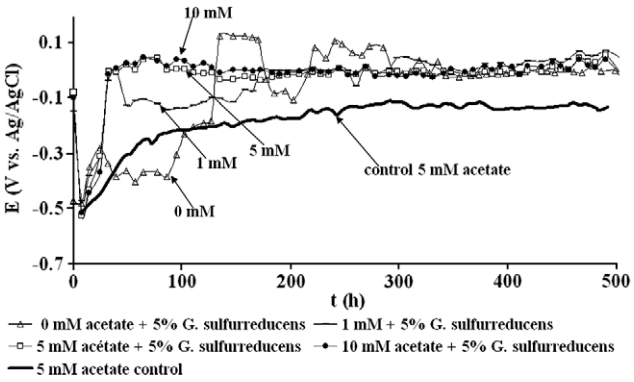

Fig. 7. Variation of E_{oc} of 304L stainless steel with time in the presence of 5% v/v *G. sulfurreducens* in a medium containing different concentrations of acetate.

Table 2
Potential ennoblement provoked by inoculation with 5% v/v *G. sulfurreducens*, 1 mM acetate; ΔE was measured 3 h after inoculation (27 h–24 h). Final E_{oc} is the open circuit potential value at t = 48 h for mild steel and at t = 150 h for ferritic steel and austenitic steels. Mild steel was not left longer in the medium because of generalized corrosion.

Types of steel	ΔE (V)	Final E_{oc} (V vs. Ag/AgCl)
Mild steel: 1145	0.31; 0.33	−0.29; −0.29
Ferritic steel: 403	0.35; 0.36	−0.29; −0.31
Austenitic steel: 304L (average value and standard deviation from 7 experiments)	0.20 ± 0.13	−0.02 ± 0.02
Austenitic steel: 316L	0.22	0.00

Table 3
Comparison of the potential ennoblement of 304L stainless steel for 3 h ΔE (24 h–27 h) and 126 h ΔE (24 h–150 h) after inoculation with 5% v/v *G. sulfurreducens* in a medium containing different concentrations of acetate. The minimum and maximum ΔE values are given for the number of experiments indicated in brackets. Absorbance and pH were measured at the end of the experiments (t = 500 h).

[acetate] (nb of experiments)	Min and max ΔE (24 h–27 h) V	Min and max ΔE (24 h–150 h) V	[CFU mL^{-1}]	pH
0 mM (3)	Fluctuations	Fluctuations	70,810	6.97
1 mM (7)	0.04; 0.37	0.29; 0.45	94,410	7.03
5 mM (7)	0.29; 0.34	0.39; 0.54	122,740	7.05
10 mM (1)	0.36	0.41	207,710	7.12

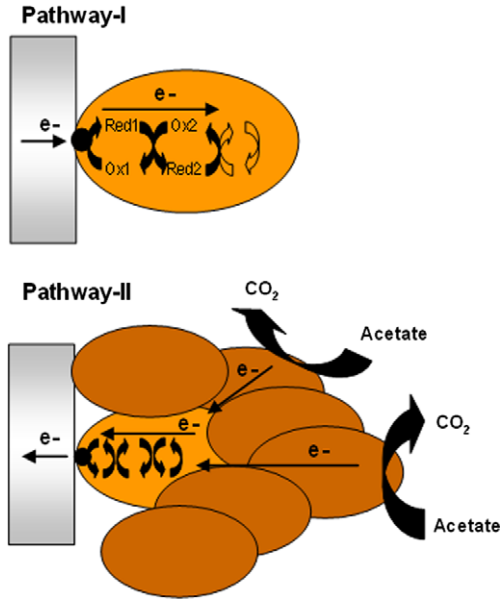

Fig. 6. Pathway I and II as described in the text [12].

