

Assessment of the corrosivity of AISI 1020 steel through microbiological analyzes and the mass loss technique in a clayey soil

Avaliação da corrosividade do aço AISI 1020 por meio de análises microbiológicas e técnica de perda de massa em solo argiloso

Evaluación de la corrosividad del acero AISI 1020 mediante análisis microbiológico y técnica de pérdida de masa en suelos arcillosos

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Abstract

This search aims to evaluate the corrosivity of AISI 1020 steel in clayey soil through microbiological analyzes and the mass loss technique. Through the results obtained, according to the identification methodology of the Bergey manual, the presence of two microorganisms was verified, Acidithiobacillus thiooxidans and ferrooxidans, responsible for the biocorrosion process, in addition to filamentous fungi. The presence of these bacteria does not generate a classification for the soil, however, it is known that they can accelerate the corrosion process when in contact with a metallic structure. Regarding the mass loss rate, a criterion used to simulate the assessment of corrosivity in pipes, NACE Standard RP-07-75 was adopted, which defined the intensity of the corrosive process, obtaining as a result for the sterilized sample a value considered low, since it was free of microbial activities and any other contaminating factor, but for the sample without sterilization, the soil was classified as having severe potential. Therefore, this research sought to correlate the characteristics of the soil representative of the Amazon with a process of corrosion of buried pipes due to the presence of microorganisms, which would correspond to a microbiological corrosion. Although, in many cases, there is suspicion of the accuracy of corrosion monitoring techniques, mass loss and microbiological identification techniques were used, which had a positive result in relation to the microbiological one.

Keywords: Clay soil; Corrosion; AISI 1020 steel; Mass loss rate; Microbiological analysis.

Resumo

Esta pesquisa tem como objetivo avaliar a corrosividade do aço AISI 1020 em solo argiloso por meio de análises microbiológicas e da técnica de perda de massa. Através dos resultados obtidos, de acordo com a metodologia de identificação do manual de Bergey, foi verificada a presença de dois microrganismos, Acidithiobacillus thiooxidans e ferrooxidans, responsáveis pelo processo de biocorrosão, além de fungos filamentosos. A presença dessas bactérias não gera uma classificação para o solo, porém, sabe-se que podem acelerar o processo de corrosão quando em contato com uma estrutura metálica. Em relação à taxa de perda de massa, critério utilizado para simular a avaliação da corrosividade em tubulações, foi adotada a Norma NACE RP-07-75, que definiu a intensidade do processo corrosivo, obtendo como resultado para a amostra esterilizada um valor considerado baixo, já que estava livre de atividades microbianas e qualquer outro fator contaminante, mas para a amostra sem esterilização, o solo foi classificado como de potencial severo. Portanto, esta pesquisa buscou correlacionar as características do solo representativo da Amazônia com um processo de corrosão de tubulações enterradas devido à presença de microrganismos, o que corresponderia a uma corrosão microbiológica. Embora, em muitos casos, haja suspeita da precisão das técnicas de

monitoramento da corrosão, foram utilizadas técnicas de perda de massa e identificação microbiológica, que tiveram resultado positivo em relação à microbiológica.

Palavras-chave: Solo argiloso; Corrosão; Aço AISI 1020; Taxa de perda de massa; Análise microbiológica.

Resumen

Este busca tiene como objetivo evaluar la corrosividad del acero AISI 1020 en suelos arcillosos mediante análisis microbiológicos y la técnica de pérdida de masa. A través de los resultados obtenidos, según la metodología de identificación del manual de Bergey, se verificó la presencia de dos microorganismos, *Acidithiobacillus thiooxidans* y *ferrooxidans*, responsables del proceso de biocorrosión, además de hongos filamentosos. La presencia de estas bacterias no genera una clasificación para el suelo, sin embargo, se sabe que pueden acelerar el proceso de corrosión al entrar en contacto con una estructura metálica. En cuanto a la tasa de pérdida de masa, criterio utilizado para simular la evaluación de la corrosividad en tuberías, se adoptó la Norma NACE RP-07-75, que definió la intensidad del proceso corrosivo, obteniendo como resultado para la muestra esterilizada un valor considerado bajo, ya que estaba libre de actividades microbianas y cualquier otro factor contaminante, pero para la muestra sin esterilizar, el suelo se clasificó como de potencial severo. Por lo tanto, esta investigación buscó correlacionar las características del suelo representativo de la Amazonía con un proceso de corrosión de tuberías enterradas por la presencia de microorganismos, lo que correspondería a una corrosión microbiológica. Si bien en muchos casos se sospecha de la exactitud de las técnicas de monitoreo de corrosión, se utilizaron técnicas de pérdida de masa e identificación microbiológica, las cuales tuvieron un resultado positivo en relación con la microbiológica.

Palabras clave: Suelo arcilloso; Corrosión; Acero AISI 1020; Tasa de pérdida de masa; Análisis microbiológico.

1. Introduction

For Gentle (2022) Corrosion can be defined as the deterioration or even destruction of a material, usually metallic, by chemical or electrochemical action of the environment, due or not to mechanical efforts.

