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Biofilm Formation and Corrosion on Carbon Steel API 5LX60 in Clayey Soil

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Corrosion of buried pipelines is a matter of concern to the oil and gas industry since the time when carbon steel began to be widely used in these pipelines for the transportation of fluids. The microbial communities associated with biofilms promote modification in the surrounding environment and may accentuate the degradation of oil and gas pipelines causing leaks or even accidents. This work aimed to evaluate corrosion and biofilm formation in carbon steel API 5LX60 coupons buried in clayey soil from an industrial region in north-eastern Brazil. The average corrosion rates were determined by gravimetric test and the quantification of bacteria and fungi were using the Most Probable Number (MPN) and Colony Forming Units (CFU) techniques respectively. The results showed a great influence of clayey soil on corrosion rates and time of adherence for microorganisms on metal surfaces.

Keywords: *biofilm, corrosion, pipelines, soil, microorganisms.*

1. Introduction

Most oil, gas and sanitation companies use thousands of kilometres of steel pipes to transport crude oil, natural gas, fuel, chemicals, fresh water and seawater [1]. The corrosion of buried pipelines can result in their rupture, causing severe environmental damage and economic loss[1,2].

Soil can be defined as a superficial layer of the Earth's crust formed by organic and mineral compounds in which there are several microbial communities. Most of the time, microorganisms are in the sessile state (solid surface cells), resulting in the formation of ecosystems with different complexities, composing dynamic structures called biofilms[2,3]. Due to the complexity and heterogeneity of soils, it is fundamental to study their physical, chemical and microbiological characteristics, as well as the influence of these characteristics on the material used in pipe manufacture[1,2,4].

Biofilms are highly complex heterogeneous ecosystems composed of microbial cells inserted in an extra cellular polymer matrix (EPS) of microbial origin[2]. The presence of biofilms causes changes in the metal/environment interface, favouring the corrosive process [2,5]. Biofilm formation and bacterial adhesion are phenomena that occur in natural environments and in several kinds of industry[5,6]. Carbon steel has been the most used material in most of the industrial segments (widely used in gas pipelines and civil construction) due to its properties, practical utility and low cost when compared to other types of materials[7,8]. However, carbon steel has certain limitations in its use, especially when resistance to corrosion, heat and wear, as well as particular electrical or magnetic characteristics and exposure to the microbial environment are required[8].

The major responsibility for microbiologically induced corrosion (MIC) is attributed to sulphate-reducing bacteria (SRB), since during the metabolism of this bacteria there is a release of high amount of sulfuric acid a reactive, toxic, and corrosive agent[9,10,11]. Other microbial populations contribute to SRB activity and to create anaerobic conditions, such as acid-producing bacteria and iron-precipitating bacteria [12], respectively.

Due to the economic losses and environmental damage caused by the deterioration of steel pipes installed under the ground, there is a great incentive to investigate the physicochemical and microbiological characteristics of the soil, showing that microbial groups affect the studied area. Thus, this work was designed to assess the biofilm formation and corrosion of carbon steel coupons API5LX60 for period 15 days in clayey soil samples collected in Pernambuco and used in the Abreu and Lima Refinery. The results were expected to make it possible to set up strategies for monitoring and controlling corrosion.

2. Methodology

2.1. Coupons

We used coupons API 5LX60 steel with dimensions of 25x25x3 mm in experimental tests with chemical composition of 99.38% Fe, 0.21% C, 0.46% Mn, 0.23% Si, 0.005% S, 0.022% P and 0.024%Al, 0,06% Cr, 0,01% Ni, 0,02% Mo, 0,001%V, 0,002%Ti and 0,001% Nb. To standardize the metal surface by eliminating corrosion products, preferential corrosion areas, sharp edges, scratches, and other heterogeneities that may enhance corrosion processes, the metal coupons

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were blasted with glass beads. Then, they were immersed in isopropyl alcohol and acetone, dried in an oven at 70°C for 30 minutes, taken to a desiccator for 20 minutes, and then weighed. Only after this process, the coupons were considered suitable for the experiments. Some coupons were observed by SEM (scanning electron microscopy) using a Hitachi TM 3000 scanning electron microscope.

