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# Biocorrosion of carbon steel alloys by an hydrogenotrophic sulfate-reducing bacterium *Desulfovibrio capillatus* isolated from a Mexican oil field separator

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#### Abstract

The hydrogenotrophic sulfate-reducing bacterium (SRB) *Desulfovibrio capillatus* (DSM14982<sup>T</sup>) was isolated from an oil field separator with serious corrosion problems; this is the study of its role in the corrosion of carbon steels under anaerobic conditions. Immersion tests with two steel alloys, St-35.8 (typical carbon steel employed in European naval industry), and API-5XL52 (weathering alloy steel employed in Mexican oil industries) were performed. Total exposure was 45 days and different concentrations of thiosulfate as electron acceptor for bacterial growth were employed. The samples immersed in media with SRB undergo fast activation and numerous active sites form on the surface. Microscopic observations were made by environmental scanning electron microscopy (ESEM). Weight loss and electrochemical testing included open circuit potential ( $E_{corr}$ ), polarization resistance ( $R_p$ ), electrochemical impedance spectroscopy (EIS) and electrochemical noise (EN) were measured with and without bacteria in the culture medium in order to determine corrosion rates and

\* Corresponding author. Tel.: +34 956 016 154; fax: +34 956 016 746. *E-mail address:* manuel.bethencourt@uca.es (M. Bethencourt). mechanisms. All electrochemical techniques have shown that after the end of the exponential phase the corrosion activity notably increased due to the high concentration of bacterial metabolites. Finally, the corrosion behavior of API-5XL52 was worse than St-35.8. © 2005 Elsevier Ltd. All rights reserved.

Keywords: Carbon steel; Biocorrosion; Sulfate-reducing bacteria; Electrochemical impedance spectroscopy; Electrochemical noise analysis

## 1. Introduction

Biocorrosion or microbially influenced corrosion (MIC), is the damage caused or accelerated by the presence of bacteria and other microorganisms and their metabolic activities. The main types of bacteria associated with metals in terrestrial and aquatic habitats are sulfate-, iron- and CO<sub>2</sub>-reducing bacteria, sulfur-, iron- and manganese-oxidizing bacteria [1]. Among them, sulfate-reducing bacteria are recognized as a major group of microorganisms linked to anaerobic corrosion. These latter microorganisms can coexist in naturally occurring biofilms with a wide bacterial community including fermentative bacteria, often forming synergistic communities (consortia) that are able to affect electrochemical processes through co-operative metabolism [2].

The biocorrosion process may be recognized by a combination of observations: corrosion, presence of microbial slime masses, presence of hydrogen sulfide and ferrous or ferric hydroxide as observed in anaerobic systems [3]. This process is of considerable concern to the power-generating, chemical-processing, oil and shipping industries as well as to the military. Bacterial activity and mainly sulfate reductive activity, is thought to be responsible for >75% of the corrosion in productive oil wells and for >50% of the failures of buried pipelines and cables [4].

In the petroleum industry, engineers have faced problems caused by microorganisms, since the beginning of commercial oil production. A wide variety of bacteria have been isolated or detected in these environments by molecular techniques, but because of their detrimental effects, sulfate-reducing bacteria (SRB) have been the most commonly studied group. The SRB presence in oil environments was detected in 1926 [5] and was rapidly recognized as responsible for the production of hydrogen sulfide, which is a toxic and corrosive gas responsible for a variety of environmental and economic problems including reservoir souring, contamination of natural gas and oil, corrosion of metal surfaces, and the plugging of reservoirs due to the precipitation of metal sulfides and the consequent reduction in oil recovery [6,7]. Beside sulfide production from sulfate reduction by SRB, most of them are also known to reduce thiosulfate into sulfide. This latter process, performed not only by SRB, was reported as a major risk factor increasing biocorrosion processes [8,9]. The SRB are most probably a subject of preoccupation in the oil industry not only because of sulfide production, but also due to their ability, for some of them, to (i) oxidize hydrogen, (ii) use  $O_2$  and  $Fe^{3+}$  [10,11], (iii) their capacity for aliphatic and aromatic hydrocarbons utilization [12], (iv) their capacity to connect sulfate reduction to the magnetite intracellular production [13] and (v) their aptitude to compete with nitrate-reducing/sulfur-oxidizing bacteria (NRB-SOB) since they may have a nitrite reducing activity [14].

