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Study on Biofilm Forming Microorganisms Associated with the Biocorrosion of X80 Pipeline Steel in Produced Water from Oilfield

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Biocorrosion is the main process that causes pipeline damages and losses in the oil industry. The objective of this work was to investigate the influence of biofilm forming microorganisms on the biocorrosion of X80 steel exposed in produced water through microbiological characterization, film and biofilm analysis by optical microscopy and scanning electron microscopy, weight loss and surface analysis by laser confocal microscopy. Changes in produced water after 360 days were attributed to planktonic cells, temperature conditions, contact with air, photo-oxidation, biodegradation, and seasonality. The total aerobic bacteria presented sessile cell concentration of 7.39 x 10^4 CFU/cm^2, while the other investigated groups showed lower concentrations. The micrography of the film showed salt crystals, whereas in the biofilm microorganisms, exopolysaccharides and corrosion products were observed. Weight loss after 360 days for the abiotic and biotic systems was 0.0222 g/cm^2 and 0.3039 g/cm^2, respectively, showing that microorganisms accentuated the corrosion of X80 steel.

Keywords: *Biocorrosion, biofilm, pipeline, produced water, X80 steel.*

1. Introduction

The produced water is the largest waste volume generated by the oil and gas industry during the production, recovery and transportation phases of crude oil[1]. Although the composition of the produced water varies from field to field, it consists essentially of dissolved organic and inorganic compounds, dispersed hydrocarbons, dissolved gases, chemicals, suspended solids and microorganisms[2].

This environment of complex chemical composition promotes the growth of different groups of microorganisms[3]. Precipitating iron bacteria, acid-producing, sulfate reducing (SRB), sulfur oxidants, exopolysaccharide (EPS), fermenting, methanogenic, *Pseudomonas*, *Bacillus spp*., *Archaea* and fungi can be isolated from the fluids of the oil industry[4,5]. On top of that, bacterial genera belonging to sulfate reducers (*Desulfomicrobium*, *Desulfovibrio*, *Desulfohalobium*, *Desulfococcus*, *Desulfosarcina*, *Desulfobacter*, *Desulfobacterium* e *Desulfobulbus*) and iron reducers (*Desulfuromusa*, *Pelobacter*, *Malonomonas* e *Desulfuromonas*) have been identified in samples of produced water[3].

Different microbial species coexist in a mixed consortium leading to corrosive processes synchronized with the interaction among cells[6,7]. The deposition of existing macromolecules in the fluid and the subsequent transport of planktonic cells to the metal surface initiate biofilm formation[8]. In this microenvironment, microorganisms release toxic substances, produce exopolysaccharides, corrosive acids (organic and inorganic) and volatile compounds (ammonia and hydrogen sulfide), and depolarize the corrosion cell using hydrogen, oxygen or iron in the environment[9-12]. The gradual development of biofilm along with the consequent production of microbial metabolites significantly affect cathodic and anodic reactions on the metal surface, causing or accelerating localized corrosion, also called pitting corrosion[13,14]. This type of corrosion is more intense and dangerous than uniform corrosion in pipelines, due to the difficulty in predicting its appearance and preventing this corrosive process from starting and compromising the functionality of the structure, causing accidents[15]. Some published studies show pits of corrosion on the surface of X80 steel from the physiological activity of microorganisms[16-20].

The interaction among biofilms, microorganisms and their metabolites, abiotic corrosion products and the metal surface is called biocorrosion or microbiologically induced corrosion (MIC)[9]. Biocorrosion is responsible for causing about 40% of all the internal corrosion events in oil industry pipelines[21]. Previous studies have shown that the fluid speed inside the pipes affects the biofilm formation. At low speeds, biofilm is formed and steel becomes susceptible to MIC and pitting corrosion, while at high speeds a layer of corrosion products is formed on the steel surface[21]. The effect of Fe ions on biofilm formation and EPS production was studied by Jin and Guan[22]. The researchers found that in moderate concentrations of Fe there were both formation of biofilms and production of EPS. In the initial stage, EPS accelerated corrosion by bonding with iron ions causing anodic dissolution. Postliminary, corrosion was inhibited due to reduced oxygen and phosphorus availability of EPS.

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Changes in the crystalline phases of the corrosion products were observed by the presence of EPS. Cote et al., observed a decrease in bacterial diversity after the cultivation of pigging debris in artificial seawater and produced water mixed with seawater, to which low-alloy carbon steel was exposed[23]. Therefore, corrosion in steel did not occur solely due to the presence of SRB and other bacteria of the taxonomic order *Clostridiales*. Liu et al., found that the nitrate-reducing bacteria *Brevibacterium frigoritolerans* can increase the depth of the pits in X80 steel, destroy the stable layer of corrosion products and act as a biological cathode within the biofilm by reducing nitrate[24].

