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Microbiologically-Influenced Corrosion of 1020 Carbon Steel in Artificial Seawater Using Garlic Oil as Natural Biocide

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This work aims evaluate the use of biocides in the microbiologically-influenced corrosion (MIC) of AISI 1020 carbon steel by sulfate-reducing bacteria (SRB) in artificial seawater. A natural biocide (garlic oil) and a commercial biocide (glutaraldehyde) were used to control the corrosion caused by these bacteria in artificial seawater. Microbial growth on the steel surface was evaluated by quantifying the sessile SRB using the most probable number (MPN) method. The action of biocides in the biocorrosion process was studied by electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization. The biofilm formation and the corrosion products on the steel surface were observed by scanning electron microscopy (SEM). The results showed that, although it was not able to inhibit the growth of sessile SRB completely, garlic oil showed a greater reduction in the corrosion process when compared to glutaraldehyde, indicating its possible application as a natural biocide under these conditions.

Keywords: *Microbiologically-influenced corrosion, sulfate-reducing bacteria, electrochemical techniques, garlic oil.*

1. Introduction

Microbiologically-influenced corrosion (MIC) is a term used to designate corrosion due to the presence and activities of microorganisms. Their ability to adhere onto metallic surfaces and form biofilms can influence corrosion by changing the electrochemical conditions at the metal/solution interface [1]. Corrosion caused by microorganisms can lead to a high economic loss in the oil and gas, power generation and maritime industries, for example [2].

Carbon steel is the most widely used material in the oil and gas industry, as well as in structures present in marine environments (bridges, piers, pipelines and platforms). Sulfate-reducing bacteria (SRB) are the main group of microorganisms involved in the biocorrosion of carbon steel in these applications because they are normally found in marine environments, in coastal clay soils, and in off-shore oil extraction equipment [2].These bacteria use sulfate as a final electron acceptor in their bio-energetic process, which results in the production of sulfide ions, hydrogen sulfide and iron sulfides [3,4].

Methods for the prevention and control of MIC are necessary to avoid or reduce the effects of microbial activity on metals and alloys, such as carbon steel. Most of the time microorganisms are in the sessile state, thus composing biofilms [5]. Sessile cells, which are attached to the steel surface, are directly responsible for MIC because they can utilize extracellular electrons or secrete highly concentrated corrosive metabolites underneath the biofilms [4,6]. Thus, as the control of problematic biofilms is necessary to mitigate MIC, the generally used methods include cleaning procedures, application of organic coatings, cathodic protection and chemical treatments with biocides [7].

Biocides are used to prevent, inhibit or eliminate the growth of microorganisms [8]. Chloride, glutaraldehyde and quaternary ammonium compounds are the biocides most widely used in the control of biocorrosion [9-11]. However, sessile cells are very difficult to mitigate because of their diffusional barrier (which slows down the penetration of biocides), intentionally low metabolic rate (which reduces the biocide intake), preservation of persistent cells, up-regulation of resistance genes that code for proteins that degrade antimicrobials and presence of efflux pumps to remove harmful chemicals [6,12]. Therefore, due to the various defense mechanisms employed by biofilms, high dosages of biocides must be used in field applications to eliminate the sessile cells. Moreover, the repeated treatment cycles usually needed to avoid the formation of new biofilms on the metallic surfaces may create resistant microorganisms [6,13].

The toxicity and residual concentration of the most-used commercial biocides in industrial effluents are of high environmental concern [14], increasing the process cost and, sometimes, causing operational problems. Glutaraldehyde, for example, is acutely toxic to aquatic organisms, mainly to freshwater fishes [15], although it is usually considered a biodegradable biocide [4,6]. For these reasons, the use of alternative, environmentally friendly biocides is of great

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industrial interest. Currently, tetrakishydroxymethylphosphonium sulfate (THPS) is the green biocide generally used in the oil and gas industries [4], either alone or in mixtures with aminoacids [6,12,16]. Recently, glyceryl trinitrate (GTN) and caprylic acid (CA), two biodegradable biocides with potential for field applications, were also evaluated for mitigation of *D. vulgaris* biofilms on C1018 carbon steel coupons [17].