Diverse mechanisms of performance have been described to explain the SRB contribution in the corrosion processes, which include: cathodic depolarization, anodic depolarization, sulfide production and extracellular polymeric substances (EPS) [2]. A wide variety of microscopic and electrochemical techniques, have been reviewed by several authors in relation to their use in biocorrosion evaluation [15,16]. However, it is necessary to take particular care with the interpretation of data supplied by electrochemical methods, because biocorrosion is rarely interpreted by a single mechanism or seldomly caused by a single species of microorganisms. It is rather a complex process in which an infinity of factors intervene, and generally it is necessary to employ other types of additional tools. Recent progress in microscope (ESEM) and confocal laser microscope (CSL) have allowed biofilm observation in real time and without introducing an alteration of the samples. There is an increasing number of references using these innovative technologies in recent biocorrosion literature [1,17–19].

Most of the basic theories on electrochemical corrosion are valid in biocorrosion and could be employed to interpret the acceleration of the corrosion process by microorganisms in different media both in anaerobic or aerobic conditions. The main difficulties in understanding the phenomenon arise from the complexity of the chemical composition in cultures of sulphate-reducing bacteria. Apart from chloride-ions, sulphide present in SRB cultures is of particular importance in pitting formation.

The SRB *Desulfovibrio capillatus* is a hydrogenotrophic microorganism isolated from an oil field separator in the Gulf of Mexico which had undergone biocorrosive damage. Besides hydrogen, it oxidized a limited range of substrates including lactate and pyruvate. However *D. capillatus* may use a wide range of sulphur compounds (thiosulfate, sulphate, sulphite and elemental sulphur) as electron acceptors, which are reduced to sulphide. It is slightly halophilic, growing optimally at NaCl concentration of 3%, thus suggesting its marine origin [20]. Cells of *D. capillatus* tend to aggregate during exponential growth [20] and this physiological trait might facilitate *D. capillatus* to form biofilms. This bacterium was therefore studied for its ability to corrode carbon steel alloys in the presence of thiosulfate as the electron acceptor, which was shown to increase its growth and biocorrosion risks [8,9].

### 2. Materials and methods

*D. capillatus* was isolated from a water/oil mixture from Samaria III oil field separator with serious corrosion problems, near of the Gulf of Mexico [20]. The immersion tests were conducted in 60 mL glass vials at 40 °C, in anaerobic medium containing (per L distilled water) 1 g NH<sub>4</sub>Cl, 0.3 g K<sub>2</sub>HPO<sub>4</sub>, 0.3 g KH<sub>2</sub>PO<sub>4</sub>, 0.1 g KCl, 0.1 g CaCl<sub>2</sub> · 2H<sub>2</sub>O, 0.5 g MgCl<sub>2</sub> · 6H<sub>2</sub>O, 30 g NaCl, 0.5 g cystein–HCl, 2 g yeast extract, 1 mg resazurin, and 10 mL trace mineral element solution [21]. The pH was adjusted to 7 with 10 M KOH and the medium was boiled under a stream of O<sub>2</sub>-free N<sub>2</sub> gas and cooled to room temperature. Forty milliliter aliquots were dispensed in serum bottles, under a stream of N<sub>2</sub>–CO<sub>2</sub> (80:20, v/v) gas. Once metallic samples were introduced, the vessels were sealed and autoclaved for 45 min at 110 °C. Prior to bacteria inoculation, NaHCO<sub>3</sub> was injected from anaerobic sterile stock solutions to final concentrations of 0.02% (w/v). Lactate (20 mM) as electron donor and sodium thiosulfate (0 and 20 mM) as electron acceptor were likewise introduced. Sterile abiotic mediums with both concentrations were used as

control to distinguish the effects of the culture medium and the electron acceptor in the biocorrosion process.