fluctuated for days and finally stabilised around 0.00 V vs. Ag/AgCl. Seven experiments performed with 304L stainless steel in the presence of 5% v/v *G. sulfurreducens* gave the same general behaviour: for five experiments E_{oc} ennoblement in 3 h (from inoculation at time = 24 h to 27 h) was in the range between 0.21 V and 0.37 V; the other two experiments gave E_{oc} ennoblement of around only 0.04 V in 3 h but it reached 0.34 V 12 h after inoculation. Injection of 0.5% v/v *G. sulfurreducens* increased E_{oc} also by 0.34 V (from −0.33 V to +0.01 V vs. Ag/AgCl) but with slower kinetic, similarly to what was observed with mild steel. 50 h after inoculating, E_{oc} always reached similar values, regardless of the quantity of bacterial cells (5% or 0.5% v/v). No significant jump of E_{oc} was observed in the absence of bacteria but E_{oc} increased smoothly over several days in the control experiments (Fig. 5).

E_{oc} ennoblements recorded 3 h after inoculating (from t = 24 h to t = 27 h) are reported in Table 2 for the four materials tested. In the case of mild steel 1145 and ferritic steel 403, the free potential reached a stable plateau after t = 27 h. In contrast, the free potential fluctuated and reached stability only around t = 150 h in the case of 304L and 316L steels. Three hours after inoculation, the standard deviation for the potential ennoblements ΔE (24 h–27 h) obtained from different experiments was low for 1145 or 403 steels (of the order of +0.01), expressing fine reproducibility of the measures, while the deviation was one order of magnitude greater for austenitic steels (±0.1). The coupons were immerged

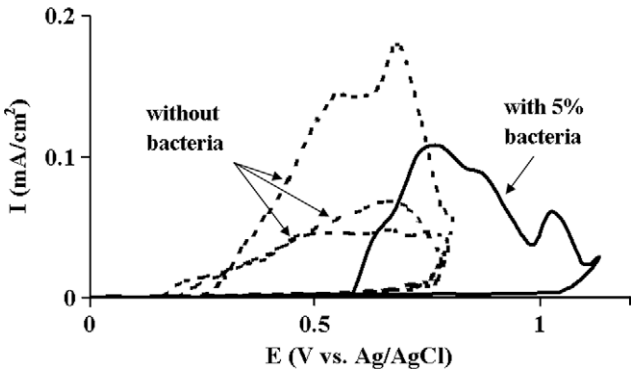

Fig. 8. Pitting curves (scan rate 0.5 mV s^{-1}) after 20 days' immersion in a medium inoculated with 5% v/v bacteria or in the absence of bacteria.

Table 4
Pitting potential (E_{pit}) and repassivation potentials of 304L stainless steel in the presence and absence (control) of *G. sulfurreducens* (5% v/v) with different concentrations of acetate. The average value, [the maximum and minimum values] and (the number of independent experiments) are given in each case.

[acetate]	E_{pit} *Geobacter* (V vs Ag/AgCl)	E_{pit} control (V vs Ag/AgCl)	$E_{repassivation}$ *Geobacter* (V vs Ag/AgCl)	$E_{repassivation}$ control (V vs Ag/AgCl)
1 mM	0.93 [0.84; 1.09] (3)	0.80 [0.79; 0.80] (3)	0.49 [0.39; 0.60] (3)	0.23 [0.17; 0.28] (3)
5 mM	1.09 [1.07; 1.11] (7)	0.84 [0.76; 0.95] (5)	0.64 [0.62; 0.65] (7)	0.35 [0.26; 0.47] (5)
10 mM	1.05 (1)	0.86 (1)	0.60 (1)	0.30 (1)

into the solution just after polishing. In the case of stainless steels the concomitant formation of the passive layer and its evolution was probably the source of the fluctuations of the free potential. At 150 h, when the free potential of stainless steels reached a stable plateau, the standard deviation decreased indicating stabilization in the passive layer: for seven independent experiments performed with 304L, ΔE (24 h–150 h) was 0.32 V with a standard deviation of only +0.06 V.

