ABSTRACT
This study evaluates the soil corrosivity of API5LX60 carbon steel buried in two different soil textures from the area of Port of Suape - PE / Brazil under the influence of the soil’s associated microbial community. The evaluation of the corrosivity of both types of soil was based on the Steinrath Index. The clayey soil was considered highly aggressive and the sandy soil of low aggressiveness. The evaluation of pitting corrosion showed similar average pit depth for both soils. The DGGE / PCR technique indicated that the clayey soil presented greater microbial diversity.

KEYWORDS
biocorrosion; soils; corrosivity; microorganisms; 5LX60 API
1. INTRODUCTION

The industries of oil, gas, and sanitation have many metallic infrastructures buried, such as tanks and pipes. Soil is one of the most complex corrosion agent and influences the corrosion resistance of buried metallic structures (Afonso et al., 2009). Soil corrosion has a major contribution to overall corrosion costs, related to the enormous Brazilian pipeline network that extends over 27,000 kilometers (CIA Factbook, 2016).

The deterioration processes of these structures are influenced by several factors, such as the type of material, chemical and microbiological characteristics of the soil, interference currents, conductivity, and the presence of anions that increase the risk of corrosion, such as Cl\(^-\), S\(^2-\) or SO\(^4^2-\) (Lopes et al., 2006; Albertini, 2008). Drainage and aeration can also influence soil corrosion aggressiveness due to dissolved oxygen (Jones, 1992; Afonso et al., 2009).

Therefore, the evaluation of soil corrosiveness is considered important and supports decisions regarding the application of corrosion protection methods, such as cathodic protection or the application of protective coatings. Some parameters such as the presence of salts, resistivity, pH, moisture, redox potential, and concentration of sulfate-reducing bacteria (SRB) are taken into account in the evaluation of soil corrosiveness based on Steinrath or modified Steinrath Indexes (Trabanelli et al., 1972; Silva & Brasil, 2010; Pereira et al., 2015).

There are several studies available in the area of soil corrosion, but there is still a lack of information and many aspects need to be examined further. It is therefore necessary to find a correlation between the degree of aggressiveness of the soils, such as the ones given by the Steinrath index, and the corrosion rates obtained from gravimetric experiments (Afonso et al., 2009). Pinning corrosion also should be investigated, as it is an important aspect affecting carbon steel corrosion. Carbon steel account for 90% of industrial pipes, and it is considered a low cost material that is easy to weld and shape, and presents excellent mechanical properties (Bueno, 2007). Despite the advantages mentioned, carbon steel has low corrosion resistance.

In this context, it is critical to understand the problems caused by the deterioration of metals, especially in areas where the soil creates a corrosive medium. The aim of this study is to evaluate soil corrosivity of API5LX60 carbon steel buried in two different soil textures, and under the influence of the soil’s associated microbial community.

2. MATERIALS AND METHODS

2.1 Soil samples

The soil was collected at the port complex of Suape, in the Brazilian State of Pernambuco. The experiments used two bioreactors. These bioreactors consisted of plastic boxes with dimensions 52x36x18cm, and a nominal capacity of 100 kg. The two types of soil textures (clayey and sandy) were tested in two different boxes and all analysis were performed for both sample types.

2.2 Soil characterization

Several criteria for the evaluation of soil aggressiveness were proposed. One of the most comprehensive criteria to evaluate soil corrosivity is the Steinrath Index, which is based on the determination of resistivity, redox potential, pH, chloride ions, sulfate ions, and sulfide ions as parameters (Trabanelli et al., 1972). The modified Steinrath index (Souza & Olivier, 2002), is also very important for replacing redox potential by SRB concentration. SRB concentration is also a very relevant parameter in soil corrosivity studies. Chemical, physicochemical and microbiologic parameters were determined. The contents of both sulfates and chlorides were obtained using the methodology described in Vogel (1981). The sulfide content was followed by an adaption of the colorimetric method using N, N-dimethyl-p-phenylene diamine and ferric chloride, as described by Jacobs, Braverman, and Hocheiser (1957). Resistivity measurements were performed according to GCOI technical / SCM / 95 (GCOI/SCM/95, 1995), using the plotting of the resistivity curve as a function of moisture content. At a first stage, the soil was thoroughly dried in an oven at 80°C for 24hrs. The 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 sulfate (Cu/CuSO₄) reference electrode (Costanzo & McVey, 1958). 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). For SRB quantification, a soil sample was dissolved in a reducing solution and distributed in a modified Postgate E medium (Postgate, 1984) using the MPN technique, and was incubated for 28 days. After this time, the growth of these bacteria was identified by the turbidity of the culture medium caused by the growth of microbial cells and the release of metabolites.