Samples of two steel alloys: St-35.8 (a typical carbon steel alloy employed in European naval industry), and API-5XL52 (a weathering steel alloy employed in Mexico oil facilities). Their compositions were determined using a Spectrometer (Spectrolab Jr model Spark) (Table 1). The samples used for immersion tests were sized  $10 \times 10 \times 1$  mm<sup>3</sup>. They were perforated, sanded with SiC paper of 80, 120, 220 and 500 grit, rinsed with distilled water, degreased using acetone, dried in a current of air, and weighed in an analytic balance (±0.1 mg). Total areas were measured with a digital gauge (Mahr model 16ES) (±0.1 mm). Sixty-four coupons of both alloys were exposed during 7, 15, 30 and 45 days to assess the microbial growth on the coupons and the corrosion phenomena.

Corrosion products were observed and analyzed by SEM–EDS in an environmental electron microscope QANTA 2000. They were then eliminated by immersion of the samples in hydrochloric acid–hexamethylene tetramine for 5–10 min. General damage was observed by SEM–EDS and in a metallographic microscope (Nikon-104), and the final weight was measured after hydrochloric acid immersion.

The electrochemical measurements were made in an ASTM cell, coupled to a potentiostat (Solartron-1287). An Ag/AgCl electrode (Crison 52-40) was used as the reference electrode (0.207 V vs NHE). Once filled, the electrochemical cell was purged with nitrogen to maintain an anaerobic environment. Because of the long-term nature of the study, nondestructive electrochemical techniques have been employed. Open circuit measurements of corrosion potential ( $E_{corr}$ ) was selected. Monitoring  $E_{corr}$  can yield qualitative information on the contribution of anodic and cathodic processes to the observed mixed potential [22]. In addition, lineal polarizations were performed regularly to determine the polarization resistance ( $R_p$ ), with amplitude of  $\pm 10$  mV around the corrosion potential. The polarization rate was 10 mV min<sup>-1</sup>.  $R_p$  is a good indicator of the overall rate of electrochemical activity on metal surface [23].

The impedance spectra were obtained using the potentiostat coupled to a Solartron 1255 FRA. Both devices were controlled by a PC through the CorrWare and Zplot Scribner programmes. In general, the amplitude of the AC signal applied was 5 mV. The Fit-Engine routine of the equivalent circuit option of the ZView Scribner program was used to construct the equivalent circuit models. Frequencies sweep the range from  $10^4$  Hz to  $10^{-3}$  Hz.

EN records, containing 2048 data points, were collected at 2.16 points per second. Both potential and current noise signals were measured simultaneously, at open circuit potential, using the potentiostat Solartron SI 1287 controlled by CorrWare software. In EN, two working electrodes are necessary which were made from the same alloy plate. The area of each of these electrodes was 5.1 cm<sup>2</sup>.

## 3. Results and discussion

Curves of weight loss vs time of samples of API-5XL52 and St-35.8 alloys, immersed in different culture media, were recorded (Fig. 1). In both cases, clear differences can be noted between the samples exposed to medium with bacteria and thiosulfate and those immersed in sterile medium or in medium with bacteria but without the electron acceptor. Thus, the average slope of the trace of the first treatment in API-5XL52 and St-35.8 was about three times that observed in others. Both alloys in medium with bacteria and thiosulfate showed

Table 1   Composition (%) of St-35.8 and API-5XL52 alloys																	
Element	С	Si	Р	S	Mn	Al	Cr	Мо	Ni	V	Co	Nb	Ti	Ν	Cu	Sn	Fe
% St-35.8 % API-5XL52	0.126 0.18	0.188 0.45	0.017 0.025	0.009 0.020	0.667 1.4	0.028 0.03	0.023 0.30	0.001 0.10	0.009 0.30	0.011 0.05	0.011	0.04	0.03	0.012	0.020 0.25	0.008	rest rest



Fig. 1. Weight loss-time curves of full immersion of St-35.8 and API-5XL52.