Explanations reported in the literature for potential ennoblement in aerobic environments are commonly based on the enhancement of the cathodic reaction of proton reduction. All experiments performed here showed a clear E_{oc} ennoblement. In the case of 304L stainless steel, this E_{oc} ennoblement has been explained in our previous work by the capacity of *G. sulfurreducens* cells to implement direct electron transfer from stainless steel. This reaction involves only the redox compounds that constitute the electron transfer chain of the cell and can thus be implemented as soon as the cells settle on the material surface (pathway I of the scheme of Fig. 6). The same phenomenon is demonstrated here to occur on mild steel and ferritic steel. This cathodic reaction results in two groups of values for the final E_{oc}, corresponding to mild steel and ferritic steel on one hand, and austenitic steels on the other hand (Table 2). The E_{oc} value results from the balance between the cathodic reaction (extracting electrons from the material) and the anodic process (providing the bulk material with electrons). The anodic process is controlled by free corrosion for

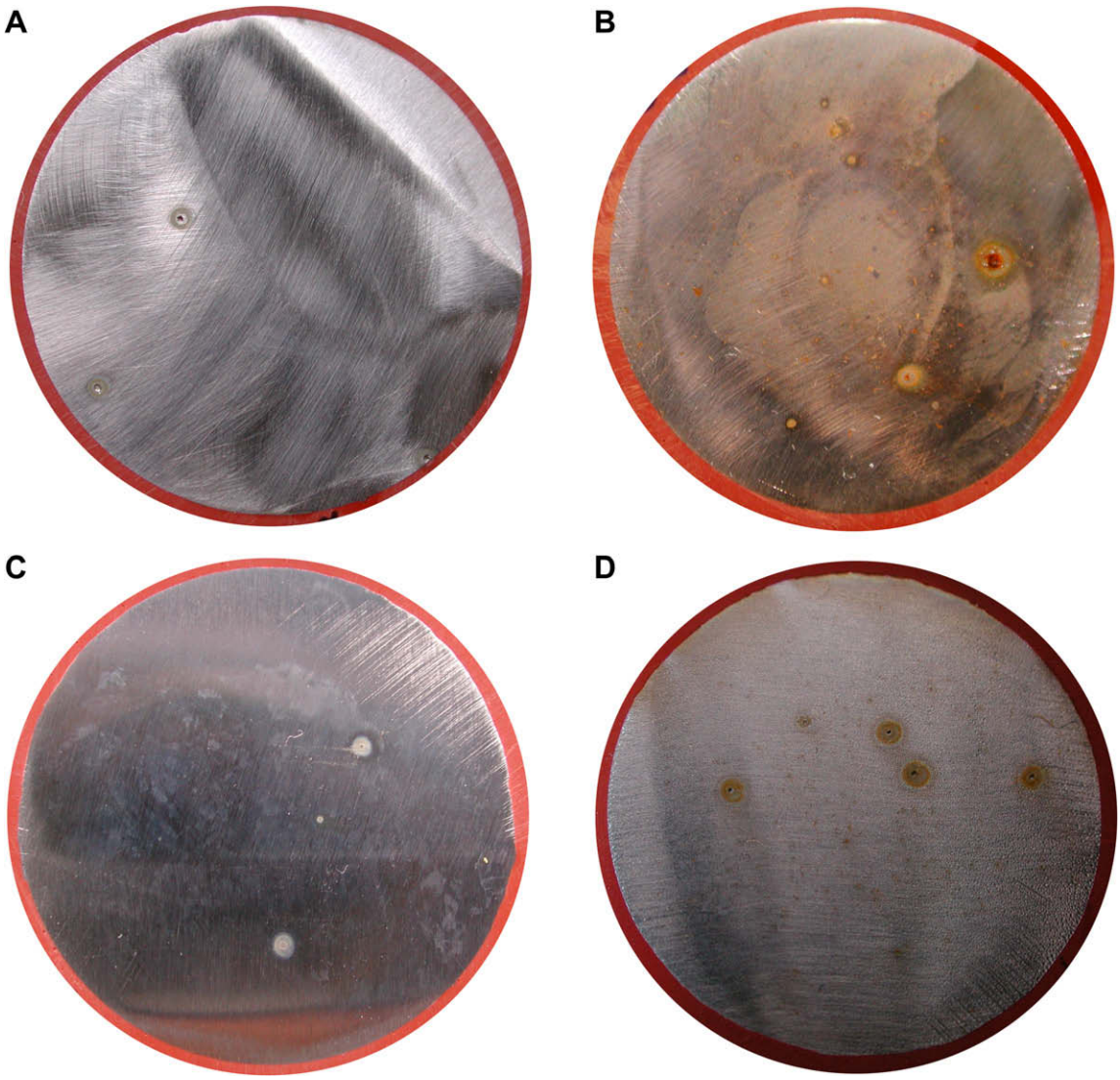

Fig. 9. 304L stainless steel coupons after 500 h immersion and after recording of the pitting curve; (A) acetate 1 mM no bacteria; (B) acetate 1 mM, 5% v/v *G. sulfurreducens*; (C) acetate 5 mM no bacteria; (D) acetate 5 mM, 5% v/v *G. sulfurreducens*.

mild and ferritic steels, and it is due to the development of the passive layer for 304L and 316L. The anodic currents linked to free corrosion have higher values than the currents related to a passive layer. The cathodic reaction created by the presence of *G. sulfurreducens* induced the greatest shift of E_{oc} towards positive values for the materials that had the smallest anodic current. This is fully consistent with the different values of the final E_{oc} recorded here.

The *G. sulfurreducens*-driven cathodic reaction was not strong enough to initiate corrosion of austenic steels in the conditions of the experiment. In contrast, *G. sulfurreducens* shows here that it is able to modify the corrosion features of mild steel and to increase local corrosion of ferritic steel. It can be concluded that the cathodic reaction can be created by *G. sulfurreducens* cells independently of the kind of steel, with important consequences on corrosion in the case of less alloyed steels.

