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Carbon steel corrosion induced by sulphate-reducing bacteria in artificial seawater: electrochemical and morphological characterizations

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ABSTRACT

In this work, the corrosion behavior of carbon steel AISI 1020 was evaluated in artificial seawater in the presence of mixed sulfate-reducing bacteria (SRB) culture isolated from the rust of a pipeline. The corrosion evaluation was performed by electrochemical techniques (open circuit potential (E_{ocp}), polarization curves and electrochemical impedance spectroscopy (EIS)), while the formation of a biofilm and corrosion products were observed by scanning electron microscopy (SEM) and X-ray energy dispersive spectroscopy (EDS). The presence of SRB in the medium shifted the open circuit potential to more positive values and increased the corrosion rate of the steel. Electrochemical and morphological techniques confirmed the presence of a biofilm on the steel surface. EDS spectra data showed the presence of sulfur in the corrosion products. After removing the biofilm, localized corrosion was observed on the surface, confirming that localized corrosion had occurred. The biogenic sulfide may lead to the formation of galvanic cells and contributes to cathodic depolarization.

Keywords: sulphate-reducing bacteria, biofilm formation; carbon steel, electrochemical impedance spectroscopy, morphological characterization.

1. INTRODUCTION

Corrosion, in its various forms, can cause damages to bridges, ships, platforms and pipelines. It has been estimated that approximately 20 % of corrosion cost is due to microbiologically influenced corrosion (MIC), and a significant part of this biocorrosion process can be induced by sulphate-reducing bacteria (SRB) [1, 2]. Biocorrosion is an electrochemical process of metal dissolution initiated or accelerated by bacteria and other microorganisms through their metabolic activities [3]. The interaction of bacteria with the metal surface may also result in the formation of biofilms, which can severely affect the kinetics of cathodic and/or anodic reactions in an electrochemical process [4]. Moreover, a synergistic interaction of microorganisms may occur, resulting in biofilms and metabolic products that enhance corrosion processes [5].

The main characteristic of SRB is the use of a sulphate ion as the final electron acceptor in their bioenergetic process. In their metabolic process, sulphate ions are reduced to sulphide ions, which can be present in three forms: H_2S (soluble), HS^- and S^{2-}, depending on the pH of the environment [6]. SRB are anaerobic microorganisms generally found in anoxic environments, such as soil sediment, oil fields and the anaerobic reactors used in wastewater treatment [7]. In the oil, gas and shipping industries, SRB are particularly aggressive, generally causing pitting corrosion in metal equipment, which results in high corrosion costs [8].

To investigate SRB induced corrosion, an understanding of its corrosion mechanism is very important. A theory named “Cathodic Depolarization” or “Classic Theory” suggests that only SRB that are hydrogease-positive are able to consume protons to generate molecular hydrogen and accelerate direct electron transfer between the hydrogenase and the metal [9]. An alternative mechanism considers the formation of an iron/iron sulphide galvanic cell, in which the iron sulphides (FeS_x) formed by the precipitation of the biogenic sulphide

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with ferrous ions act as sites for the reduction of H^+ ions to molecular hydrogen, enhancing the corrosion [10, 11].

Currently, researchers are investigating the formation of biofilms on the corrosion of carbon steels using various species of SRB and microbial consortia [12, 13, 14, 15]. In these cases, artificial seawater is the main incubation medium for these bacteria [16]. Therefore, in this work, the corrosion of carbon steel AISI 1020 in artificial seawater was evaluated under anaerobic conditions in the presence of a mixed culture of SRB using an experimental exposure time of 35 days. The corrosion process was investigated by open circuit potential (E_{ocp}), polarization curves, electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM) and X-ray energy dispersive spectroscopy (EDS). The aim of this study was to investigate the electrochemical and morphological impacts of a mixed culture of SRB, obtained directly from a real marine environment, in the MIC process on carbon steel in an artificial saline medium using a longer experimental time.