For a better understanding of biocorrosion processes, it is necessary to study the microorganisms, and also the formation and morphology of biofilms in substrates. There are few studies on mixed species biofilms that influence corrosion in produced water systems. The current work aims to investigate the influence of biofilm forming microorganisms on the biocorrosion of X80 steel after 360 days of exposure to produced water in abiotic and biotic static systems. X80 steel was characterized by analysis of chemical composition, metallography and grain size. Physico-chemical analyzes were performed on the produced water. The influence of microorganisms was evaluated by exposing X80 steel coupons to systems composed of sterile (abiotic) and non-sterile (biotic) produced water, and subsequent sessile microbiological quantification for 360 days. Optical Microscopy (OM) and Scanning Electron Microscopy (SEM) images were used to observe the morphologies of the film and biofilm. Accumulated weight loss tests were used to assess corrosion. A three-dimensional topography of the surface was used to measure the size of the pit by means of Confocal Laser Microscopy (CLM).

2. Experimental Procedure

2.1. Material

X80 steel coupons with dimensions of 30 mm x 10 mm x 5 mm were used in the tests. Their chemical composition (%) was analyzed, and it is given in Table 1. Metallographic characterization was carried out according to the methodology described in the previous paper[25]. Subsequently, the microstructure and the grain size of the coupons were analyzed by SEM HITACHI® TM 3000. Figure 1 shows the microstructure of X80 steel, consisting of acicular ferrite (AF) and polygonal ferrite (PF). Figure 2 shows the microstructure with the respective grain size distribution graph within 4 µm and 11.5 µm. Before being inserted into the abiotic and biotic systems, the coupons were blasted to obtain the appearance of white metal (grade Sa 3), ensuring a uniform metallic color surface, free of visible contaminants, lamination scale and corrosion[26]. Then, each coupon was washed with isopropyl alcohol and acetone, and subsequently sterilized through exposure to ultraviolet light for 30 min prior to utilization.

2.2. Produced water sampling and characterization

The produced water samples (60 L) were collected in a refinery located in the Northeast of Brazil in sterile plastic drums and immediately transported to the laboratory. The samples were subjected to physico-chemical analyzes initially (zero time) and after 360 days, according to the Standards Methods[27]. The parameters of pH, electrical conductivity at 25 ºC, salinity, sulfates, total sulfide, nitrates, dissolved iron, chlorides, oils and greases, total dissolved solids and total suspended solids were evaluated.

2.3. Bioreactors

Two static aerobic experimental systems containing produced water under sterile (abiotic) and non-sterile (biotic) conditions were used. The produced water used in the abiotic system was filtered through sterile membranes with a porosity of 0.22 µm and diameter of 47 ± 0.5 mm and 1 mg/L of sodium hypochlorite was added to maintain the medium sterility. Whereas, for the biotic system, produced water in natura was used, without any additional treatment. Both systems were maintained at room temperature (~ 25 ºC).

2.4. Microbiological analysis of sessile microorganisms

Bacterial cells were quantified in the period of 15, 30, 45, 60, 120, 240 and 360 days of exposure to the biotic system. The Most Probable Number (MPN) method was used to count the total anaerobic, acid-producing aerobic and anaerobic bacteria and sulfate reducing bacteria (SRB)[28] and the group's total aerobic and precipitating iron bacteria were quantified by the Colony Forming Unit (CFU) method[29,30]. This article is part of a series of studies, the quantification

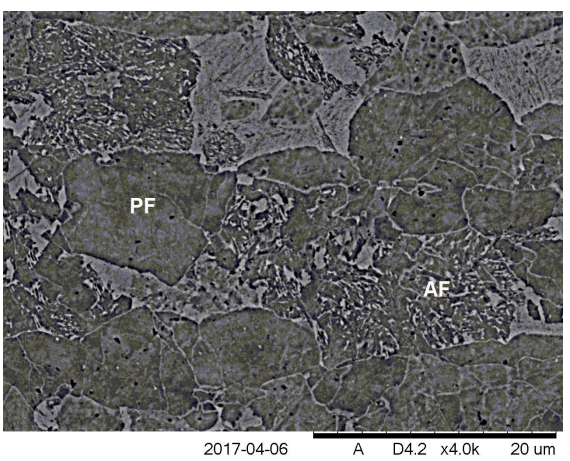

Figure 1. Microstructure showing acicular ferrite (AF) and polygonal ferrite (FP) of X80 steel (magnification 4000x).

Table 1. Chemical composition of X80 steel (%).

Element	C	Si	Mn	P	S	Cr	Ni	Mo	Cu	Al	V	W	Ti	Nb	Fe
X80 steel (%)	0.08	0.3	1.82	0.009	0.001	0.17	0.01	0.20	0.01	0.037	0.024	0.003	0.021	0.081	balance