3.2. Influence of acetate concentration on the electrochemical behaviour of 304L stainless steel

The impact of varying the concentration of the electron donor (acetate) was studied on 304L stainless steel in the presence of 5% v/v *G. sulfurreducens* (Fig. 7).

When the medium did not contain any acetate (0 mM), no potential ennoblement was observed in the three hours following the injection of bacterial cells, but E_{oc} fluctuated with no reproducible pattern. Actually, it was very difficult for the bacteria to grow in these very stressful conditions. In fact a very low concentration of acetate was present due to the amount that had not been consumed in the volume of the inoculum. This low concentration was enough for the cells to survive, as confirmed by the measurement of CFU at the end of the experiment (Table 3), but the stress induced required several days acclimation, before an effect could be observed on the E_{oc}. With 1 mM acetate in the medium, the potential ennoblement was slower than with higher concentrations nevertheless, after 150 h, E_{oc} values were similar whatever acetate concentration. Even with 10 mM acetate, the potential increase was identical, indicating that it was not absolutely necessary to diminish the concentration of electron donor and provoke an imbalance in the redox state of the cells to promote a cathodic reaction. Absorbance and pH were measured at the end of each experiment. Absorbance and pH increases were directly related to planktonic bacterial growth in the reactors. With 1 mM acetate, planktonic growth was not significantly higher than without acetate but it was sufficient to stabilize the electrochemical results. Planktonic growth directly depended on the acetate concentration, which confirmed a limitation related to the electron donor. In contrast, varying the acetate concentrations from 1 mM to 10 mM did not affect the electrochemical behaviour of the material. E_{oc} increase was consequently not directly controlled by the planktonic bacterial population.