2. EXPERIMENTAL

2.1 Metal coupon preparation

Rectangular carbon steel AISI 1020 coupons (1.5 cm x 3.5 cm x 0.1 cm) were used for cellular quantification and morphologic evaluation. The composition of the carbon steel was (% mass): C 0.18, Mn 0.63, P 0.035 max, S 0.035 max [17].

Coupons of the same type of steel were used in the electrochemical tests. First, they were attached to a copper conductor wire and then embedded in epoxy resin, so that only one face (with an area of 2 cm^2) would be exposed to the solution.

In all cases, the coupons were sequentially polished with a series of sandpaper (grades 100, 200, 300, 400, 500 and 600), washed with distilled water and ethanol, and dried in a current of air. The coupons were polished on both sides for the cellular quantification and morphologic evaluation.

2.2 SRB cultures

The mixed culture of SRB used for the study was isolated from the surface of a rusty pipeline that was submerged in Guanabara Bay, Rio de Janeiro, Brazil. The layer of rust was removed and then inoculated into flasks containing 50 mL of culture medium (0.5 g KH_2PO_4, 1.0 g NH_4Cl, 4.5 g Na_2SO_4, 0.04 g $CaCl_2.2H_2O$, 0.06 g $MgSO_4.6H_2O$, 9.4 mL sodium lactate (50 % m/v), 1.0 g yeast extract, 0.3 g sodium citrate, 0.004 g $FeSO_4.7H_2O$, 1.9 g agar, 4.0 mL resazurin) in 1 L of artificial seawater (0.003 g NaF, 4.0 g Na_2SO_4, 0.02 g $SrCl_2.6H_2O$, 10.78 g $MgCl_2.6H_2O$, 0.03 g H_3BO_3, 23.5 g NaCl, 0.1 g KBr, 0.02 g $NaSiO_3.9H_2O$, 0.7 g KCl, 0.001 g Na_2EDTA, 1.113 g $CaCl_2$, 0.2 g $NaHCO_3$) [18].

The medium was prepared anaerobically under nitrogen purge due to the reductive metabolism of the SRB. The pH was adjusted to 7.6 using 1 mol L^{-1} NaOH, and the medium was then autoclaved at 121 °C for 15 min. The samples were incubated at 30 °C for 7 days.

2.3 Test conditions

Biocorrosion tests were conducted in 100 mL bottles containing 80 mL of the culture medium previously described in the absence or presence of the mixed culture of SRB (10 % v/v) isolated in this study. Nitrogen gas was used to purge the medium and remove the dissolved oxygen (O_2) at the exact moment that the specimens were immersed in the reactors. Although the medium composition did not perfectly resemble the natural conditions of the MIC process, it may be interesting for inducing biofilm production and accelerating the MIC testing as performed by PÉREZ et al. [19].

All of the tests were performed for 35 days, and the data were collected at intervals of 7 days, considering the time zero after inoculation of the medium.

2.4 Enumeration of SRB

The SRB were enumerated using the most probable number (MPN) technique (n=3) in Postgate E semi-solid medium (0.5 g KH_2PO_4, 1.0 g NH_4Cl, 1.0 g Na_2SO_4, 0.67 g $CaCl_2.2H_2O$, 1.83 g $MgCl_2.6H_2O$, 7.0 mL sodium lactate (50 % m/v), 2.0 g sodium acetate, 1.0 g yeast extract, 0.1 g ascorbic acid, 0.5 g $FeSO_4.7H_2O$, 1.9 g agar and 4.0 mL resazurin in 1 L of artificial seawater) for both sessile and planktonic bacteria. The extrac-

tion of the biofilm from the steel coupon surface was carried out by transferring the coupon to a flask containing a dilution solution (0.124 g sodium thioglycollate, 0.100 g ascorbic acid, 4.0 mL resazurin in 1 L of artificial seawater), shaking it on vortex, and then sonicating it under ultrasound. After the removal of the biofilm, aliquots of the suspension were taken for quantification. The inoculated tubes were incubated at 30 °C for 28 days, and the growth of the SRB was indicated by the formation of a black FeS_x precipitate.