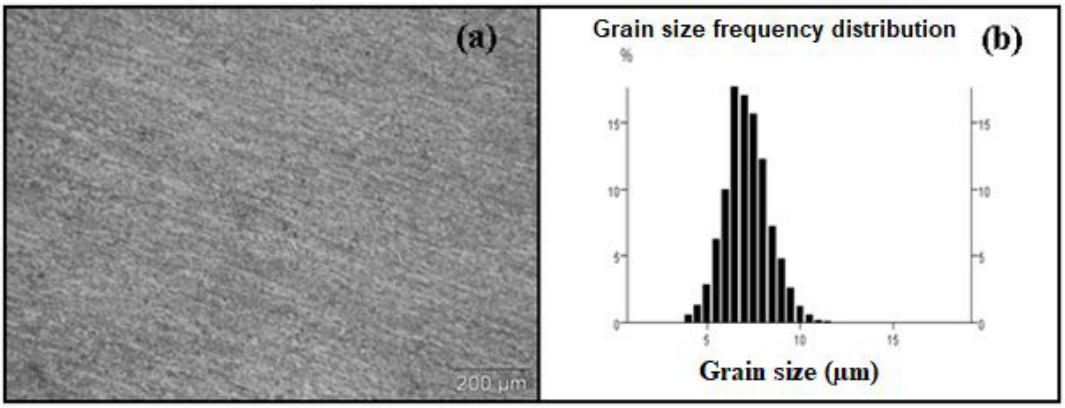

Figure 2. Grain size distribution of X80 steel. In (a) micrograph of the coupon with 100x magnification and (b) grain size graph versus percentage referring to the grain size.

Table 2. Planktonic microorganisms in the produced water[31].

Groups of microorganisms	Total aerobic	Total anaerobic	Acid-producing aerobic	Acid-producing anaerobic	Precipitating iron bacteria	Sulfate reducing bacteria
Cell concentration	1.2 x 10^4 (CFU/mL)	1.1 x 10^3 (MPN/mL)	7.0 x 10 (MPN/mL)	2.5 x 10^2 (MPN/mL)	Undetected	4.5 x 10^3 (MPN/mL)

Table 3. Culture medium composition.

Microorganisms	Composition (g/L)
Total aerobic bacteria	22.5 plate count agar (PCA) (pH 7.0)
Total anaerobic bacteria	30.0 fluid medium to thioglycolate (pH 7.0)
Acid-producing aerobic and anaerobic bacteria	10.0 sucrose, 10.0 tryptone, 1.0 beef extract and 0.018 phenol red (pH 7.2)
Sulfate reducing bacteria	0.5 KH_2PO_4, 1.0 NH_4Cl, 1.0 Na_2SO_4, 0.67 $CaCl_2.2H_2O$, 1.68 $MgCl_2.6H_2O$, 7.0 mL sodium lactate (50%), 1.0 yeast extract, 0.1 ascorbic acid, 1.9 agar-agar, 4.0 mL resazurin (0.025%) and 0.5 $FeSO_4.7H_2O$ (pH 7.6)[27]
Precipitating iron bacteria	30.0 NaCl, 0.5 $(NH_4)_2SO_4$, 0.2 $CaCl_2.2H_2O$, 0.5 $MgSO_4.7H_2O$, 0.5 $NaNO_3$, 10.0 ferric ammonium citrate and 0.5 K_2HPO_4 (pH 7.0)[28]

of planktonic microorganisms was published previously and it is shown in Table 2[31].

2.5. Microorganisms cultivation

Initially, dilution solutions (saline and reducing for aerobic and anaerobic microorganisms, respectively) were prepared. The saline solution was prepared with a concentration of 30.0 g of NaCl in 1 L of distilled water, and the reducing solution was prepared with 0.124 g of sodium thioglycolate, 0.1 g of ascorbic acid, 4 mL of 0.025% resazurin, 30.0 g of NaCl in 1 L of distilled water. All the culture mediums received 30 g of NaCl in 1 L of distilled water and the compositions used are shown in Table 3. The culture medium and the dilution solutions were autoclaved at 1 atm, 121 ºC for 15 minutes. The coupons were removed from the biotic system and placed in bottles containing 30 mL of reducing solution. Then, the biofilm was removed from the surface of the coupons using a sterile spatula. Subsequently, 1 mL of this solution was inoculated into the dilution solutions, followed by a reinoculation in the respective culture medium. All groups of microorganisms were incubated at 35 °C.

2.6. Film and biofilm characterization

After 15 days, the coupons were removed from the systems, the films and biofilms formed on the surface were analyzed by OM using the ZEISS® AXIO Zoom. V16 stereomicroscope. Then, they were immersed in bottles containing 5% glutaraldehyde solution in sodium cacodylate buffer (0.1 M) for 24 hours. After this period, the coupons were washed in 0.1 M sodium cacodylate for 30 minutes, so as to fix the films and biofilms formed on the surface, and dehydrated using acetone of different concentration (30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% v/v). The coupons had their surfaces metallized with gold (40 nm) in the Quick Coater metallizer, and then they were analyzed in the SEM TESCAN® MIRA 3, at 10 kV.

2.7. Weight loss measurement

Before being inserted into abiotic and biotic systems, the coupons were weighed on an analytical balance. After 15, 30, 45, 60, 120, 240 and 360 days of exposure to static systems, the coupons were removed and the films, biofilms and corrosion products were scraped from the surface with

a non-metallic spatula. The coupons were immersed in Clark solution, followed by ultrasonic cleaning in distilled water. Afterwards, they were cleaned in isopropyl alcohol and acetone, dried and weighed. The X80 steel corrosion behavior was assessed from the specific weight loss based on the coupon exposed surface area according to ASTM G1-03 standard[32].