At the end of each experiment, pitting curves were recorded at 0.5 mV s^{-1}. Fig. 8 presents an example of the pitting curves obtained after 20 days' immersion in the absence and in the presence of bacteria with 1 mM acetate. As observed in Fig. 8, and confirmed by all experiments reported in Table 4, the presence of the bacteria delays the occurrence of pitting. Varying the concentration of acetate from 1 mM to 10 mM does not have any significant effect on E_{pit}. It has been shown that *G. sulfurreducens* biofilms catalyse the electrochemical oxidation of acetate on stainless steel anodes polarized at potentials higher than around 0.2 V vs Ag/AgCl [12]. Actually, the bacterial cells that settle first on the surface can achieve direct electron transfer with the material. At low potential value this process leads to the cathodic reaction that was observed here at open circuit. The bacterial colonies that further develop around the first-settled cells can use acetate as electron source and, when the potential of the material is high enough, release the electron to the material through the first-settled cells (Pathway II of Fig. 6) [12]. Here, this reaction occurred during the potential scan and gave a supplementary source of electrons that changed the behaviour of the passive layer at high potential and resulted in delaying the pitting potential for more than 0.2 V. The oxidation reaction also helps the passive layer to rebuild during the reverse potential scan, resulting in an increase of the repassivation potential of 0.2 V with low acetate concentrations and up to 0.3 V with the higher acetate concentrations (Table 4). For both pitting and repassivation phenomena, the occurrence of the biofilm-catalysed acetate oxidation finally results in a significant shield effect against corrosion.

Pictures of the coupons after the pitting experiments showed a few small pits in the absence of bacteria (Figs. 9A and C) while more marked pits were observed on the coupons immersed in the presence of bacteria (Figs. 9B and D), especially in the medium

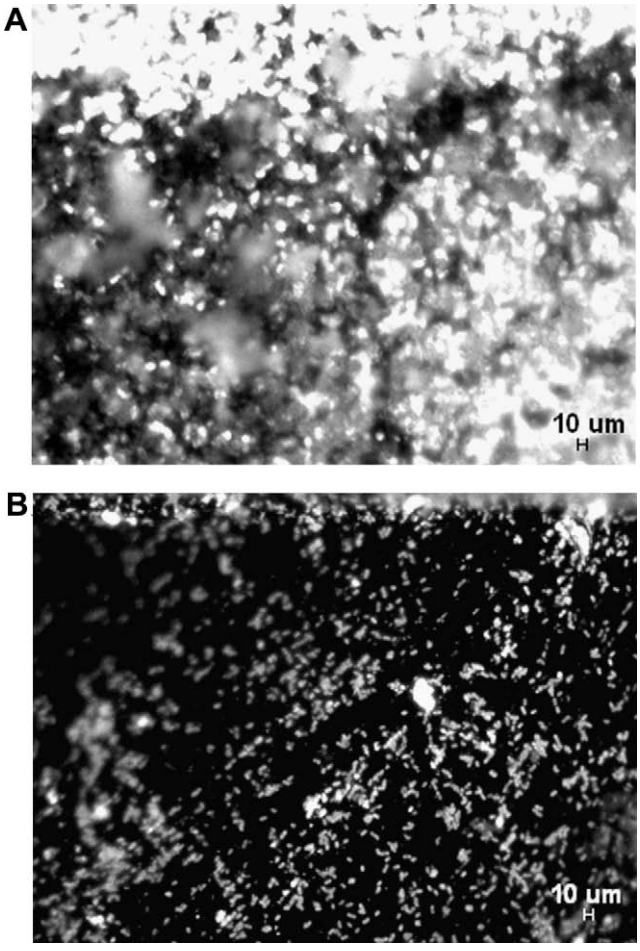

Fig. 10. Epifluorescence microscopy of the same 304L stainless steel electrode after 240 h in the presence of 5% v/v *G. sulfurreducens*, in a medium containing 5 mM acetate; (A) dense biofilm in the vicinity of a pit; (B) few bacteria randomly distributed in the zones free from pitting. (Magnification 100×).