Natural compounds, such as plant and food extracts, can also be considered as non-aggressive alternatives to commercial biocides for MIC mitigation [18,19]. Many plant/food oils and aqueous extracts are able to inhibit the action of fungi and bacteria [18]. De et al. [20] showed that compounds traditionally used as food preservatives, antiseptics and disinfectants, such as clove, cinnamon, chili, cumin, tamarind, black cumin, garlic, and onion, among others, presented antimicrobial properties. Recently, Narenkumar et al. [21] used ginger extract as a green biocide to control microbial corrosion of mild steel in cooling water systems.

Garlic (*Allium sativum*) is the oldest vegetable used, both in cooking and in medicine, due to its diversified biological activity including antimicrobial, anticancer, and antioxidant actions, among others [22]. In addition, garlic oil and garlic peal extract have also been used as a natural corrosion inhibitor for carbon steel in acidic media [23,24]. Thus, garlic presents a possible use in the prevention of biocorrosion of carbon steel, since it has bactericidal properties [25-27] and acts as a natural corrosion inhibitor.

Therefore, this work proposed the evaluation of the microbiologically-influenced corrosion (MIC) of AISI 1020 carbon steel in artificial seawater by SRB using a natural (garlic oil) and a commercial (glutaraldehyde) biocides.

2. Materials and Methods

2.1 Organisms and culture medium

The bacterial culture used in this work was a mixed culture containing the bacteria group known as sulfate-reducing bacteria (SRB). The culture was provided by the Instituto Nacional de Tecnologia (INT), Rio de Janeiro, Brazil.

The electrolyte used in all tests was artificial seawater. It was used for both the preparation of culture medium and as the electrolyte in electrochemical evaluation. The artificial seawater was composed of NaF (0.03 g), $SrCl_2.6H_2O$ (0.20 g), H_3BO_3 (0.30 g), KBr (1.00 g), KCl (7.00 g), $CaCl_2$ (11.13 g), Na_2SO_4 (40.00 g), $MgCl_2.6H_2O$ (107.80 g), NaCl (235.00 g), $NaSiO_3.9H_2O$ (0.20 g), Na_2EDTA (0.01 g), $NaHCO_3$ (2.00 g), and distilled water (10 L) [28].

The SRB were cultivated in a modified Baar's medium (adapted from ATCC medium 1249). The composition of this growth medium was $MgSO_4$ (2.00 g), sodium citrate (5.00 g), $CaSO_4$ (1.00 g), NH_4Cl (1.00 g), K_2PO_4 (0.50 g), sodium lactate 50% w/v (7 mL), yeast extract (1.00 g), resazurin 0.025% w/v (4 mL), sodium thioglycollate (0.12 g), and artificial seawater (1 L). The medium was prepared anaerobically under nitrogen purge due to the reductive metabolism of the SRB. The pH was adjusted to 7.50 (the optimal pH for growth of SRB) using 1 mol/L NaOH, and the medium was then autoclaved at 121 °C for 15 min.

2.2 Metal coupon preparation

Carbon steel AISI 1020 coupons (2.0 cm x 1.8 cm x 0.1 cm) were used for cellular quantification and morphologic evaluation. The composition of the carbon steel was as follows (% mass): C 0.180, Mn 0.630, P_{max} 0.035, S_{max} 0.035 [29].

Samples of the same type of steel were used in the electrochemical experiments. In this case, the coupons were attached to a copper conductor wire for electrical connection of the system and then embedded in epoxy resin, so that only one face (with an area of 3 cm^2) would be exposed to the corrosive environment.

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

2.3 Biocides

The natural biocide used in the present work was garlic oil (Alhonat Nativa), which was sold in capsules containing 250 mg of oil. As a comparison, a commercial biocide (glutaraldehyde 25% w/v, Sigma-Aldrich) was also evaluated. For each kind of biocide, different concentrations were used based on previously conducted experiments [30], as shown in the experimental conditions section.

2.4 Experimental conditions

All experiments were carried out in 100-mL bottles containing 80 mL of Baar's culture medium and inoculated with the mixed culture of SRB (10% v/v). First, a monitoring experiment was conducted to examine the microbial growth over 35 days in the culture medium (control tests) and set the duration of subsequent tests with the biocides (biocide tests). Before inoculation, an $Fe(NH_4)_2(SO_4)_2.6H_2O$ 0.50 g/L solution, previously filtered through a Millipore membrane (pore size = 0.45 μm), was added to the culture medium in order to provide a source of iron.

The experiments with biocides were conducted using the same procedure described above. The biocide (commercial or natural) was added to the culture medium before inoculation. The concentration used for the commercial biocide (glutaraldehyde) was 0.01% w/v, and for the natural biocide (garlic oil), it was 3.00% w/v. A surfactant (potassium laurate, 10^{-3} mol/L) was also added to the culture medium in the experiments containing garlic oil to enhance the solubility of the oil in the aqueous solution.