2.5 Surface analysis

The surface morphologies of carbon steel 1020 specimens after 35 days of exposure to the culture medium were examined with a scanning electron microscope (SEM; LEO 1450VP SEM) coupled to an energy dispersive X-ray spectroscopy (EDS) system (IXRF-EDS 2000 Microanalysis System). The specimens were visualised in variable pressure mode (low vacuum), making fixation and dehydration steps unnecessary. The metal coupons were washed carefully with distilled water and ethanol and then conditioned in a desiccator for 3 hours prior to coating with gold. Only one side of the coupon was covered with gold for morphological evaluation; EDS analysis was performed on the reverse side of the gold-coated specimen due to the overlap of the gold and sulphur spectral lines [20].

After 35 days-biofilm evaluation, the corrosion products of the specimens were pickled in Clark solution for 1 minute, and the naked corroded surfaces were also observed by SEM.

2.6 Electrochemical Techniques

The electrochemical experiments, consisting of open circuit potential measurements (E_{OC}), polarization curves and electrochemical impedance spectroscopy (EIS), were performed in a three-electrode cell using a computer-controlled potentiostat/galvanostat (Autolab PGSTAT302N). The three electrodes consisted of a carbon steel AISI 1020 coupon as the working electrode, a saturated calomel electrode as the reference electrode, and a platinum wire as the counter electrode.

All the experiments were conducted in the culture medium earlier described, either with or without SRB, in triplicate. The open circuit potential of the steel was measured after 15 minutes of stabilization. Polarization curves were then performed and the Tafel curves were recorded by scanning the potential from -1.2 V to 0.0 V (SCE) with a rate of 20 mV s^{-1}. A rate above 10 mV s^{-1} was chosen based on the literature [11, 21]. The corrosion current density (I_{corr}) and the corrosion rate were obtained by extrapolation of the Tafel curves.

EIS measurements were carried out in duplicate using a frequency range of 100 kHz to 0.001 Hz with an AC wave of ±10 mV after 30 minutes of stabilization in the open-circuit potential. The experiments were performed in the same electrolytic media earlier used for the E_{ocp} measurements and polarization tests.

3. RESULTS AND DISCUSSION

3.1 SRB Growth

The results for the enumeration of planktonic and sessile bacteria using the MPN technique are shown in Figure 1.

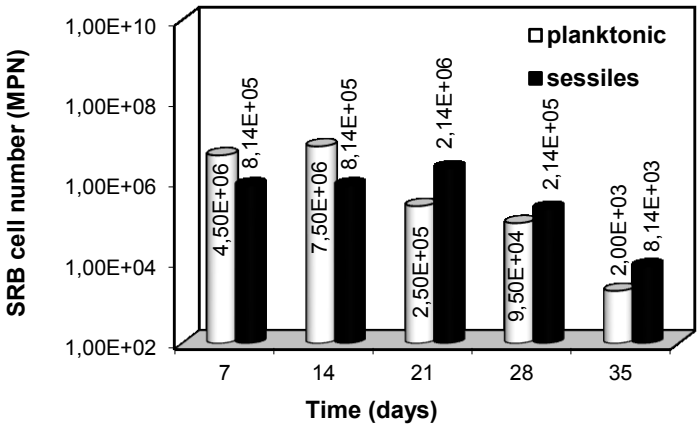

Figure 1: Quantification of planktonic (mL^{-1}) and sessile (cm^{-2}) SRB cells by the most probable number (MPN) method.

The initial concentration cell in the inoculum was 10^5 cells mL^{-1}. The maximum planktonic cell density (7.50×10^6 cells mL^{-1}) was reached at 14 days of exposure, when an intense blackening of the medium was observed, most likely due to the precipitation of iron sulphide. From the 21st day onward, the number of planktonic cells decreased by an order of magnitude every 7 days, maintaining this trend until the end of the test. During this same interval, visual inspection of the biofilm on the metal surface revealed that it had become thicker.