2.8. Surface topography of X80 steel coupon

To examine the corrosion morphology, the coupon was removed from the biotic system after 45 days. Subsequently, the same procedure for cleaning and removing biofilm and corrosion products from the coupon surface was performed in the analysis of mass loss, previously described according to the ASTM G1-03 standard[32]. Then, two-dimensional (2D) and three-dimensional (3D) measurements were performed on the coupon surface using CLM ZEISS® Axio Imager Z2m.

3. Results and Discussion

3.1. Physico-chemical analysis of produced water

The physico-chemical characterization of produced water at zero time and after 360 days is given in Table 4.

It was observed that there was a variation in the parametric values with time (Table 4), and that can be related to the initial quantification of planktonic microorganisms presented in Table 2. In Table 4, after 360 days of exposure, a small reduction in the pH value was observed, due to the release of acidic substances by microorganisms. According to Borenstein[33] and Xu et al.[34], acid-producing bacteria release volatile acids (organic and inorganic) that cause acid corrosion. The sulfate content decreased due to the reaction of reduction of this substance by SRB transformed into sulfide, which increased in concentration. SRB obtain energy by reducing sulfates resulting in the release of sulfides or H_2S[35]. The nitrate content was also possibly reduced by the use of this compound as an alternative substrate to SRB. According to AlAbbas et al. and Dall'agnol et al., some SRB species, such as *Desulfovibrio desulfuricans*, can reduce nitrate as a substrate[36,37]. The chloride content showed a small increase, due to water evaporation and to the consequent concentration of dissolved salts since the static system was open. It is worth mentioning that chloride and sulfate ions can accelerate corrosion in pipelines[38]. The concentration of dissolved iron practically doubled, due to the oxidation reactions in the coupon, which reflected on the increase in the parameters of total dissolved and suspended solids. According to Igunnu and Chen, the solids produced in the produced water include corrosion products, precipitated solids, carbonates, among others[39]. The accumulation of these produced solids can cause serious problems such as the formation of emulsions and clogging of pipelines. The oil and grease content decreased due to the microbiological action on the biodegradation of hydrocarbons in the produced water. Some authors have reported that biodegradation is one of the biological treatment methods used to remove hydrocarbons in the produced water. Ergo, it has been considered an effective method of removing dissolved oil, although it fails when the salinity of the water is very high[40-43]. The rate of hydrocarbons biodegradation in the produced water is slow, requiring days to carry out the long period of chemical reactions[43]. Thus, the results show that the produced water is a dynamic medium of complex chemical composition, subject to variation as a function of time, due to ambient temperature conditions, contact with air, photo-oxidation, biodegradation, as well as other factors inherent in the studied system[2]. The literature states that variations in the physical-chemical parameters of produced water in oilfields may be related to seasonal aspects[44].

Table 4. Physicalchemical analysis of the produced water.

Parameters	Zero time	After 360 days
pH	6.82	5.91
Sulfates (mg/L)	230.00	28.80
Total sulfide (mg/L)	0.60	8.40
Nitrates (mg/L)	0.42	0.02
Chlorides (mg/L)	56.28	72.31
Salinity (mg/L)	88.70	-
Dissolved iron (mg/L)	5.78	10.60
Oils and greases (mg/L)	79.00	<10
Total dissolved solids (mg/L)	76.80	130.06
Total suspended solids (mg/L)	239.00	670.00

3.2. Cell concentrations of sessile microorganisms

Figure 3 shows the cellular concentration of sessile microorganisms after 15, 30, 45, 60, 120, 240 and 360 days of exposure to the biotic system.

Comparing the Table 2 and Figure 3, it can be seen that planktonic and sessile total aerobic bacteria was the group that reached the highest cellular concentration in the produced water (1.27×10^4 CFU/mL) and in the biofilm (7.39×10^4 CFU/cm^2), respectively, within 120 days of exposure to the biotic system. After this period, the cellular concentration of the total aerobic bacteria decreased significantly. Aerobic bacteria consumes oxygen dissolved in the fluid, which provides an anaerobic environment conducive to the growth of anaerobic bacteria[45,46]. Total anaerobic, acid-producing aerobic and anaerobic bacteria and SRB present initially in the produced water (Table 2), were also present in the biofilm (Figure 3). Thence, it indicates that planktonic bacteria promoted an effective adhesion, with the development of biofilm along with a high microbial diversity. SRB are primarily responsible for microbiologically induced corrosion in the oil industry's operating systems. The planktonic and sessile precipitating iron bacteria were not detected at 15 days by the CFU technique shown in Table 2 and Figure 3, respectively. This was probably due to the low concentration of iron (5.78 mg/L) in the produced water and the relatively short time for the iron degradation in the coupons. Hereupon, the iron is a compound that generates energy for the metabolism of the precipitating iron bacteria. The biomineralization of iron by the precipitating iron bacteria occurs through oxidation reactions converting the ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) and oxygen as the final electron acceptor[47]. According to Bazaka et al. the adherence of live cells to surfaces depends on the properties of this surface and the