Table 5
Ratio of the surface area covered by adherent cells in the vicinity of pits and in zones away from pits. Each value is the average of twenty different spots. Same samples as in Table 4.

[acetate]	Vicinity of a pit	Away from pits
1 mM	61 ± 19	16 ± 7
5 mM	64 ± 17	16 ± 7
10 mM	60 ± 29	16 ± 3

that contains 5 mM acetate. This difference may be explained by the higher potential values at which pitting finally occurred in the presence of bacterial cells. The presence of *G. sulfurreducens* delays the pitting potential by more than 250 mV but, in turn, when the passive layer is finally disrupted, pitting finds higher energy available for its propagation.

Observing the coupons by epifluorescence microscopy showed that pitting always occurs in zones that exhibits dense biofilm coverage (Fig. 10A), whereas the zones with only a few, randomly distributed bacteria are free from significant pitting (Fig. 10B). The epifluorescence pictures were numerically processed to measure the surface coverage ratio of bacterial cells. Each value given in Table 5 is averaged on 20 different spots on each coupon. The existence of two distinct types of bacterial settlement is confirmed, with dense zones that exhibits a biofilm coverage ratio higher than 60%, and zones presenting only scattered cells with a coverage ratio of approximately 16%. The less covered surface zones seem to result from cells that adhere to the surface and do not develop further. In contrast, the densest zones clearly show biofilm development with formation of a matrix of exopolymeric substances (EPS). The concentration of acetate from 1 mM to 10 mM does not affect the coverage features. This is in full agreement with the independence of the potential ennoblement with regard to acetate concentration (Table 4). The electrochemical behaviour of the material was not affected by the concentration of planktonic cells, which depended on acetate concentration (Table 3), but it confirmed to be controlled by the adherent cells, the configuration of which was not affected by acetate concentration (Table 5).

SEM pictures show large, deep pits and confirm the presence of bacteria in their close vicinity (Fig. 11), whereas no pits are observed far from the zone of dense biofilm coverage. The presence of *G. sulfurreducens* biofilm delays the occurrence of pitting but pit propagation proves to be intimately linked to the densest bacterial coverage: the significant pits are always observed in the vicinity or beneath the dense biofilm zones. Pitting does not occur in the same range of potential values in the control experiments and in the experiments performed in the presence of bacteria, it is consequently not possible to choose formally between two different hypotheses to explain the link between biofilm and pitting location:

i) either the biofilm really promotes pit propagation, in this case it would be directly responsible for pitting propagation in its vicinity,
ii) or the biofilm only develops preferentially on the sites that are then the most sensitive to pit propagation, in this case the bigger pits observed in the presence of bacteria would only be due to the highest potential values that were reached, the biofilm only detects the zones that are the most sensitive to further corrosion, because of surface defects or inclusions for example.

4. Conclusions

Geobacter sulfurreducens cells adhering to the surface of the steels induce a free potential ennoblement of the order of 0.35 V in only a few hours on 1145 mild steel and 403 ferritic steel, and of 0.35 V on 304L and 316L austenitic steels. The kinetics of E_{oc} ennoblement on the first hours following bacterial injection depends on the amount of bacteria in the inoculum, but the final potential value depends mainly on the nature of the material. The corrosion features of mild steel and ferritic steel are modified by the presence of bacteria with corrosion attacks gathered into determined zones of the material surface. Local corrosion is significantly enhanced on ferritic steel. Potential ennoblement is not sufficient