According to GAYOSSO et al. [8], the kinetics growth is different for planktonic microorganisms and for those established at the metal surface (*i.e.*, sessile microorganisms). Thus, it is also important to quantify the number of sessile cells, and this result is also presented in Figure 1. The sessile cell quantification showed maximum and minimum cell densities of 2.14×10^6 cells cm^{-2} and 8.14×10^3 cells cm^{-2}, respectively. It is important to note that the highest concentration of sessile bacteria occurred on the 21st day of immersion, when the reduction in the number of planktonic cells began. From the 28th day onward, both the number of sessile bacteria cells attached to the biofilm and the number of planktonic cells decreased. This behaviour may be attributed to a lack of nutrients and/or to the generation of biogenic hydrogen sulphide as the metabolic product of SRB, which is considered a growth inhibitor [22].

3.2 Surface analysis

Morphological analysis of the steel coupons in the presence of SRB is shown in Figure 2.

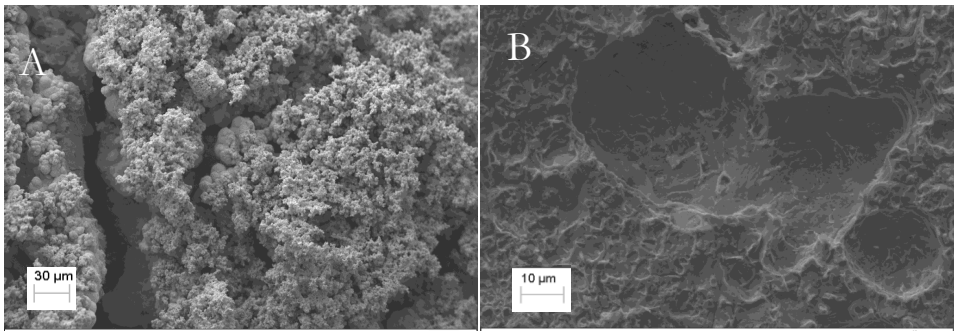

Figure 2: SEM micrograph of the steel coupon surface exposed to culture medium with SRB (993x) after 35 days of exposure (A). The same coupon after pickling the biofilm and corrosion products (4000x) (B).

As expected, the micrograph in Figure 2A shows a nonhomogeneous, porous biofilm on the steel surface, confirming that the biofilm was not a protective layer and that it most likely accelerated the metal corrosion. After removal of the biofilm (Figure 2B), localized corrosion can be observed. This result confirms that inoculating the medium with SRB can result in harmful corrosive processes.

The EDS spectrum of the steel surface covered with a biofilm (Figure 3) shows the presence of sulphur and iron, indicating the formation of FeS_x in the corrosion product, as observed by MIRANDA et al. [3]. The formation of FeS_x may be the result of a reaction between the sulphides generated by SRB metabolism and the Fe^{2+} ions produced by the oxidation of the substrate [7]. Additionally, the spectrum in Figure 3 shows an intense carbon line, which is most likely due to extracellular products (EPS) and colonies of bacteria attached to the substrate. Therefore, this result suggests that the film observed on the carbon steel in Figure 2A is mainly formed by both organic and inorganic matter, the last one originated from the corrosion process of steel in a SRB medium.