Fig. 2. SEM image of deposits on the surface of API-5XL-52 alloy and EDS spectra of these deposits, at: (a) 7 days and (b) 45 days of immersion in medium with bacteria and thiosulfate.

quite different plots for the whole exposure time. API-5XL52 steel showed worse behavior than St-35.8. In all cases, the addition of bacteria together with the optimal concentration



Fig. 3. SEM micrograph of typical pits after removing the corrosion products in API-5XL52 alloy at (a)  $2000 \times$  and (b)  $6000 \times$ ; 45 days of immersion in medium with bacteria and thiosulfate.

of electron acceptor resulted in a significant increase in corrosion rates. Minimal variations in the pH of the culture medium were observed at the end of the experiments ( $\pm 0.2$  units).

After the immersion tests, samples of both alloys were studied using light microscopy, SEM and EDS. Deposits on the surface of API-5XL52 samples between 7 and 45 days of immersion were observed in coupons with bacteria and 20 mM sodium thiosulfate (Fig. 2(a) and (b)). The EDS reveals that this deposit mostly consisted of Fe and S (probably iron sulfide). Bunches with similar composition were observed in St-35.8 alloy as well.

After removal of the corrosion products, similar sized pits appeared on the surface of both alloys (Figs. 3 and 4). Pits density was greater in API-5XL52 than in St-35.8 (Fig. 3(a) vs Fig. 4(a)). These results were correlated with weight losses showed in Fig. 1.

In order to identify the cause of the relatively high weight loss found in API-5XL52 steel, which is generally used in the Mexican oil industry, additional electrochemical measurements were made with this alloy.  $E_{\rm corr}$  measurements were monitored continuously during 7 days in sterile culture medium with sodium thiosulfate (20 mM) (M1) and culture medium with the same acceptor concentration and SRB (M2) (Fig. 5).



Fig. 4. Typical pits seen after removal of corrosion products in St-35.8 alloy at (a)  $2000 \times$  and (b)  $6000 \times$ ; 45 days of immersion in medium with bacteria and thiosulfate.



Fig. 5. Ecorr evolution with and without bacteria.

In either case, during the first 48 h, marked diminutions in the potential were observed, until reaching a stable  $E_{corr}$  about -550 mV vs Ag/AgCl in M1 and -570 mV vs Ag/AgCl in M2. The fluctuations were more evident in M2, with a steep drop between the first 40–54 h. This drop could have resulted from: (a) establishment of a biofilm, (b) metabolic processes associated with microbial colonization and growth (i.e. production of metabolites that influenced the electrochemical behavior at the metal surface) or (c) establishment of a cathodic reaction whose reversible potential was low [24].

During the first 48 h of incubation, *D. capillatus* formed a biofilm on the work electrode. This is the same theoretical time needed for this strain to reach the end of the exponential growth phase, when grown in the absence of iron on media containing lactate as



Fig. 6.  $R_p$  evolution with and without bacteria in medium.

electron donor and thiosulfate as electron acceptor. Interestingly, thiosulfate is highly reduced to sulfide by *D. capillatus* at the end of this exponential growth phase thus suggesting that corrosive activity could act before significant sulfide production by this bacterium. Recently it has been shown than some strains of SRB are able to oxidise metallic iron using sulfate [11] as an electron acceptor but, in this respect, the direct oxidation of Fe(II) by *D. capillatus* is not confirmed. It is unknown at present how widespread this metabolic pathway is.

 $E_{\rm corr}$  was typically 20 mV lower in M2 than M1, showing a slightly higher electrochemical activity on the metal surface probably due to the biological activity. Between 24 and 48 h in experiment M2, a thick biofilm covered the coupon was observed. The reduction reactions could be modified by the transformation of thiosulfate to H<sub>2</sub>S, due to the generation of an acid microenvironment within the biofilm and in contact with the metal surface.