2.3 Corrosion studies

2.3.1 Weight loss

The coupons were treated before determining their initial mass. After each period (7, 15, 30, 60, and 100 days) they were weighted again to obtain the weight loss. The calculation of corrosion rate was carried out according to the classification of standard NACE- RP-0775.

2.3.2 Characterization of the metal surface

Coupons API 5LX60 steel with dimensions of 25x25x3 mm were used in this study. The preparation of the coupons for the evaluation of pitting corrosion was performed using Clark Solution, as recommended in NBR6210 /ABNT. To examine the pits along the corroded metal surface, an Axion Plan 2 - Zeiss Microscope System optical microscopy was used, at a magnification of 10X (100X), and of 50X, 100X, and 200X for pit depths. Following, a program which correlates the difference between the focus of the fine-tuning on the metal surface and the base of the pit was used. This analysis was performed with 2 coupons for each soil sample.

2.3.3 DNA of microbial community associated to the soils

The total DNA of the clayey and sandy soils was extracted by mechanical lysis. The total DNA of the soil was extracted from 0.5g of fresh soil, using the Fast DNA Spin Kit for soil (BIO101, CA, USA), with its principle outlined below. Cells were mechanically lysed for 1min at room temperature in the presence of buffer and glass beads in 40s, at 5.5 m/s¹, using FP120 Fast Prep Cell Disruptor (BIO101). The DNA extraction was performed according to the manufacturer’s recommendations for each kit (Abaim et al., 2004). DNA concentration and integrity were estimated by means of agarose gel electrophoresis on 0.8% (w/v), and by running 5 µl of the final extraction product together with 5 µl of solution for DNA electrophoresis, in TBE 1X buffer. The molecular weight marker used was a 1kb ladder (Fermentas). At the end of the run, the gel was kept at room temperature for about 20 minutes in a staining solution containing red gel. Then, the gel was observed and photographed under UV light in the imago image analysis system (BEL, Netherlands) (Abaim et al., 2004).

After the extraction and purification of the DNA samples, the amplification of the 16S rDNA genes was carried out using a pair of universal primers; gene fragments encoding the 16S subunit of the bacterial rRNA were amplified by PCR, starting from the total DNA extracted from soil samples. The selected primers were: U968f- GC1 ("clip" + 5'AAC GCG AAG AAC CTT AC 3') and L1401r (5'GCG GTA CAA GAC TGT CC 3'). This step was verified by electrophoresis on 1.2% agarose gel in 0.5X TBE. The electrophoresis using the PCR products was performed in the same electrophoresis pattern used with the products of the DNA extraction. The 90V magnetic field was applied for 1 hour (Abaim et al., 2004).

The denaturing gradient Gel Electrophoresis technique (DGGE) allowed the separation of the PCR products (the DNA strands), according to the G + C content of their sequence of base pairs. It excluded the sizes of the DNA fragments, different to most techniques, such as genetic fingerprinting. The DGGE gels were prepared with a polyacrylamide solution (6%) in a Tris-acetate buffer (pH 8.3), with a denaturing gradient of urea and form amide ranging from 40% to 65% of bacteria and 40 to 70% of Archaea, respectively. 40 µl of the PCR product were applied on the gel, with the running time at 75 V, at 60°C. The gels were stained with a SYBR Gold solution (Invitrogen), and diluted at a ratio of 1: 5: 10,000 (v/v) in a Tris-Acetato-EDTA (TAE) 1X buffer, for about 40 minutes in the dark. Subsequently, the gel was observed and photographed under UV light using a STORM apparatus (Amersham Pharmacia Biotech, Munich, Germany). The dendrograms were made from the image using the Jaccard correlation.
coefficients (r) and a cluster analysis was performed, using the unweighted pair-group mean arithmetic method (UPGMA) using BioNumerics software (Aboim et al., 2004).