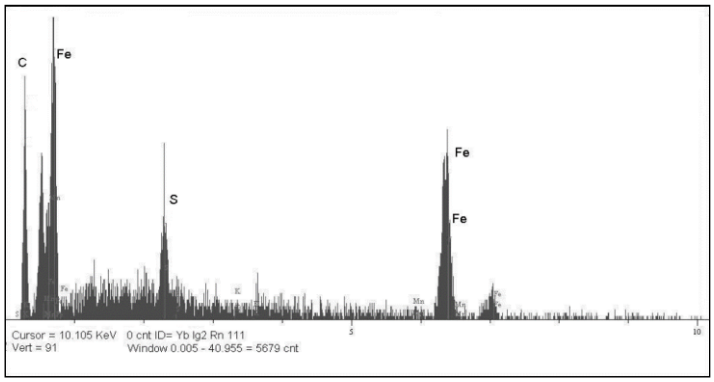

Figure 3: EDS spectrum from the carbon steel surface after 35 days of exposure in the presence of SRB.

3.3 Electrochemical Analyses

3.3.1 Open circuit potential (E_{ocp})

Figure 4 shows the variation of the measured E_{ocp} with the exposure time of the steel sample immersed in the culture medium. It can be observed that there were no significant changes in the E_{ocp} values with the exposure time, for both the media with or without SRB. The presence of SRB in the medium shifted the E_{ocp} values in the positive direction by approximately 120 mV, causing the so-called ennoblement of the substrate due to the biofilm formation [5]. Therefore, the E_{ocp} was affected by the microbial activity produced by the SRB, which were attached to the coupon surface to form a biofilm. However, as already shown in Figure 2A, the steel surface was covered by a porous biofilm, which means that this layer may not be considered as protective.

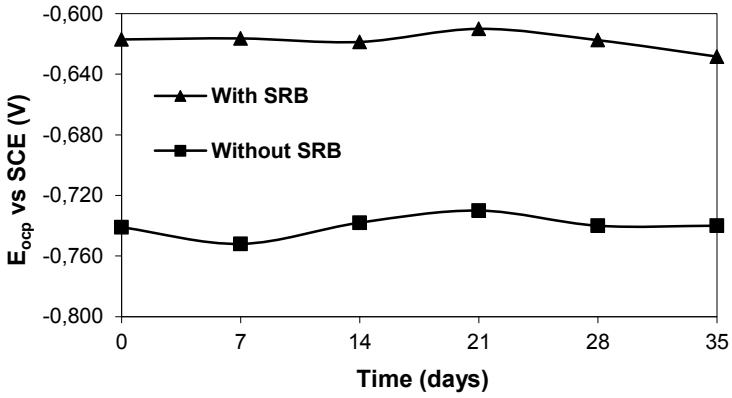

Figure 4: Variation of the E_{ocp} with the duration of immersion of the steel sample in the culture media with and without SRB.

3.4 Polarization curves

The Tafel plots of the polarization curves of the steel electrodes immersed in the culture medium either in the absence or in presence of SRB at 7 and 35 days are shown in Figures 5A and 5B, respectively.

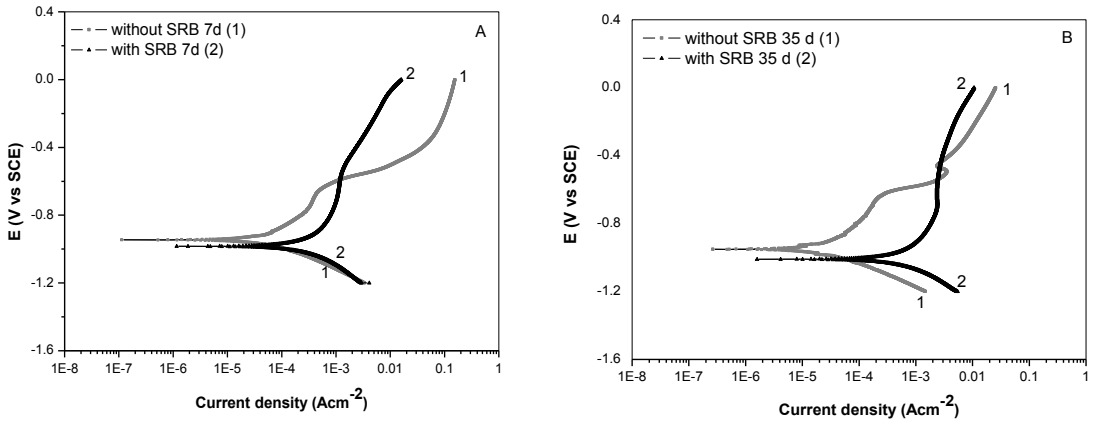

Figure 5: Polarization curves of carbon steel in the culture medium in the absence and presence of SRB after 7 days (A) and 35 days (B) of exposure.