All steel specimens were sterilized in laminar flow by the action of ultraviolet light (UV) before their immersion

into the culture medium. 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 bottles. After preparation of the system, the bottles were incubated at 30 °C during the incubation period. Based on the control experiment, the biocide tests were performed over 7 days.

2.5 Cellular quantification

Cellular quantification of the sessile cells was performed after each seven days for the control tests (total exposition = 35 days) and after seven days for the biocide-containing experiments. In all cases, the metallic coupons were aseptically removed from the medium, thoroughly washed by soaking in distilled water to remove free cells, and transferred to a bottle containing the dilution solution (sodium thioglycollate (0.124 g), ascorbic acid (0.100 g), resazurin 0.025% w/v (4 mL), and artificial seawater (1 L)). In order to remove the biofilm attached to the steel surface, the bottle containing the coupon was shaken on a vortex for 4 minutes and further sonicated for 2 minutes.

After removal of the biofilm, aliquots (1 mL) of the suspension were taken for quantification of the cells. The culture medium used in this procedure was Postgate's E medium [31], composed of KH_2PO_4 (0.50 g), NH_4Cl (1.00 g), Na_2SO_4 (1.00 g), $CaCl_2.2H_2O$ (0.67 g), $MgCl_2.6H_2O$ (1.83 g), sodium lactate 50% w/v (7 mL), yeast extract (1.00 g), ascorbic acid (0.10 g), $FeSO_4.7H_2O$ (0.50 g), agar (1.90 g), resazurin 0.025% w/v (4 mL), and artificial seawater (1 L). The quantification was performed using the most probable number (MPN) method [32,33], and the inoculated tubes were incubated at 30 °C for 28 days.

The reduction of the sessile bacterial population after addition of the biocides was calculated by equation 1[30]:

$$\%reduction = \frac{\log(MPN_{control}) - \log(MPN_{with\ biocide})}{\log(MPN_{control})} \times 100 \quad (1)$$

2.6 Electrochemical experiments

Open circuit potential (OCP) measurements, electrochemical impedance spectroscopy (EIS) and potentiodynamic polarization (PP) of the carbon steel were performed in artificial seawater after previous exposure for 7 days in the medium without (control test) and with the studied biocides (biocide test). The experiments 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 (SCE) as the reference electrode, and a platinum wire as the counter electrode.

All electrochemical tests were performed after stabilization of the system at its open circuit potential for 1 h. The EIS experiments were performed at the OCP, using a frequency range from 10^{-3} to 10^5 Hz and an AC wave of 10 mV. In the potentiodynamic polarization experiments, the applied potential ranged from -1 to 1 V, with a scan rate of 1 mV/s. All the electrochemical tests were performed in duplicates.

2.7 Scanning electron microscopy (SEM)

SEM analysis was performed for the specimens exposed for 7 days to the control medium, as well as for those inserted into the media with biocides during the same period. The coupons were washed carefully with distilled water to remove free cells and then dried using a heater at 37 °C for 1 h. The samples were metalized with gold using sputtering (DENTON VACUUM DESK V) before being analyzed by a scanning electron microscope (JEOL model JSM G510 LV) using the high vacuum mode, an SEI detector and 20 kV of voltage.

In order to perform SEM analysis after removing the biofilm, the coupons were pickled in Clark solution (HCl (100 mL), Sb_2O_3 (2.00 g) and $SnCl_2$ (5.00 g)) for 2 minutes. Then, the coupons were washed with distilled water and dried in a current of air.

3. Results and Discussion

3.1 SRB growth

The concentration of the inoculum used in all assays was $9.10x10^6$ cells/mL. The SRB enumeration was taken only including cells adhered to the metal substrate (sessile cells) at 7, 14, 21, 28 and 35 days. The results of the enumeration of sessile bacteria using the MPN technique are shown in Figure 1.