2.2. Characterization of the soil samples

Microbiologic soil parameters: For SRB characterization, reducing solution and modified Postgate E medium were used[13] Total anaerobic bacteria quantification also used reducing solution and fluid thioglycollate[14] These bacteria were incubated for 28 days. The heterotrophic aerobic bacteria were grown in a saline solution[15], incubated for 48 hours. The precipitating iron bacteria was prepared used ferric ammonium citrate green[16] culture medium and were incubated for 15 days. The acid producing bacteria were quantified in a series of three tubes containing phenol red broth[17] and were incubated for 24/48 hours. The analysis for identification of bacteria aerobes and anaerobia used the MPN technique. The filamentous fungi were quantified by counting colony forming units (CFU), using the technique "pour-plate" in Petri dishes containing agar sabouraud[15] and incubated at 30°C for 5-7 days.

Physicochemical soil parameters: The contents of both sulphates and chlorides were obtained using the methodology described in Vogel[18] (1981). Resistivity measurements were performed according to GCOI technical / SCM / 95 (1995)[19]. Resistivity was measured using a standard soil cash box (6.2 cm long, 2.5 cm, and 2.5 cm wide), with a Digimed meter mark DM- 3P model. Distilled water was added to the sample in the ratio of 5% (v / v), in relation to the mass of dry soil, and the resistivity was measured continuously. Soil redox potential was determined using a platinum counter-electrode and copper copper-sulphate ($Cu/CuSO_4$) reference electrode (Costanzo & McVey, 1958)[20]. The methodology described in Fries & Getrost[21]was used for iron oxide analysis. Geotechnical characterizations were performed according to standard procedure[22]. Soil pH was determined by the potentiometric method for soil suspension, in distilled water (ratio 1: 2.5), using a pH meter (Model Mark Quimis Q400MT) and the determination of organic carbon in the samples were carried out according to the methodology of EMBRAPA soils[23]. An appropriated instrument for soil measurement (FALKER HFM 2010) was used to monitor the humidity. In addition, using the results of the above analyses, the corrosion rate was measured according to the classification of the standard NACE- RP-07-75[24].

2.3. Experimental tests for the kinetics of biofilm formation

The experimental tests were carried out in bioreactor consisting of a plastic box with dimensions: 52x36x18cm, and nominal capacity of 100 kg. Test coupons were placed in this box separated by 5 cm. The decision to do this was based on the water retention capacity and the textural characteristics of the soils. The tests were performed over 15 days, with the removal of the coupons every 48 hours. It should, however, be noted that the first sampling was performed within the first 24 hours. Only after the second removed the interval was increased to 48 hours. For the quantitative determination of sessile microorganisms and determine the corrosion rates by gravimetric analyses, the coupons were removed, shaved and cleaning by a chemical stripping procedure according to standard procedure[25]. Figure 1 shows the experimental system with the four buried carbon steel coupons.

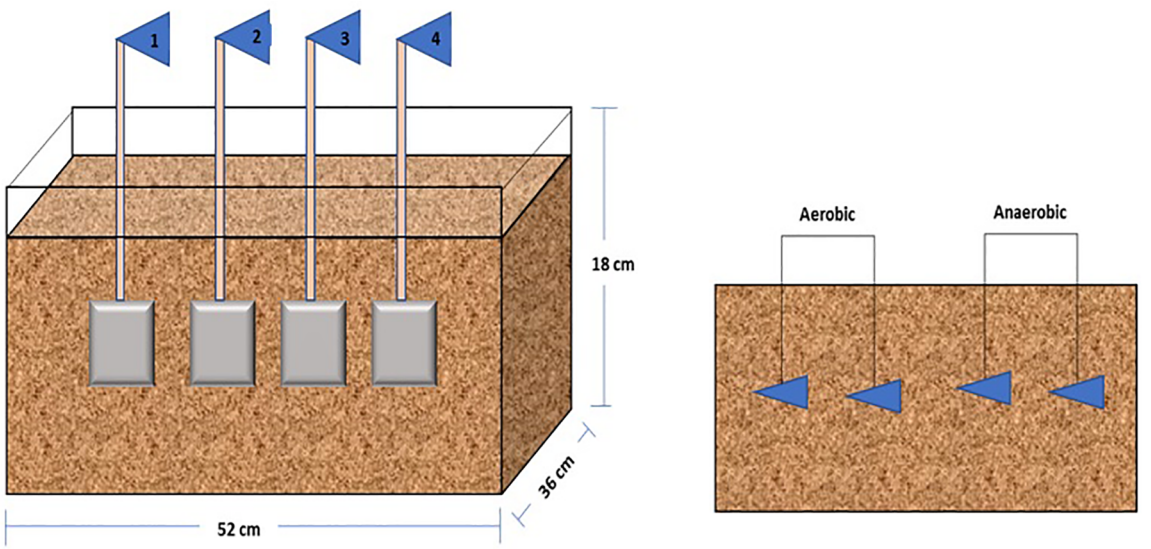

Figure 1. a) Illustration showing bioreactor with clayey soil and four buried carbon steel API 5LX60 coupons; b) Four coupons are removed and divided for aerobic and anaerobic analysis