Both polarization curves were obtained by scanning the potential from -1.2 V to 0.0 V (SCE). The curves of carbon steel exposed to the culture medium containing SRB were shifted to more negative potentials and to higher current density values independent of the exposure time. These results indicate that SRB accelerated the corrosion process, as observed by ANANDKUMAR et al. [21], acting mainly by enhancing the anodic branch. This behaviour could be attributed to the fact that the SRB activity can produce H_2S as a secondary metabolite, increasing the corrosive ability of the solution [23]. Even though, it is important to mention a small cathodic depolarization observed for the experiments performed after 35 days of exposure. This result suggests that the corrosion process in SRB medium may have changed with the exposure time. It is known that the iron sulphides (FeS_x) formed by the precipitation of biogenic sulphide with ferrous ions can act as a cathode in the galvanic couple [11]. Therefore, the presence of biogenic sulphide and the FeS_x precipitated on the steel surface could likely contributed to the depolarization observed for the cathodic branch of the polarization curve after 35 days of exposure, enhancing the corrosion process.

The highest differences between biotic and abiotic experiments were observed for 35 days of exposure (Figure 5B), where the E_{corr} and the corrosion current density (I_{corr}) in the absence and presence of SRB, varied from - 0.951 V to -1.010 V and from 3.16 x 10^{-6} A cm^{-2} to 2.87 x 10^{-4} A cm^{-2}, respectively. Comparing the results obtained for the substrate exposed to a medium containing SRB (Figures 5A and 5B), there was an increase in the corrosion process with the exposure time (I_{corr} = 1.26 x 10^{-4} A cm^{-2} and I_{corr} = 2.87 x 10^{-4} A cm^{-2}, for 7 days and 35 days, respectively). However, the anodic branches observed in the SRB curves present a narrow potential range (from -0.7 V to -0.5 V). It may indicate passivity behaviour, probably due to the formation of the corrosion products (mainly FeS_x) and biofilm on the steel surface, as shown in Figure 3. ZHAO et al. [24] suggested that FeS_2 and other non-stoichiometric polysulphide layers, which are initially protective, could become loose, porous and easily desquamated as the time goes on. Therefore, the integrity of the total biofilm and corrosion products layer could have been affected, exposing the steel substrate to the electrolytic medium and accelerating the corrosion process [25]. Moreover, the SRB activity under this film continued, which can lead to the acceleration of the corrosion rate [23].

The corrosion rates as a function of exposure time, obtained from the polarization curves, are shown in Figure 6.

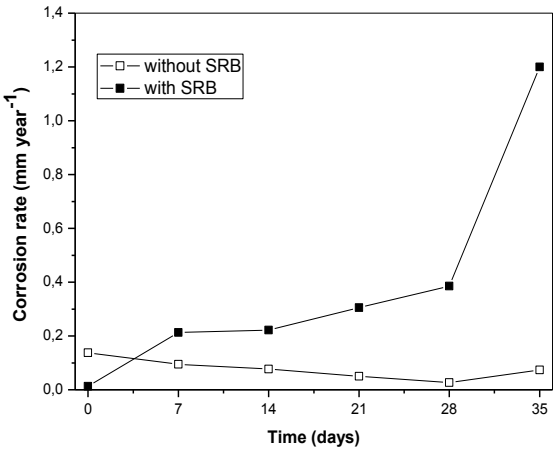

Figure 6: Corrosion rates as a function of exposure time obtained from the polarization curves

The presence of SRB in medium largely increased the corrosion rate of the carbon steel relative to the medium without this microorganism, reaching 1.2 mm year^{-1} after 35 days of exposure. In general, when the corrosion rate increases, it can be related to the breakdown of the passivity film, composed by the corrosion products and biofilm [23]. ALABBAS et al. [5] also found a high corrosion rate (approximately 1.8 mm year^{-1}) for high-strength steel after 30 days of exposure in modified Baar's medium.