Quantification of the growth of sessile SRB cells during the 35-day assay presented values ranging from $1.40x10^5$ to $1.40x10^3$ cells/cm^2. The maximum number of SRB cells/cm^2 had already been reached at 7 days of growth. No significant differences were observed up to 21 days and then the SRB number decreased. This result confirms the ability of the SRB to colonize and adhere to surfaces. SRB are capable of producing extracellular polymeric substances (EPS), which favors this adhesion [34-36]. From the 21st day onward, the number of SRB adhered to the surface decreased, which

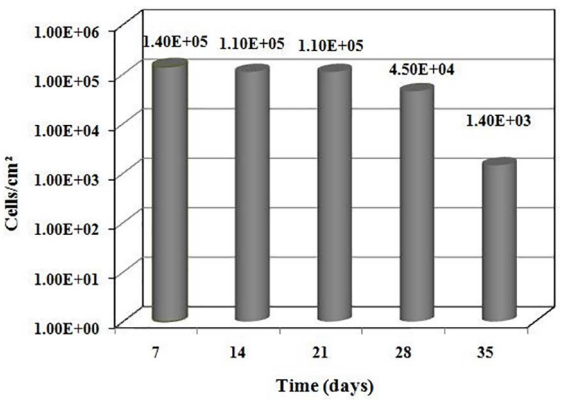

Figure 1. Quantification of sessile SRB cells by the most probable number (MPN) method

can be attributed to the lack of nutrients associated with the increase of toxic metabolites (H_2S) in the medium [37].

3.2 Biocide tests

The duration of the experiments in the biocide-containing media was 7 days, based on the highest number of sessile cells obtained during the SRB growth control experiment (Figure 1). Sessile SRB cells obtained in both the biocide and the control tests, grown over the same period, are shown in Figure 2.

The presence of the compounds studied (garlic oil and glutaraldehyde) in the growth medium decreased the number of sessile cells on the steel substrate, indicating that both compounds can be applied as biocides. No growth of sessile SRB cells was observed in the glutaraldehyde-containing medium, while the sessile SRB population was reduced by 30% ($4.50x10^3$ cells/cm^2) in the garlic oil one.

Although highly efficient as a biocide, glutaraldehyde is toxic to the environment, mainly to aquatic ecosystems [15,23,38]. Thus, the lowest possible concentration of this reagent is required to prevent the growth of the microorganisms. The minimum value depends on whether the SRB cells are planktonic or sessile [9,39,40]. In this study, 0.01% w/v (100 mg/L) of commercial biocide was sufficient for the inhibition of SRB biofilm establishment.

Compounds extracted from garlic are known to have antimicrobial and bactericidal characteristics [25-27], antioxidant properties and the capability of inhibiting corrosion [23,24]. However, to the best of our knowledge, there are no studies that discuss the action of garlic and its compounds on SRB growth. In the present study, the amount of garlic oil added (3.00% w/v) to the medium was able to reduce the sessile SRB growth from $1.50x10^5$ to $4.50x10^3$ cells/cm^2 after 7 days of incubation, indicating its potential as a natural biocide for SRB. According to Zalepugin et al. [27], allicin, diallyl disulfide, and diallyl trisulfide are the main garlic compounds presenting antimicrobial activity.

3.2.1 Surface analysis

The micrographs presented in Figure 3 were obtained with a 7-day assay of the specimen immersed in the medium containing SRB (control), which exhibited microbial adhesion throughout the biofilm formation (Figure 3a), and also for the specimens in the media containing SRB and biocides, garlic oil (Figure 3c) or glutaraldehyde (Figure 3e), added to avoid biofilm formation. To verify the final condition of the steel substrate immersed in these media, the micrographs performed after biofilm removal are also shown (Figures 3b, 3d and 3f, respectively).

The micrograph in Figure 3a shows irregularly distributed cell agglomerates on the surface of the carbon steel, exhibiting both rods and cocci. Some flakes, which are most likely due to the extracellular products (EPS) of the SRB metabolic activity, were also observed. EPS can create conditions for non-homogeneous and porous biofilm formation on the metal surface, modifying the physicochemical environment to favor the electrochemical reactions responsible for localized corrosion [41]. Under these conditions, the presence of localized corrosion (pits) and intergranular corrosion after biofilm removal (Figure 3b) was observed. Bhola et al. [18] and AlAbbas et al. [42,43] also verified pitting corrosion in their works, confirming that the SRB metabolic activity on the steel surface leads to localized corrosion increase. Although the sulfide ion concentration has not been determined in this work, it is possible that a large amount of this metabolite was produced, contributing to the formation of intergranular corrosion. Similar results were obtained by Lee and Characklis [44] when evaluating the corrosion of mild steel in the presence of a mixed-population biofilm including SRB, showing intergranular and pitting attacks in the localized corrosion area.