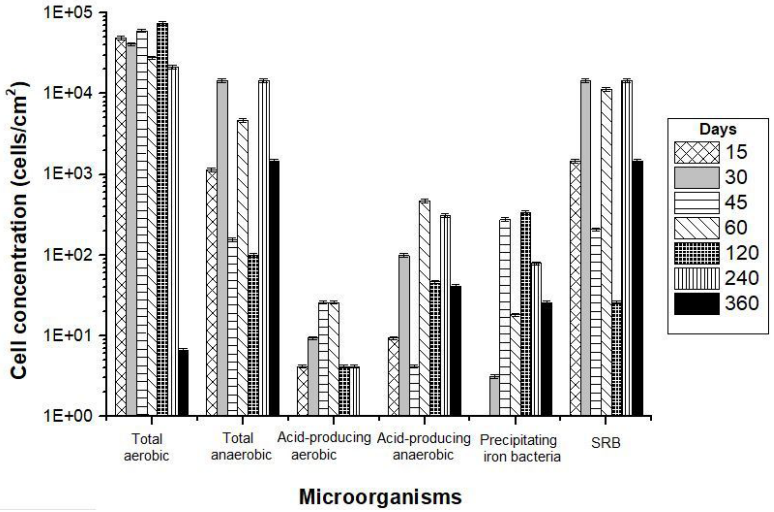

Figure 3. Cellular concentration of sessile microorganisms quantified by the Colony Forming Unit (CFU) and Most Probable Number (MPN) techniques.

characteristics of the medium (chemical composition, availability of nutrients, presence of other colonies, temperature, concentration of antimicrobials and metabolic products)[48]. Another probable cause for the non-detection could be a limitation of the plate counting technique, that is, the bacterial colonies did not develop due to the culture conditions that were not suitable for microbial growth[29]. After 30 days of exposure to produced water, sessile precipitating iron bacteria were detected in the biofilm, with an estimated cell concentration of 3.11 CFU/cm^2. After the formation of corrosion products from the oxidation reactions of iron, there was a greater availability of this compound in the fluid, which led to the growth of these bacteria. After 45 days of exposure, there was a decrease in cell concentration, of two orders of magnitude, in all groups of sessile anaerobic microorganisms, probably due to the lack of nutrients and/or the formation of toxic metabolites such as H_2S in the system. Among the various sulfur compounds produced by sulfate reducing bacteria after metabolizing sulfate, H_2S is also produced. In this regard, it is considered the most toxic form of sulfide. This molecule can penetrate the plasma membrane and react with cellular components within the cytoplasm of microorganisms. In addition, it causes serious damage to oil production systems[3,35,49]. Besides hydrogen sulfide, other toxic metabolites can be produced such as: enzymes, exopolysaccharides, organic and inorganic acids, and ammonia[50]. At 60 days there was a decrease in the cellular concentration of the groups of aerobic sessile microorganisms, except for aerobic acid-producing bacteria, and an increase in the cellular concentration of all groups of anaerobic sessile microorganisms. The biofilm for being a dynamic microenvironment provides conditions that may be favorable or unfavorable to the growth of a given microbiological group. The consumption of oxygen by aerobic bacteria establishes the formation of anoxic regions within the biofilm, which favors the colonization of anaerobic bacteria and forms cells of differential aeration[13,36,51]. According to Liu et al., the iron oxidizing bacteria inhibited the growth of planktonic SRB in different produced water systems, but they promoted the growth of sessile SRB due to their oxygen consumption after biofilm formation in the biocorrosion of Q235 carbon steel[45]. After 120 days, there was an increase in the cellular concentration of total aerobic bacteria and sessile iron precipitants. The precipitating iron bacteria increased by an order of magnitude, possibly due to the large amount of iron dissolved in the produced water from the oxidation of the metal. In the same period, there was a reduction in the cellular concentration of aerobic acid-producing bacteria and of all groups of sessile anaerobic microorganisms, caused by the decrease of nutrients in the produced water. After 240 days, all groups of aerobic microorganisms decreased their cellular concentration of sessile, except for aerobic acid-producing bacteria that had a concentration similar to the period of 120 days. Meanwhile, all groups of anaerobic microorganisms increased their concentration, in relation to the analysis made at 120 days of exposure to produced water. At 360 days, all groups of bacteria showed a reduction in cell concentration, except for aerobic acid-producing bacteria that did not survive the system conditions. Among the microorganisms investigated, the interaction between aerobic bacteria and SRB was explicit, with concentrations around 10^4 cells/cm^2. SRB can be related to acid-producing bacteria through a synergistic effect, where the secreted acids can be metabolized by SRB[52]. This behavior throughout the exposure period reflects the adaptation of the bacteria to the produced water medium. Furthermore, it showed the interaction and cooperation among different groups of microorganisms, which allowed the biofilm to survive.

3.3. Analysis of the film and biofilm by OM and SEM

Figures 4 and 5 show images by OM and SEM, respectively, of the film and biofilm formed on the surface of the X80 steel coupons after 15 days of exposure to the abiotic and biotic systems, respectively.