3. Results and Discussion

3.1 Granulometric and physicochemical characterization of the soil

Table 1 shows the physical-chemical analysis and grain size of clayey soil, relevant parameters for microorganism activity. According to the soil particle size, this soil possesses 35% of clay content, classifying it as clayey soil in accordance with the Feret triangle[26]. The soils with clay content near 12% maintain the microorganisms and substrates within the pore space, favouring enzymatic activity[27]. The percentage of fine particles in a soil matrix affects its water retention capacity; the higher the percentage of fine particles, the higher the water retention[28]. The clayey soil contained 57% fine particles, giving it higher water retention capacity. The organic carbon content in clayey soil was 0.325 mg/kg. Carbon variations due to the microbial activity that was used for maintenance and the low amount of carbon suggests that the microbial metabolism would be restricted. The clayey soil was characterized as acid; its pH was around 4.4 ± 0.2. The pH of most soils is around 4.0 and 8.5[29] and factors such as acid rain, soil fertility and biological nitrogen fixation can lower soil pH[30]. Soil pH below 5.5 is generally a sign of low availability of calcium, magnesium and phosphorus. Nitrogen content was 11.15 mg/kg[31,32,33]. Sulphur content is important because if sulphate-reducing bacteria are present in the soil, their metabolism is stimulated since the microbial species in this group are able to assimilate a wide variety of carbon sources. There was an absence of chloride ions. It is known when there is chloride, the aggressiveness of an environment is greater because this ion has the ability to adsorb at points of greater instability in the passive layers that form on the metal surface, causing rupture of this layer and, consequently local metal attack (localized corrosion)[9]. A correlation can still be made with respect to the relationship between moisture and resistivity. Higher percentages of water favour the electrical conductivity in the soil, which consequently decreases its resistivity[9]. The positive measure of the redox potential shows that the medium has more oxidizing than reducing elements. From these characterizations, the soil corrosivity can be evaluated. The clayey soil was evaluated according to the criteria of the *Steinrath Index*, which is evaluated by parameters such as resistivity, redox potential, pH, chloride ions, sulphate ions, and sulphide ions[34,35]. The parameters analysed in Table 1 showed that the clayey soil was highly aggressive to the metal alloy.

3.2 Microbiological characterization of the soil

Density of the microbial population is one of the main factors for the colonization of solid surfaces, since the greater the number of microorganisms, the greater the probability of their contact with the material and, consequently, of its colonization, initiating the process of microbiologically induced corrosion (MIC)[9]. Figure 2 show a diverse microbial population of bacteria and fungi, and there was no correspondence to the nutritional conditions of the soil.

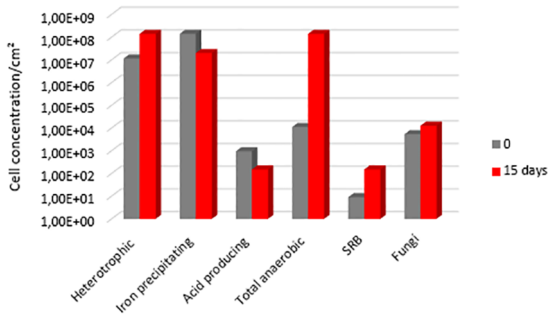

Figure 2. Microbial population of clayey soil at the beginning and the end of 15 days

There was the predominance of heterotrophic bacteria, precipitating iron bacteria and total anaerobic bacteria in the samples of clayey soil. The presence of acid producing bacteria, SRB and fungi could also be visualized. In general, at the beginning of the experiments the values of cell concentrations were smaller than after 15 days of process except for the acid-producing bacteria and iron precipitating bacteria that had a slightly higher value.

Heterotrophic bacteria are important in the corrosive process because they favour microbial adhesion to a metal surface due to increased extracellular polymeric substances (EPS)[9]. The showed that these bacteria were present during all the time in big amount. The SRB also were important because they promoted the process of MIC.

3.3 Corrosion test

3.3.1 Biofilm formation in carbon steel API 5 LX 60

Figure 3 shows the graphics of evolution kinetics with the cellular quantification of bacteria and fungi related to time. Microbiological analysis in the biofilms revealed the presence of all group of bacteria and filamentous fungi that were shown in the microbiological analysis of soil. In Figure 3, it is possible observe distinct variation in microbial populations, both qualitatively and quantitatively.