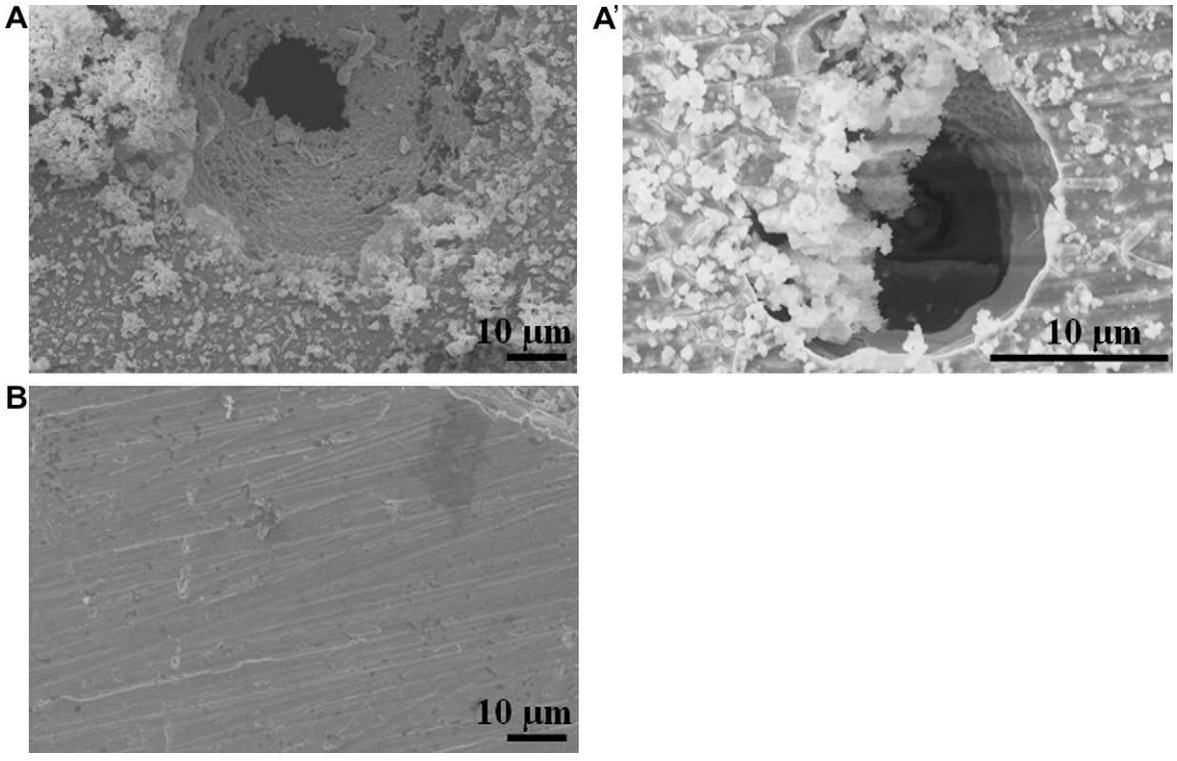

Fig. 11. SEM picture of the same 304L stainless steel electrode after 240 h in the presence of 5% v/v *G. sulfurreducens* in a medium containing 5 mM acetate, showing a high biofilm coverage in the vicinity of pits (A, A') while only scattered bacteria cells are observed far from pits (B). Magnification 1000× for pictures (A and B) and 1000 K× for pictures A'.

to induce corrosion on austenitic steels. In contrast, during the pitting polarization test, the capability of the mature *G. sulfurreducens* biofilms to oxidize acetate at high potential values modifies the behaviour of the passive layer resulting in delaying the occurrence of pitting and improving repassivation. Nevertheless, the propagation of pits proved to be intimately linked to the local presence of dense biofilm on the steel surface.

It is demonstrated here that *G. sulfurreducens* plays a major role in the control of the electrochemical behaviour of different kinds of steels, with different mechanisms at free corrosion potential and at more positive potential values. It modifies the corrosion features for the less alloyed steels and changes the resistance to pitting and the pit propagation rate for austenitic steels. As *Geobacter* sp. are ubiquitous species in sediments and soils, with the capability for some species to survive in oxic zones, they should now be considered as a serious source of microbial corrosion for buried equipment. Their presence should now be sought in any field case dealing with microbial corrosion of steels in sediments or soils.

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