3.5 EIS analysis

The EIS data were also analysed to verify the influence of SRB on the corrosion of carbon steel, and the results are shown in Nyquist plots for 7 and 35 days of exposure (Figure 7). It is evident that the polarization resistance of carbon steel was reduced in the presence of SRB, independent of the exposure time. This result confirms that the metabolic products of the SRB and extracellular polymeric substances (EPS) caused a change in the electrochemical properties of the systems [26].

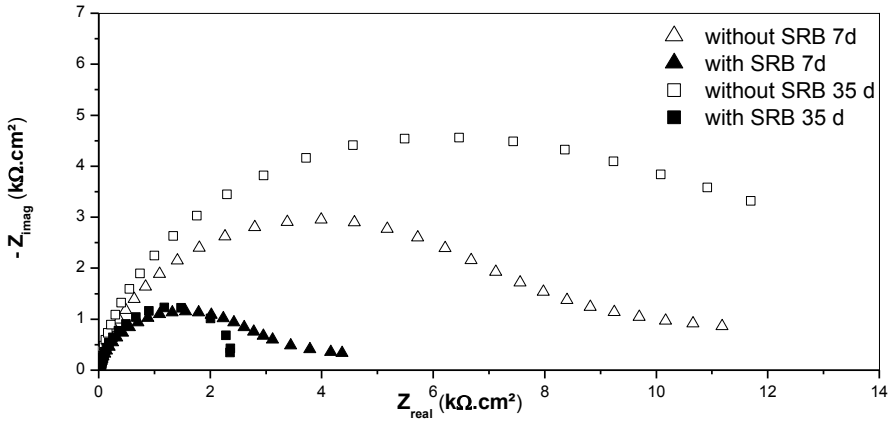

Figure 7: Nyquist plots of the carbon steel in the media in the absence and presence of SRB cells for 7 and 35 days of exposure

All of the curves in the diagrams present only one capacitive loop. The diameter of the capacitive semicircle increased with the immersion time of carbon steel in the sterile medium. This behaviour could be attributed to the formation of a surface layer due to the oxidation of steel and the precipitation of corrosion products on steel surface. DUAN et al. [1] and HEITZ et al. [27] suggested that this corrosion product becomes more compact on the surface of the substrate with the exposure time.

Different results were observed for the steel substrate exposed to the medium in the presence of SRB (Figure 7). The impedance represented by the diameter of the semicircle decreased slightly from 7 to 35 days, probably due to the rupture of the biofilm formed on the steel surface. WAN et al. [15] proposed that this

behaviour could be induced by changes in the microenvironment and in the biofilm, as well as by the corrosion of the steel substrate, that resulted from the decay of the SRB number.

4. CONCLUSIONS

A non-homogeneous and porous film, probably composed by a biofilm and corrosion products (mainly FeS_x), was observed on the steel surface in the biotic medium, as a result from the SBR action. The E_{ocp} measurements, the polarization curves and the EIS analysis indicated the presence of this film on the surface of the steel exposed to the biotic medium. However, the polarization and EIS results showed that it was not stable enough to act as a protective layer. Localized corrosion on carbon steel surface was observed when this film was removed, corroborating that the corrosion process continued under the biofilm/corrosion products layer.

It is important to note that although we used a mixed culture found in a natural marine environment in Brazil, the MIC processes observed in this study were not different from those described in works using isolated SRB cultures or any other natural marine consortium from anywhere in the world.

5. ACKNOWLEDGMENTS

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