Table 1. Physical-chemical analysis and grain size of clayey soil.

Soil type	Resistivity (ohm.cm)	Redox (mVenh)	Humidity (%)	pH	Chemical composition (mg/kg soil)					Grain size of clayey soil (%)		
					Cl^-	SO_4^{-2}	C	P	N	Silt	Clay	Sand
Clayey	2000	110.5	17.4	4.55	abs.	1.09	0.325	0.06	11.15	8	35	57

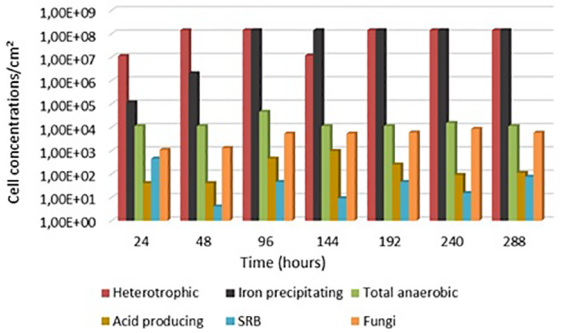

Figure 3. Cell concentrations of heterotrophic aerobic bacteria, iron-precipitating bacteria, total anaerobic bacteria, acid producing bacteria, SRB and fungi in clayey soil with the time

The clayey soil showed high cellular concentrations for heterotrophic aerobic and iron precipitating bacteria with values in the range of 10^7- 10^8 and 10^5- 10^8, respectively over the period of 15 days. For the same bacteria, over 24 hours there were lower cell concentrations, as expected, due to the low adhesion of the microbial groups to the metal surface. The producing acid bacteria, SBR and total anaerobic bacteria also were identified over the 24 hours, but with cell concentrations in the range of 10^1-10^2, 10^0-10^2 and 10^4, respectively. The presence of fungi also was detected in the range of 10^3 cell concentration/cm^2.

The total anaerobic bacteria and fungi had constant behaviour over all the times. Fungi are very important because their presence favours the consolidation of biofilm growth, with their hyphae forming structures to keep bacteria adhered to the biofilm as a mesh[36]. The SRB and producing acid bacteria varied during the process; in the beginning, the cell concentration of SRB was higher than acid producing bacteria. After 48 hours, the cell concentration changed and the acid producing bacteria enlarged and remained like this until the end of the process. Is important to clarify that the producing acid bacteria possess a relationship with the SRB, the acid secreted by them can be metabolised by the SRB. Thus, the synergistic effect of these two groups of bacteria can enhance biocorrosion[37]. The SRB is widely distributed in nature and are by far one of the most widely reported and studied bacteria in relation to MIC[38,2]. According to Moreira and Siqueira[29] the microorganisms interact and can have positive or negative effects. The negative effect is in relation to the consumption of nutrients necessary for survival; and the positive effect would be the prevention of growth of a single niche.

The initial concentrations of SRB and aerobic heterotrophic bacteria promoted the formation of biofilms. The concentrations of bacteria colonizing the coupons (sessile bacteria) was proportional to those present initially in the soil. Fassarela et al. (2007)[39,9] related that in general the number of microorganisms present in newly formed biofilms is correlated to the concentration of the respective microorganisms in the plankton phase, as is in the case of aqueous media. This information can be validated for soils, but should be considered that in soils, the water circulation is greatly reduced, even in moist soils[9].

With the results, it is possible to understand the dynamics of biofilm formation and observe that the early days of exposure in soil were sufficient to enable the adhesion of microorganisms to metal surfaces. In general, the microbial growth peaked at 96 hours. We can see also that with respect to the buried coupons colonization was related to quantity and dynamics of microorganisms in the soil.

3.3.2 Corrosion rate analysis

Figure 4 presents an image of the metallic surface of the API 5L X 60 after 15 days of contact with clayey soil. Without the particles adhered, the product of corrosion can be seen with green, brown and red brick colours, characteristics of iron oxide and iron hydroxides on the surface of the sample and some pitting attacks. The formation these colours on the surface of the metal is due the presence of oxygen traces. After contact with the oxygen, it becomes greenish. The oxidation process is fast, also being able to generate products of brown coloration, according to the iron (III) content[40]. The pitting attacks shown on the metallic surface in the presence of SRB is similar to the shapes of pitting commonly attributed to MIC[11].