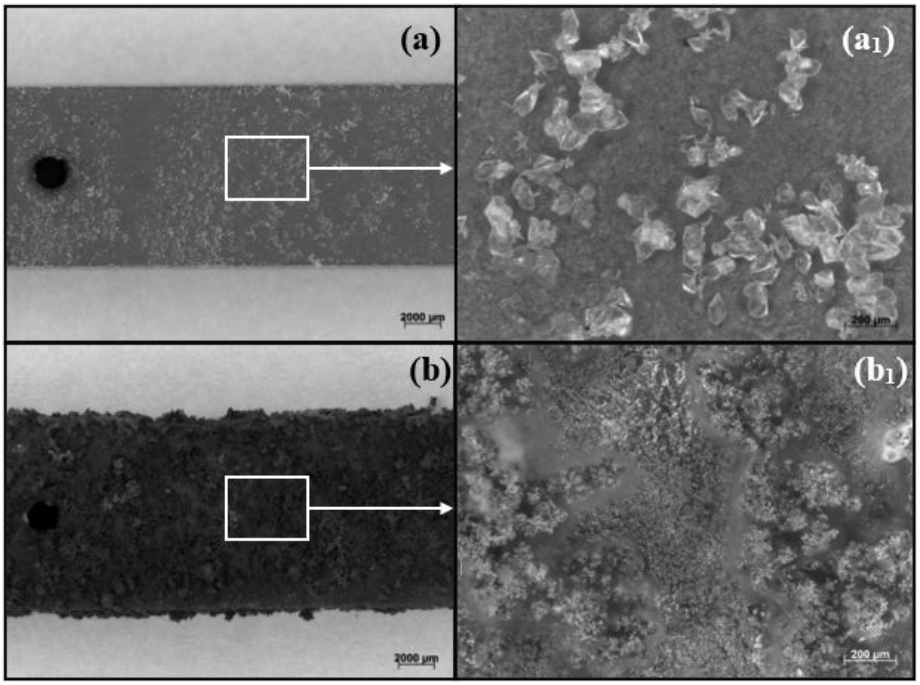

Figure 4. Macrographs of the film and biofilm formed on the coupon surface after 15 days of exposure to abiotic (a and a_1) and biotic (b and b_1) systems, respectively. Magnification of 7x (a and b) and 100x (a_1 and b_1).

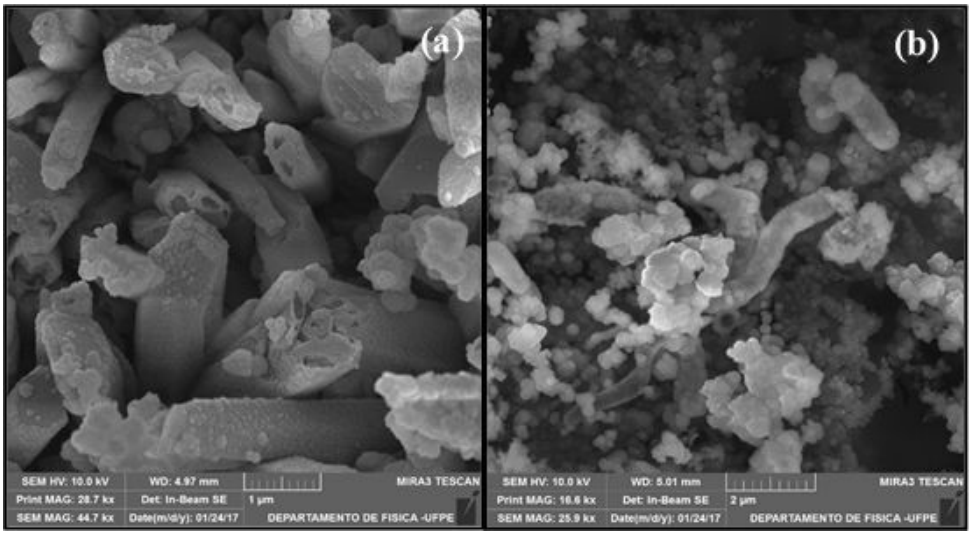

Figure 5. Micrographs of the film and biofilm formed on the coupon surface after 15 days of exposure to (a) abiotic and (b) biotic systems. Magnification of 44.700x (a) and 25.900x (b).