### 3. RESULTS AND DISCUSSION

#### 3.1 Evaluation of soil corrosivity

The results from the Steinrath index using the seven parameters described previously are presented in Table 1. Partial and total indexes for each parameter observed were analyzed at the beginning of the experiments.

Both soils presented a resistivity in the order of 2–3k Ω cm, and obtained a partial contribution for the Steinrath index of (-4). Concerning the pH, the clayey soil obtained a partial contribution of (-1) while the sandy soil the contribution was null. Redox potential is one of the parameters that may have a strong influence on the total Steinrath index, and both soils obtained a partial contribution of (-1). The soils showed an absence of chloride concentrations. The sulfide presence was bigger in clayey soil, with a partial index of (-4); while the sandy soil was of (-2). The presence of sulfate ions was very low, having obtained a null partial index for both soils. According to the corrosivity evaluation proposed by Steinrath, clayey and sandy soils had high and low aggressiveness, respectively. In the clayey soil, the overall rate of aggressiveness was (-11), and (-6) for the sandy soil.

Considering the modified Steinrath index (Souza & Olivier, 2002), which replaces redox potential by SRB count, for the sandy soil the total index was of -6 on the first assessment and of -4 on the second one, classifying it as a low aggressive soil. For the clayey soil, the total index was higher than (-10) on both assessments, having been classified as highly aggressive. The concentration of SRB was considered high (1.2x10^3 MPN/g soil), and contributed to soil aggressiveness.

Videla (2003) and Videla and Herrera (2005) showed that in the presence of sulfur species, a film is developed in the carbon steel through various chemical and electrochemical pathways leading to more stable iron sulfides. The biocorrosion process was developed from the disruption of this layer by corrosive metabolic products generated by SRB. Other studies also related the corrosion of steel influenced by SRB to the nature and structure of sulfide films produced during metal dissolution.

#### 3.2 Weight loss

The average corrosion rates of the API5LX60 coupons exposed in clayey and sandy soils, during a 100-days period, can be observed in Figure 1. The sandy soil showed a value of 0.15 mm/year, being classified as moderate corrosion. The clayey soil corrosion reached a value of 0.31 (mm/year), being considered as severe corrosion, in agreement to NACE RP-0775. In the absence of the microbial

<table>
<thead>
<tr>
<th>Soil Parameters</th>
<th>Clayey soil</th>
<th>Sandy soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistivity (Ohm.cm)</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>pH</td>
<td>4.4</td>
<td>6.8</td>
</tr>
<tr>
<td>Redox potential (mVen)</td>
<td>110.15</td>
<td>58.45</td>
</tr>
<tr>
<td>Chloride (mg/kg)</td>
<td>Absence</td>
<td>Absence</td>
</tr>
<tr>
<td>Sulfide (mg/kg)</td>
<td>0.051</td>
<td>0.13</td>
</tr>
<tr>
<td>Sulfate (mg/kg)</td>
<td>0.2</td>
<td>0.24</td>
</tr>
<tr>
<td>Total aggression Index</td>
<td>-11</td>
<td>-6</td>
</tr>
<tr>
<td>SRB (MPN/g soil)</td>
<td>1.2x10^3</td>
<td>Absence</td>
</tr>
<tr>
<td>Soil classification</td>
<td>High</td>
<td>Low</td>
</tr>
</tbody>
</table>

Table 1. Soil aggressiveness assessment based on Steinrath Index. SRB was used for modified Steinrath Index.
population, the galvanic corrosion rate was of 0.00083 mm/year for the sandy soil and of 0.0066 mm/year for clayey soil, respectively, indicating that microorganisms contributed actively for corrosivity.

Comparing mass loss test results and the evaluation of soil aggressiveness (Steinrath Index), there was an agreement between the classifications. The sandy soil was classified as moderate, according to mass loss tests, and of low aggressiveness, based on the Steinrath Index. The clayey soil matched the high and severe classifications presenting high aggressiveness.

3.3 Analysis of characterization of metal surfaces for the API 5LX60 steel

3.3.1 Evaluation of pitting corrosion

Localized corrosion was not visible at first sight, but it was possible to detect the presence of a large number of pits under the microscope (see Tables 2 and 3).