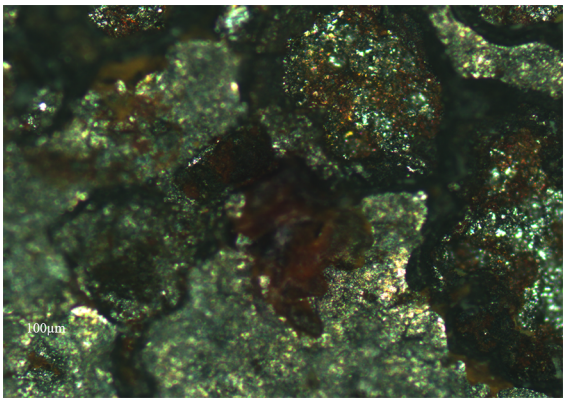

Figure 4. Image of the metallic surface of the API 5L X60 alloy in clayey soil with formation of corrosion products and pits formed on the metal surface

Figures 5(a) and 5(b) show the results of corrosion rate obtained by gravimetric tests, using four coupons per measurement point over the 15 days; also, the results of iron oxide analysis that was realized to quantify the oxide formed on the metallic surface, respectively. According to NACE-RP-07-75[23], the corrosion rate of soil was considered to be high and severe; the graphic shows a decay of corrosion rate of steel coupons. This decrease is due to the formation of biofilms on the metallic surface. At the beginning, the corrosion rate is higher due the fact that the metal was clean and the reactions are favoured.

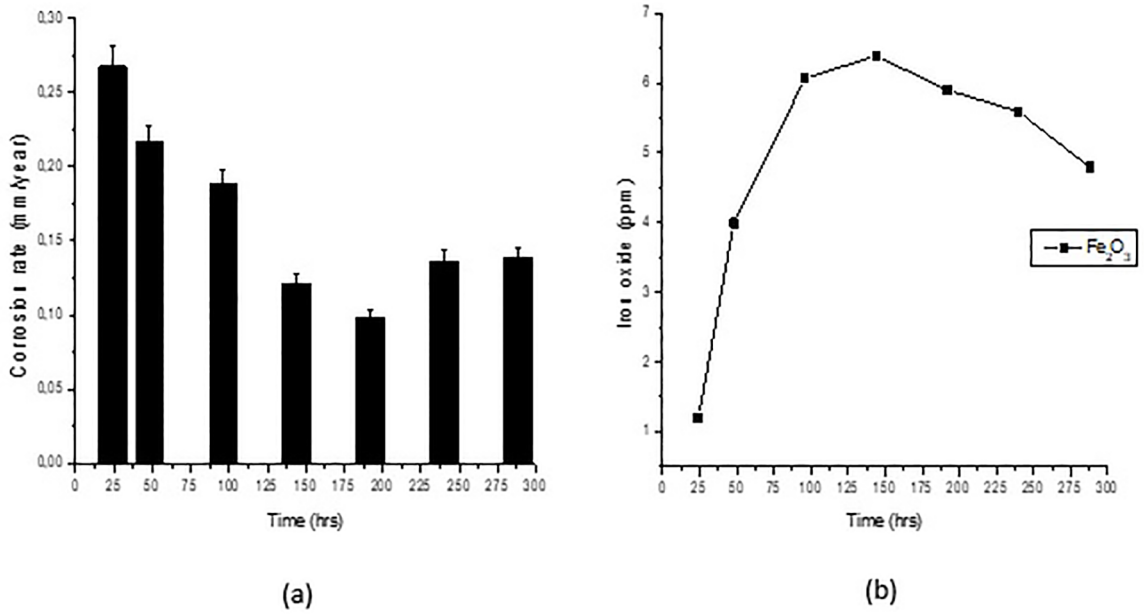

Figure 5. a) Corrosion rate with exposure time of API 5LX60 carbon steel coupons in clayey soil b) Quantifying the oxide formed on the metallic surface

After some time, the corrosion rate stabilizes and this can be seen over times of 240 and 288 hours. The values of iron oxide analysis showed that in 144 hours there was an increase of the iron oxide to maximum concentration of 6.3 ppm. With the formation of iron oxides and iron hydroxides a passivation layer can be generated, protecting the metal from the corrosion process[41]. Previous studies also have shown that the iron sulphide film formed in the presence of SRB can be protective in some cases and provide inhibiting effect on the corrosion of carbon steel coupons[11,42].