In Figure 4a and (a_1) it can be seen that there was a deposit of salts (crystals) not uniformly distributed on the surface of the coupon exposed to the abiotic system. The micrograph of this surface shown in Figure 5a shows the morphology of the salts without the presence of microorganisms. The produced water is generally acidic in nature and it has a high concentration of dissolved salts that are responsible for increasing corrosion in pipelines. According to the literature, usually the chloride anion is usually in greater quantity, whereas sulfate (SO_4^{2-}), carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) are in small amounts in the produced water. The most commonly found cations are sodium (Na^+), potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+})[53-56]. In Figure 4b and (b_1), corrosion products with non-adherent and porous aspect are observed on the coupon surface exposed to the biotic system. Generally, the upper part of the corrosion products has compounds such as ferric hydroxide ($Fe(OH)_3$) and iron oxide (Fe_2O_3) because it is an area of

greater contact with oxygen. At the bottom of the corrosion product, which is a less oxygenated area, compounds such as ferrous hydroxide ($Fe(OH_2)$), anhydrous magnetite (Fe_3O_4) and mackinawite (FeS) are often found[57]. In this case, due to the presence of microorganisms, the formation of FeS is typically associated with the production of sulfide by SRB metabolism[58,59]. The formation of deposits is one of the most common damages within pipelines, injection wells and the production of produced water in the oil industry. In addition to various salts, oxides, silicates and phosphates, the deposits may consist of calcium and iron carbonates, barium and strontium sulphates, and compounds insoluble or poorly soluble in water[56]. Figure 5b shows the heterogeneous and porous biofilm with rod-shaped bacterial cells surrounded by exopolysaccharides and corrosion products. The non-uniformity of this biofilm generates cells of differential aeration that favor the growth and adhesion of different groups of microorganisms (aerobic and/or anaerobic). Additionally, the differential ionic concentration, through the access of the electrolyte to certain areas of the biofilm, can cause localized corrosion[47]. The development of biofilm is an extremely variable process controlled by hydrodynamics. The interaction with the substrate through the adhesion of microorganisms, the development of the thickness of the biofilm, as well as its detachment are the steps of structuring the biofilm in pipelines under the strong influence of the flow. Inside the pipelines, the flow speed influences the mass transfer and, consequently, the shear stress on the wall, facilitating or making it difficult to fix the biofilm to the substrate[21,60].

3.4. Weight loss

Figure 6 shows the weight loss data of the coupons exposed to the abiotic and biotic systems for 15, 30, 45, 60, 120, 240 and 360 days.

In Figure 6, it can be seen that the X80 steel coupons showed lower values of weight loss when exposed to the abiotic system compared to the biotic system. During the exposure period, little variation was observed in the weight loss of the coupons of the abiotic system, while there was a gradual increase in the weight loss when the coupons were exposed to the biotic system. In the biotic system, the corrosivity of the produced water was possibly added to the microbiologically induced corrosion, potentiating the effect of corrosion on X80 steel coupons. The biodegradation of the metallic substrate probably occurred due to the physiological activity of the microorganisms that can cause different mechanisms of corrosion (cathodic depolarization, differential aeration, acid production and joint action of bacteria) varying according to the microbial species and chemical composition of the colonized metallic substrate[9,50,57]. SRB promote cathodic depolarization by removing hydrogen from the cathode region of the metal substrate through hydrogenase enzymes[10]. At the same time, dissimilar sulfate reduction occurs, in which SRB use inorganic sulfate as an electron acceptor, for the oxidation of the substrate resulting in the production of various sulfur compounds (sulfides (S^{2-}), disulfides (HS^-), hydrogen sulfides (H_2S), thiosulfates ($S_2O_3^{2-}$) and polyithionates (SnO_6^{2}))[61,62]. Other corrosion mechanisms caused by SRB are anodic depolarization, iron sulfides, volatile phosphorus compound, Fe-binding exopolymers, sulfide-induced stress corrosion cracking and hydrogen-induced cracking[50]. According to Gentil, corrosion by differential aeration occurs after the formation of the biofilm. At the base of the biofilm, the diffusion of oxygen is made difficult for it creates adverse conditions for some microorganisms, such as aerobes, and enables the development of anaerobic bacteria. This diffusional gradient allows the appearance of differential aeration cells that promote corrosion under the biofilm[57]. Corrosion due to acid formation occurs when acid-producing bacteria secrete organic acids (acetic, isobutyric, succinic, among others) or inorganic acids (sulfuric), which promote electrochemical oxidation of metals[57,63]. These metabolites can increase pitting corrosion of carbon steel materials[12,37]. Sulfur oxidizing bacteria (eg., *Acidithiobacillus caldus*, *A. thiooxidans* and *A. albertensis*) are able to oxidize sulfur or sulfur compounds (sulfite, thiosulfate and tetrationate) to sulfate, and produce sulfuric acid making the medium even more corrosive. Although most cases are related to corrosion, these bacteria can play positive roles in the oil industry by removing H_2S and acid gases in oil reservoirs, in addition to treating sulfide-contaminated water from the secondary oil recovery process[64,65]. The corrosion by joint action of bacteria occurs when simultaneous activity of different bacterial species is observed[57]. In the pipelines, iron oxidizing bacteria oxidize ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) producing precipitates of ferric oxide ($Fe_2O_3.H_2O$) or ferric hydroxide ($Fe(OH)_3$). Underneath the iron deposits, a favorable anaerobic environment is established, and SRB will colonize it later by producing S^{2-} which reacts with Fe^{2+} forming FeS[45,51,57]. In this case, the corrosion in the substrate is even greater due to the simultaneous action of bacteria that promote the dissolution of iron with the formation of pits[45].