It is possible to observe in Figure 3c the formation of cell clusters on the steel surface, although there is, apparently, a film covering the substrate. The film may be formed by the adsorption of garlic compounds, which are known to act as corrosion inhibitors [23,24]. After the biofilm removal (Figure 3d), localized corrosion was not observed and only a uniform corrosion process could be seen on the surface. This result suggests that, garlic oil may have contributed to the reduction of the corrosive process on the steel in the studied medium, since it also acted as a natural corrosion inhibitor. In Figure 3e, it is possible to observe only a slightly adherent film on the steel surface. There were no cells present, confirming the ability of glutaraldehyde to inhibit SRB growth. After the biofilm removal (Figure 3f), it was possible to detect the presence of corrosion products on a cracked steel surface.

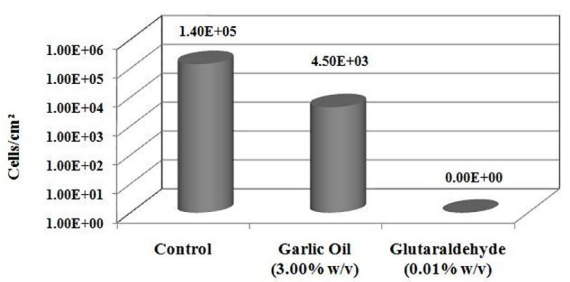

Figure 2. Effect of garlic oil and glutaraldehyde on the SRB cells after 7 days of incubation. In the control no biocide was used

3.2.2 Electrochemical experiments

Figure 4 shows the open circuit potentials (OCP) for the 1020 carbon steel samples in artificial seawater. These samples were previously immersed for 7 days in the SRB culture medium with and without the studied biocides.

The presence of biocides in the SRB culture medium caused a shift of the OCP values to more negative values, in comparison with the test without biocide (- 0.630 V). The potential reached -0.655 V and -0.692 V for the media