Examination of  $R_p$  data obtained in M1 and M2 media during 7 days revealed a direct influence of microbial activity (Fig. 6). Samples in M2 reached consistently lower  $R_p$  values than for similar samples within the control sterile medium (about 200  $\Omega$  cm<sup>2</sup> in M2 and 1000  $\Omega$  cm<sup>2</sup> in M1).

Several general statements could be made. First, the microenvironment at the metalliquid layer could have been altered by the bacterial films via accumulation of a number of organic by-products such as organic acids (i.e. acetate) and hydrogen sulfide [25]. The change in aggressiveness of the solution at the metal surface was reflected in a decrease in  $E_{\rm corr}$  accompanied by a decrease in  $R_{\rm p}$ . Based upon observed trends (decreasing  $E_{\rm corr}$ and  $R_{\rm p}$ ), bacteria may have enhanced the anodic reaction. However, it was unclear what portion of this change resulted in an increase of corrosion and what portion was due to other redox couples promoted by the bacteria.

EN measurements were carried out to compare with the  $R_p$  results. The EN technique shows the mechanism and determines the rate of corrosion reaction [26]. In addition, EN



Fig. 7. Averaged density current from ENM of API-5XL52 alloy, in function of the exposure time in presence (M2) and absence (M1) of bacteria.

is a non perturbing technique which does not produce modifications in the corrosion potential. This technique is specially suitable to measure the activity of systems showing considerable corrosion rates, such as the system studied in this paper.

The parameters studied were: the current density flowing between the two working electrodes (I), and the noise resistance ( $R_n$ ). Although I is theoretically associated with the asymmetry of electrodes, in Refs. [27–29] a relationship was found between I and the corrosion rate of carbon steel in chloride solutions. When average values of I are plotted in function of the exposure time (Fig. 7), I showed much higher values in the medium containing bacteria and its metabolites. This feature could be expected, since in this systems the high I values are experimentally associated with high corrosion rate values [27].

Noise resistance  $(R_n)$  is inversely proportional to the activity of the system [30]. As corrosion rate increase,  $R_n$  values decrease. This statistical parameter can be calculated dividing the standard deviation of the potential by the standard deviation of the current:

$$R_{\rm n} = \frac{\sigma E}{\sigma I} \tag{1}$$

 $R_{\rm n}$  values of API-5XL52, in function of the exposure time, in medium without bacteria were much higher than those measured with bacteria (Fig. 8). This can mean that the bacterium and its metabolites induce an increase in the corrosion rate of API-5XL52.

The results derived from the parameters used to interpret the EN, I and  $R_n$ , demonstrated a good agreement with the results obtained with classical electrochemical techniques ( $R_p$ ).

Finally, in this last section electrochemical impedance spectroscopy (EIS) was used to distinguish between the different processes taking place in the studied system.

Electrochemical impedance diagrams of carbon steel immersed for 7 days in M1 are presented (Fig. 9). The first arc, which appears in the Nyquist plot (Fig. 9(a)), corresponds to the impedance response of the corrosion products layer [31]. This arc slowly increases till the third day of immersion, after which, it stays more or less stable. These results match with those obtained with the open circuit potential (OCP) technique.



Fig. 8. Noise resistance values of API-5XL52 alloy, in function of the exposure time in presence (M2) and absence (M1) of bacteria.



Fig. 9. Impedance diagrams of API-5XL52 samples immersed in M1 media for 7 days. (a) Nyquist diagram, (b) Bode modulus diagram and (c) Bode phase angle diagram.



Fig. 10. Impedance diagrams of API-5XL52 samples immersed in M2 media (sterile media + SRB + thiosulfate) for 7 days. (a) Nyquist diagram, (b) Bode modulus diagram and (c) Bode phase angle diagram.