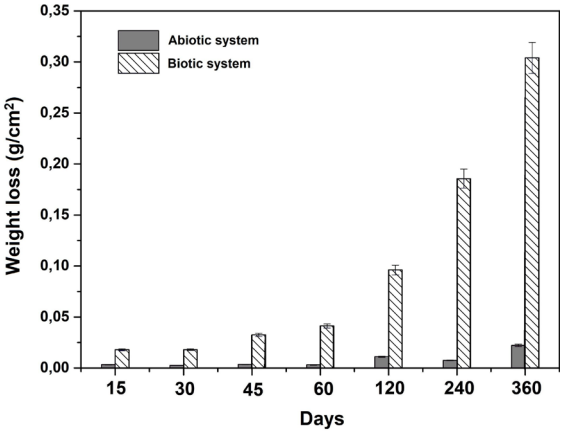

Figure 6. Weight loss of coupons X80 in produced water.

3.5. Surface topography

Figure 7 shows the pitting of corrosion formed on the surface of the X80 steel coupon after 45 days of exposure to the biotic system. The two-dimensional (2D) depth map represents the area of the pit on the surface shown in Figure 7a. The profile shown in Figure 7b indicates that the pit is 32.87 µm wide and 12.42 µm deep with elliptical shape, according to Standard ASTM G46 – 94[66]. The three-dimensional (3D) topography and the corresponding cross section of the pit

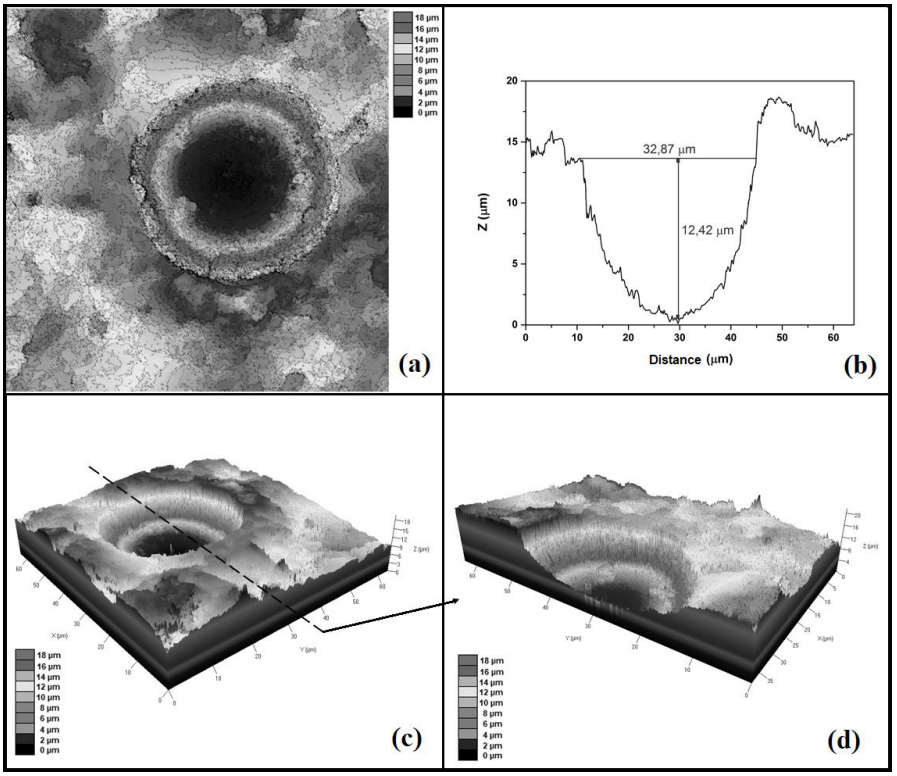

Figure 7. Pit formed on the surface of the X80 steel coupon after 45 days of exposure to the biotic system. In (a) 2D analysis, (b) profile of the pit, (c) 3D topography and (d) cross section of the pit in 3D

can be seen in Figure 7c and d, respectively. It was found that the surface of X80 steel exposed to the biotic system showed pitting, probably due to the contact with aggressive substances from the metabolic activity of microorganisms present in the biofilm. Previous results revealed that the surface of the coupons from both systems showed localized or pitting corrosion morphology[31,67]. However, the coupons from the abiotic system exhibited almost imperceptible pits, while the coupons from the biotic system showed pits containing larger diameters. In this biological system, the action of microorganisms within the biofilm accelerated localized corrosion and promoted a significant weight loss in the coupons.

4. Conclusions

A wide variety of biofilm forming microorganisms has been detected in produced water in oilfields and has been associated with the evolution of biocorrosion in X80 steel. The total aerobic bacteria were in a higher cellular concentration in the biofilm, influencing the process of microbial adhesion to the substrate and in the development of the biofilm. SRB were present in the biofilm, but at concentrations lower than the total aerobic bacteria. The images by OM and SEM associated with the measures of weight loss and depth of the pit confirmed that the corrosion of X80 steel was accelerated by the presence of microorganisms in the produced water. X80 steel was susceptible to pitting corrosion, because of the occurrence of MIC underneath the biofilm due to microbiological involvement.

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