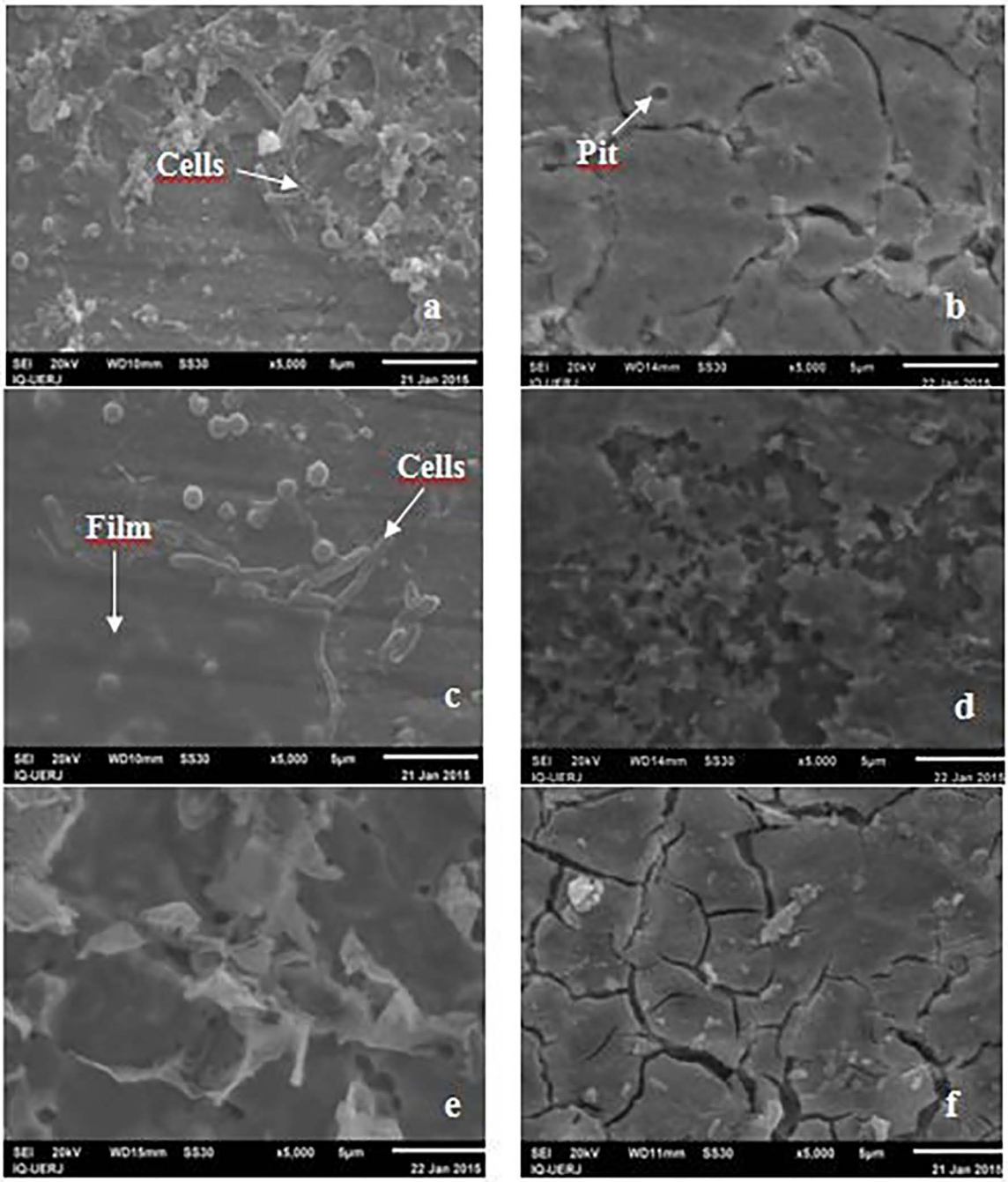

Figure 3. SEM images for the biofilm developed on carbon steel AISI 1020 surface in modified Baar's medium for 7 days. (a) Biofilm without biocides (control); (b) The same coupon (a) after biofilm removal (control); (c) Biofilm with garlic oil; (d) The same coupon (c) after biofilm removal; (e) Biofilm with glutaraldehyde; (f) The same coupon (e) after biofilm removal

containing garlic oil and glutaraldehyde, respectively. As a comparison, the OCP of the steel sample incubated for 7 days in the same culture medium without SRB (abiotic medium) was -0.720 V [30]. Thus, the addition of biocides into the samples incubated reduced the phenomenon known as "ennoblement" on the steel surface, in which the OCP of the steel substrate immersed in the medium containing SRB shifts to more positive values (nobler values), when compared to coupons of the same type exposed to abiotic medium (without SRB) [45]. Although the mechanism by which "ennoblement" occurs is not completely known yet, this phenomenon has been attributed to the reproduction and colonization of the SRB in the steel, leading to the formation of biofilm and to changes in the electrochemical

processes on the surface of the material. This phenomenon may also increase the probability of localized corrosion by pitting or cracks [42,45]. The present result indicates the reduction or absence of microorganism colonization on the metallic surface, which is in agreement with the microbial quantification (Figure 2) and the micrographs of the control, garlic oil and glutaraldehyde experiments (Figures 3a, 3c and 3e, respectively).

The Nyquist diagram of the above-mentioned 1020 carbon steel samples in artificial seawater obtained using the electrochemical impedance spectroscopy (EIS) technique is shown in Figure 5a. As a decrease in the diameter of the capacitive loop of this diagram represents an increase in the corrosion process, the Nyquist diagram indicates that the presence of biocides in the incubation medium decreased the corrosive action associated with SRB metabolism on the steel surface. This result is in agreement with the microbial quantification, the morphological analysis and the drop in the OCP values, shown in Figures 2, 3 and 4,

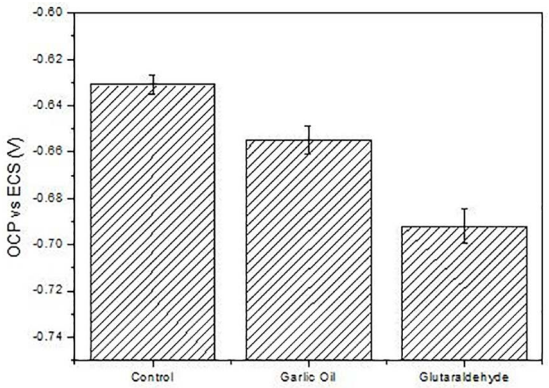

Figure 4. OCP values for the 1020 carbon steel in synthetic seawater. The coupons were previously incubated in SRB-containing culture medium in the absence and presence of the studied biocide