A white test with 0.75% sodium azide also was realized and the galvanic corrosion rate (in the absence of microbial population) reached the value of 0.0066 mm/year for clayey soil. This test confirmed that the microorganisms contributed actively to the corrosive process.

3.3.3 Surface analysis of API 5LX60 carbon steel

Figure 6 show the micrographs of the coupons with the SEM analysis. The micrograph shows clearly that the coupons display a lot of material distributed non-uniformly in the metallic surface of carbon steel API 5LX60. The presence of microbial cells was not visualized, possibly due to the large amount of clayey adhered to the surface; but the presence of polymeric material could be observed. The formation of this polymeric material is due the presence of iron. The SRB vigorously metabolize and

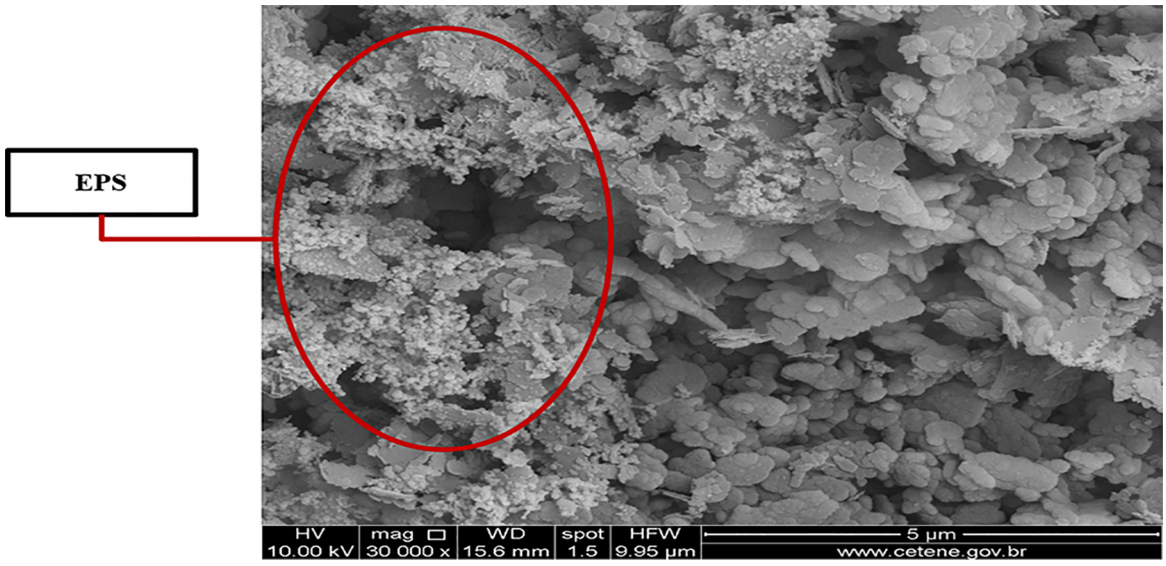

Figure 6. Microscopy of API 5LX60 carbon steel coupons with biofilm clayey soil

produce large quantities of EPS, which rapidly adhere to metal surfaces and form a dense biofilm[43].

Rodrigues (2010)[9] also showed that in the samples of the surface have a heterogeneous distribution of deposited material and the presence of microorganisms is not perceptible. Non-disclosure of adhered microbial cells is possibly due to the presence of the large amount of surface adhered material or carbon metallization.

Romero et al. (2008)[44] also studied biofilms in soil and attributed the non-visualization of adhered bacterial cells to their location, probably below the corrosion products formed. By analysing the graphs, the deposits containing the largest amounts of iron were formed on the coupons buried in soils containing the highest concentration of BRS, regardless of whether they were protected or not.

4. Conclusion

In this study, the microbial groups existing in the soil and on metallic surfaces were quantified by biofilm formation. Six microbial groups were identified in the clayey soil samples and all these microorganisms contributed to the process of bio-corrosion. In the study of biofilm formation over a period of 15 days, the first 24 hours of exposure of the soil coupons were seen to be sufficient to promote the adhesion of micro-organisms. The microbial groups studied adhered well to the surface of the metal material, promoting the formation of a dense biofilm with extracellular polymeric material production. These results lead us to conclude that the microbiota present in the studied soil contributed actively to the corrosive process of API 5LX60 steel, evidencing the need to apply some protective media, such as implementation of biofilm monitoring techniques and visual inspection of biofilm accumulation, use of biocidal agents to penetrate the biofilm and use of physical treatments. These actions can increase the useful life of this metal alloy under the conditions studied.

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