Nevertheless, although the system resistance (|Z| at slow frequencies) keeps constant, the phase associated with the coating displaces towards the slow frequencies. This might be related to the quality decrease of the corrosion products layer.

In Fig. 10 EIS diagrams of samples immersed in the M2 medium have been plotted. In the Nyquist spectra (Fig. 10(a)), the diameter of the first arc increased during the first two days, and then decreased considerably. Impedance diagrams are very similar for both media (M1 and M2) within the first hours of exposure (Figs. 9 and 10). This appreciation agrees with the above mentioned explanations, since at the beginning of the immersion, the bacteria have not developed their activities.

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Media	Time (days)	0	1	2	3	4	5	6	7
M1	$ Z _{\text{slow}f}$	1183.8	1321.6	1037.6	1472.9	1310.1	1201.5	1176.6	1154.3
M2	$ Z _{\text{slow}f}$	1934.4	2013.2	2075.7	1413.2	375.32	399.93	319.64	290.61

Impedance modulus at 0.001 frequency, obtained from Figs. 9(b) and 10(b)

In the Bode spectra (Fig. 10(c)), two time constant can be seen. The first one, which appears at high frequencies, has been related to the response of the corrosion products layer [31]. The second time constant appears at low frequencies after two days of exposure and could be related to the growth and stabilization of the biofilm.

The evolution of the resistance at highest frequencies, |Z|, can be obtained from analysis of Figs. 9 and 10. This value has been related to the electrolyte resistance [32]. The values of this parameter keep constant in the absence of bacteria growing (Fig. 9(b)) while increase with time in the presence of biofilm (Fig. 10(b)). This fact can be due to physical barriers that accompany the formation of this biofilm.

In order to compare the results obtained in OCP with those obtained by means of EIS, the module of Z at the lowest frequency (0.001 Hz) was analyzed. This parameter is theoretically equivalent to  $R_p$  measured in DC techniques [32]. So, Table 2 shows these values depending on the immersion time for both M1 and M2 media.

From the analysis of the Table 2, it can be seen that  $|Z|_{0.001\text{Hz}}$  values for M1 increases until the third day, and then decreases lightly. For the sample immersed in M2, a maximum of resistance is observed at 2 days of immersion, while after this time,  $|Z|_{0.001\text{Hz}}$  values decreases considerably. In short, the results of EIS are in good agreement with those obtained with OCP measurements,  $R_p$  values coming from linear polarization curves (Fig. 6) and ENM.

To summarize, with the EIS diagrams evaluation, we can confirm the results obtained by DC. In culture medium with bacteria, after the maximum exponential growth, and arrival at the latency stage (around 48 h), the resistance of the system diminishes, due, mainly, to the increase of the  $H_2S$  production.

In general, microorganisms may initiate or accelerate corrosion reactions by creating differences in electric potential by stimulating either the anodic or cathodic reactions at the metal surface, due to the products of their metabolic activities [2,11].

In this case, *D. capillatus* form a biofilm that may offer some mass transfer resistance to a number of substances and may establish a microelectrochemical cell. Thus, the activity of bacteria on API-5XL52 steel surface results in a deposition of ferric sulphates in discrete, raised, hard mounds. The bunches deposited by some metal-oxidizing organisms result in anaerobic microenvironments beneath the deposits [4], providing conditions for the accumulation of chloride-ions (to maintain charge neutrality), which combine to form acidic ferric chlorides. These compounds are in general highly corrosive to iron alloys. After removal of corrosion products, deep cavernous pits are often found in the metal below these bunches.

However, the bacteria presence in the media is not the one single cause of the corrosion phenomena. Thus, in the case of the API-5XL52 alloy there was also observed a certain degree of loss of weight for the sterile media, mainly in the one which contains thiosulfate. It is known that the presence of sulphides can induce pitting on steel in solutions with or without chloride-ions. Salvarezza and Videla [33] suggested that activation of the metal

Table 2

surface is caused by the  $HS^-$  ion and iron dissolution occurred through the formation of soluble FeHS<sup>+</sup> species. Moreover, in chloride containing solutions, a very small addition of sulphides can promote steel activation.