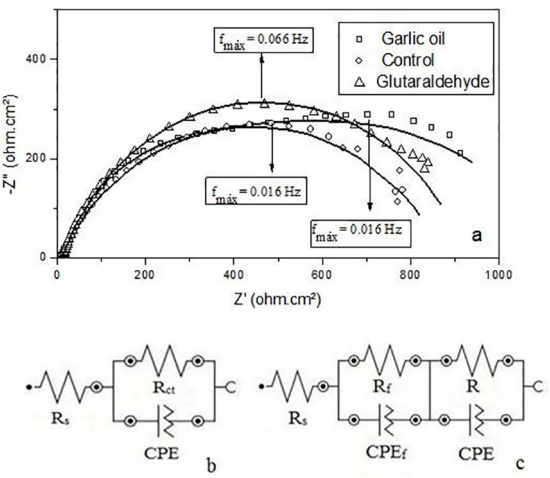

Figure 5. (a) Nyquist diagrams for the 1020 carbon steel in synthetic seawater. (b) Equivalent circuit model used to simulate the EIS data of the tests with glutaraldehyde biocide and control. (c) Equivalent circuit model used to simulate the EIS data of the test with garlic oil biocide

respectively, because the decrease in sessile cells observed in the biocide-containing media can be related to the decrease in the corrosive process induced by the SRB. Comparing the effects of the two biocides, the capacitive loop obtained for the steel sample immersed in the medium containing the garlic oil presented a larger diameter than that immersed in the medium containing glutaraldehyde.

Quantitative values of the charge transfer resistance (R_{ct}) and the double layer capacitance (C_{DL}) can be obtained by simulating the EIS experimental data using the equivalent electrical circuits shown in Figure 5b and 5c (NOVA 1.10 Metrohm Autolab software). These circuits represent the interface between the steel surface previously immersed in the SRB medium with or without the studied biocides, and the seawater electrolyte [46].

In Figure 5b, it was observed that the electrode/solution interface was composed of the steel substrate (probably covered by a thin porous layer of the corrosion product or the biofilm itself) and the corrosive medium [41]. In this circuit, R_s represents the electrolyte resistance, R_{ct} is the charge transfer resistance, and CPE represents the constant phase element associated with the electric double-layer capacitance [42].This circuit was used to simulate the control test and the biocide glutaraldehyde test. However, the data obtained from the garlic oil biocide test presented two time constants, indicating a more complex corrosion process [36]. It was not possible to fit this result adequately using the proposed simple circuit (Figure 5b), so the equivalent circuit shown in Figure 5c was used. In this case, R_f and CPE_f refer to the resistance and the constant phase element of the biofilm, respectively, while R is the substrate resistance. In this series circuit, $R_{ct} = R_f + R$, and it is therefore proposed that the surface was covered by a protective film. In both cases, the simulation fitting was considered accurate for an error value less than 1% [47]. The double-layer capacitance value (C_{DL}) was calculated using equation (2):

$$C_{DL} = (CPE)^{\frac{1}{N}} \times R_{CT}^{\left(\frac{1}{N}-1\right)} \qquad (2)$$

where N defines the equivalence degree between the CPE and the capacitive component [41].

Table 1 shows the values of R_s, R, C_{DL}, R_f, C_{DLf} and R_{ct} obtained from the simulation of the EIS data from the steel previously immersed in the experimental media for 7 days.

The results in Table 1 confirmed that, compared to those of the control experiments, the R_{ct} values increased and the C_{DL} values decreased when the EIS measurements were performed using a steel sample previously immersed in an SRB medium containing one of the biocides studied in this work. Under these conditions, an increase in the R_{ct} values is related to a decrease in the corrosion process. In addition, a decrease in the C_{DL} values can be associated with the lack of a stable biofilm and the presence of protective corrosion

Table 1. Parameters obtained by simulating the EIS data for the 1020 carbon steel (previously incubated in SRB-containing culture medium in the absence and presence of the studied biocides) in synthetic seawater, using the equivalent electrical circuits presented in Figures 5b and 5c

	$R_s(\Omega)$	R (Ω.cm^2)	C_{DL} (F/cm^2)	N	R_f	C_{DLf} (F/cm^2)	N_f	R_{ct} (Ω.cm^2)	Experimental Error (%)
Control	3.490	-	$1.670x10^{-2}$	0.711	-	-	-	744	0.045
Glutaraldehyde	6.410	-	$6.430x10^{-3}$	0.781	-	-	-	803	0.157
Garlicoil	4.430	900	$4.760x10^{-3}$	0.639	234	$3.390x10^{-3}$	0.919	1134	0.036

products (or a protective film) on the steel surface, which hinders the corrosion process [47].