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**Table 2. Characteristics of pits found in metal coupons buried in clayey soil.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total observed pitting</td>
<td>57</td>
</tr>
<tr>
<td>Maximum area</td>
<td>0.1845 mm²</td>
</tr>
<tr>
<td>Average area (standard deviation = 0.053 mm²)</td>
<td>0.038 mm²</td>
</tr>
<tr>
<td>Density</td>
<td>1.035. 10⁶</td>
</tr>
<tr>
<td>Maximum depth</td>
<td>0.085 mm</td>
</tr>
<tr>
<td>Average depth (standard deviation = 0.007 mm)</td>
<td>0.035 mm</td>
</tr>
</tbody>
</table>

**Table 3. Characteristics of pits found in metal coupons buried in sandy soil.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total observed pitting</td>
<td>36</td>
</tr>
<tr>
<td>Maximum area</td>
<td>0.1045 mm²</td>
</tr>
<tr>
<td>Average area (standard deviation = 0.053 mm²)</td>
<td>0.0405 mm²</td>
</tr>
<tr>
<td>Density</td>
<td>6.4.10⁶</td>
</tr>
<tr>
<td>Maximum depth</td>
<td>0.049 mm</td>
</tr>
<tr>
<td>Average depth (standard deviation = 0.007 mm)</td>
<td>0.035 mm</td>
</tr>
</tbody>
</table>
In general, the pits were large and deep (Figures 2 and 3 (a) and (b)). Figure 2 shows the metal surface pits found in clayey soil, and Figure 3 (a) and (b) show the evaluation of pitting in sandy soil.

The establishment of pitting corrosion is often related to the presence of certain aggressive anions and chloride ions, which are the most aggressive of all. However, sulfide inclusions are also responsible for pitting attack on carbon steel (Galvele, 1983). This observation was corroborated in this study, as only the presence of sulfide in soil was detected (without chloride ions).

Comparing both soils, the clayey one had a greater number of pits on the coupons, but the average depth of pits was the same for both soils. It is important to notice that the depths of some pits were greater in clayey soil than in sandy soil. The lower depths for sandy soil, corroborate the classification for each type of soil: low, high, and severe aggressiveness, respectively.

As mentioned, the presence of SRB contributed to clayey soil corrosion on carbon steel. Rodrigues (2010) inoculated the soil with a SRB concentration of $10^6$ (MPN/g soil) and measured an average depth of 28.36 µm and $1.1 \times 10^6$ density (pits/m²), similar to those found in the experiments of this study. In the present study, even when testing soil corrosion without SRB, it was possible to observe pits on coupons buried in sandy soil, suggesting that a minimum concentration of sulfide ions contributed to the pits appearance. Microbial activity in metallic surfaces modifies the environmental conditions and the metal/solution (Videla, 2003). The process is
particularly severe in the presence of SRB that releases sulfur compounds (sulfides, disulfide and hydrogen sulfides, thiosulfates, and polythionates) in their metabolic activity (Beech, 2003, Beech & Sunner, 2004).

3.4 Evaluation of microbial diversity using the PCR / DGGE technique

Microbial communities of sandy soil (AT) and clayey soil (AR) are distinct, and the dendrogram showed that they form separate groups (Figure 4). The samples at the end of the process selected some microbial groups and some bands were stronger after a 100-day period. More than identifying genetic variability of the consortium, the DGGE technique allows a comparison between two or more samples, analyzing band patterns and showing the changes in the diversity of bacterial communities (Muyzer et al., 1993; Jackson, 2000). DGGE analysis performed in the present study indicated that the clayey soil is likely to have higher microbial diversity than the sandy soil.

4. CONCLUSIONS

According to Steinrath Indexes and corrosion rates, the clayey soil was more corrosive (presented higher aggressiveness) and showed higher microbial diversity than the sandy soil. The pitting corrosion corroborated these results - coupons placed in clayey soil showed severe pitting corrosion compared to the sandy soil ones. SRB were not found in sandy samples, but other factors contributed to the corrosion process that was detected on coupons buried in both soils.

ACKNOWLEDGEMENTS

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5. REFERENCES


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