These results indicated that electrochemical transfer processes other than metal oxidation contributed to the electrochemical response. At this step, the consumption of hydrogen produced from iron in contact with water [11] by *D. capillatus* and its impact on the overall biocorrosive process cannot be ruled out as the latter SRB easily formed biofilms on metallic surfaces. However, more reasonable explanation is that sulfides or some other metabolic product of bacteria accelerated the anodic reaction. SRB may alter the local environment in several ways: diminution of pH and metabolizing reducing thiosulfate to sulfide. It is well established in the case of SRB that the major corrosion effect is due to the biogenic production of sulfide. Rapid corrosion occurs when sulfide is added or when SRB growth is stimulated by the addition of nutrients [34]. Sulfides then may react to form either  $H_2S$  or some metal sulfide.

### 4. Conclusion

Microbial induced corrosion of carbon steel (St-35.8) and weathering steel (API-5XL52) alloys by the hydrogenotrophic SRB *D. capillatus* has been monitored by electrochemical techniques, weight loss and SEM–EDS. MIC resistance of each of the materials was predicted by weight loss measures. The corrosion behavior of API-5XL52 was worse than St-35.8, and the weight loss was greater. SEM examination of both materials has shown pitting induced by SRB in the form of large radial growth patterns on surfaces. Analysis of SEM–EDS data did not reveal important differences between both alloys.

The electrochemical response ( $E_{corr}$ ,  $R_p$ , EIS, and EN) of API-5XL52 was influenced by the presence of bacteria.  $E_{corr}$  decreased in presence of SRB, showing a small increase of the electrochemical activity on the metal surface. The decrease in  $R_p$  indicated that electrochemical reactions other than metal oxidation occurred when bacteria were active. In any case, the pH of the medium remained approximately constant.

ENM and EIS of samples in the presence of and the absence of bacteria were carried out. The results obtained were in very good agreement with the results coming from the classical electrochemical techniques,  $E_{corr}$  and  $R_p$ . So, I and  $R_n$  have been shown to be suitable parameters to measure the electrochemical activity of metallic alloys subjected to biological corrosion. On the other hand, EIS was able to distinguish between the different processes taking place on API-5XL52 in both media. All electrochemical techniques have shown that after the end of the exponential phase, when the biofilm is formed and the metabolic activity is maximal (two days in our case), the corrosion activity of API-5XL52 notably increased, due to the high concentration of bacterial metabolites (H<sub>2</sub>S, mainly).

MIC has been recognized by a combination of observations: pitting corrosion, presence of biofilm, presence of iron sulfide, and ability of microorganisms to oxidize iron. In general, the observations tend to confirm that *D. capillatus* plays an important role in the corrosion of steel, principally in API-5XL52 alloy, in laboratory tests. This is most probably due to the capacity of *D. capillatus* to easily establish biofilms thus favoring electrochemical exchanges between the metallic surface and the culture liquid medium. Although in this experiment, lactate is the primary electron donor to reduce thiosulfate, one can expect that *D. capillatus*, an efficient oxidizer of hydrogen, may also recover electrons from iron oxidation as this metabolic feature is quite widespread within the SRB [11]. For one recent isolated SRB belonging to the genus *Desulfobacterium*, it has even been suggested that it has more direct access to electrons from iron than via hydrogen consumption [11]. Such an efficient oxidation of iron through sulfate reduction may be explained by an electron uptake linked to a cell-surface-associated redox component [11] which is not known so far. Taking into account the metabolical and physiological features of *D. capillatus*, it should be of interest to test its capacity to oxidize iron directly in the presence of sulfate as the terminal electron acceptor. Nevertheless, for now, our experiments indicate that the presence of thiosulfate, known as increasing biocorrosion risks [8,9], in the culture medium and its use by the SRB seems to be the principal factor in the corrosion process.

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