In agreement with the Nyquist diagrams shown in Figure 5a, the highest R_{ct} value was obtained from the experiment performed with the sample previously immersed in the medium containing garlic oil. Although the garlic oil could not inhibit the SRB growth completely (Figure 2), it is known that the compounds present in garlic oil or even in the aqueous extract of garlic peal may act as corrosion inhibitors [23,24] by adsorbing onto the steel surface. Therefore, it is probable that these results are not related to the biocidal action of these garlic compounds only, but also to the formation of a corrosion-inhibiting film adhered to the steel surface. This means that the double action of garlic oil, as a biocide and as a corrosion inhibitor, can protect the metal from corrosion. In fact, Figure 3d shows that only a uniform corrosion was observed in the sample immersed in the medium containing garlic oil. Similar results were obtained for the steel sample previously immersed in the medium containing the biocide glutaraldehyde: an increase in R_{ct} and a decrease in C_{DL} when compared to the control test. In this case, however, the reduction of corrosion occurred only due to the inhibition of SRB growth, as verified in Figure 2.

Potentiodynamic polarization curves (PP) were also obtained, in duplicate, for the same steel specimens in artificial seawater and are presented in Figure 6. The main electrochemical parameters were obtained by Tafel extrapolation of the curves presented in Figure 6 and the values of the corrosion potential (E_{corr}) and the corrosion current density (j_{corr}) are presented in Table 2. The profiles of the polarization curves are similar. There are no differences concerning the E_{corr} values (Table 2), obtained for the samples previously immersed in the media containing the studied biocides. Small variations between these curves were verified only in the cathodic branches, as shown in the β_c values in Table 2.

Even though very similar to each other, the small decrease occurred in the j_{corr} value of the sample previously immersed in the medium containing garlic oil may suggest that this biocide affected the biofilm formation and/or decreased the production of unstable corrosion products. This behavior agrees with the EIS result, confirming the effect of the garlic oil in decreasing the biocorrosion process. In contrast, the j_{corr} value of the steel sample previously immersed in the medium containing glutaraldehyde was very close to the value obtained for the control experiment. Although this biocide may have hindered the SRB growth on the surface of the steel coupon, this result indicates that it did not prevent corrosion of the steel in the studied medium, probably due to the presence of corrosion products on a cracked steel surface (Figure 3f).

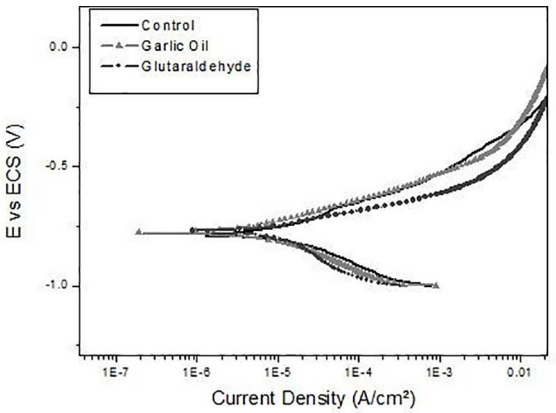

Figure 6. Polarization curves for the 1020 carbon steel in synthetic seawater. The coupons were previously incubated in SRB-containing culture medium in the absence and presence of the studied biocide

Table 2. Electrochemical parameters obtained from the Tafel extrapolation of the polarization curves presented in Figure 6

	E_{corr} (V)	j_{corr} (A/cm^2)	β_a (V/dec)	β_c (V/dec)
Control	-0.780	$2.240x10^{-6}$	0.074	0.077
Garlicoil	-0.770	$1.060x10^{-6}$	0.068	0.068
Glutaraldehyde	-0.770	$2.320x10^{-6}$	0.066	0.092

4. Conclusion

Microbial quantification of sessile SRB cells reached the maximum value after 7 days of immersion in the culture medium, confirming the ability of this group of bacteria to colonize and adhere to metallic surfaces, causing corrosion. The tests performed in the present work showed that the type of corrosion caused by SRB can be controlled by the addition of the evaluated biocides. Both garlic oil and glutaraldehyde reduced corrosion of the steel and prevented

biofilm formation. Garlic oil is presented as a promising natural biocide, which can replace more toxic commercial biocides. In addition, its action as a corrosion inhibitor also favored the decrease in corrosive processes in the studied medium, unlike the biocide glutaraldehyde.

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