Several methods have been proposed to study and monitor corrosion processes, such as mass loss tests (rate of mass loss), analysis of aqueous extract (microbiological analysis), detection of galvanic current, measurement of electrical resistance and electrochemical measurements, among others. others (Macdonald et al., 1998; Ferreira, 2005; Rodrigues, 2006; Da Silva, 2007).

The investigation of the soil corrosivity rate is of great relevance for several activities directly associated with the useful life of materials, equipment and structures, especially vital structures such as electrical energy transmission towers, which must, mandatorily, maintain their physical integrity (Labegalini et al., 1992; Sharma & Kumar, 2021).

With the increasing number of reservoirs and buried pipes, the study of soil as a corrosive agent is of great importance, since its capacity for deterioration can represent serious economic and environmental problems over the years.

The wear process in buried reservoirs and pipes is due to physical-chemical and microbiological characteristics and some superficial factors, which generally cause high costs for a variety of sectors, such as the industrial sector (Gomes, 2001; Ert hal et al., 2017).

Carbon steel is often used in the manufacture of pipelines for use in the petroleum industry. Buried pipelines traverse a variety of soils, textures, depths, and in some cases, ions increase and accelerate soil corrosivity. Thus, the useful life of the tubes depends on the thickness of the metal, the exposed area and the maintenance/repair techniques employed (Oguzie et al., 2004; Wang et al., 2020).

It is important to emphasize that microbiological corrosion can also occur, in which colonies of microorganisms chemically modify the environment, releasing products of their metabolism such as acids that accelerate the corrosive process, in addition to the formation of films adhered to the metallic surface, which promote corrosion by aeration. differential (Gentil, 2012; Gentil, 2022; EMBRAPA, 2018).

According to Oliveira (2010), there are several types of bacteria that are fully involved in the soil corrosion process, such as: iron precipitating bacteria, acid-producing bacteria, exopolysaccharide-producing bacteria (*Pseudomonas aeruginosa*) and sulfate-reducing bacteria (SRB).

Another way to assess corrosivity is through the mass loss rate, which consists of monitoring the behavior of the metallic sample as a function of time, determining the mass loss per unit surface. The test provides objective values of soil aggressiveness for cases of generalized corrosion (Trabanelli et al., 1972; Erthal et al., 2017). It is the simplest and most widely used corrosion assessment method.

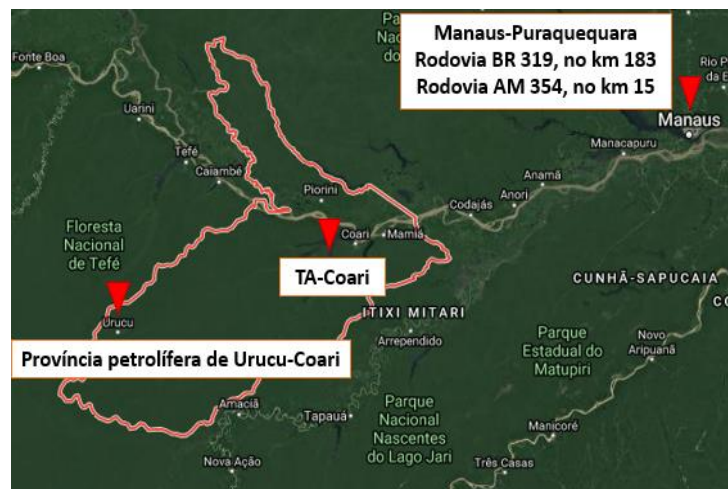
In this context, the present work aimed to study the corrosivity process in samples of AISI 1020 steel, similar to oil and gas pipelines, promoted by soils in the state of Amazonas, by the technique of mass loss/corrosion rate, by microbiological analyzes of the aqueous extract of soils and the identification of active bacteria in the process of corrosivity of this type of soil.

2. Methodology

Soil Sample Collection

Soil samples were collected at the following locations: province Urucu-Coari oil company (geographical coordinates: S 04° 59' 01.68" and W 65° 19' 59.20"); AM 354 Highway, at km 15 (S04° 59' 01.68" and W 55° 20' 60.21"); Manaus-Puraquequara (S 03° 05' 3.54" and W 59° 51' 48.60") and Rodovia BR 319, at km 183 (S 05° 43' 41.40" and W 62° 16' 35, 00"), as shown in Figure 1. The collections were carried out in two periods, being: the first, in January 2018, a very rainy period, and the second, in December 2018, a period of sparse rains according to the reports 2018 of the National Institute of Meteorology (INMET).

Figure 1 - Soil sample collection locations.



Source: Google Maps.

Each soil sample was collected in a maximum radius of 1 m from the helical metallic stake n at a depth of 0.5 to 1 m, with the aid of an auger in the amount ~1 kg, transferred to a plastic bag, sealed with adhesive tape and coded.

Samples A1 (oil receiver), A2 (GLP), A3 (oil shipment) and A4 (GLP) were taken to the laboratory for drying, sieving and maceration, for further analysis.

Test Body Samples (AISI 1020 Steel)

The AISI 1020 steel samples, in the cylindrical format of 2.54 cm in diameter and 3 cm in height, were preserved and kept in a dry environment. This type of steel consists of carbon steel alloy with chemical properties similar to metallic tubes used in the oil industry.

Microbiological Characterization

Aqueous extracts of collected soils were used to obtain bacterial samples according to the procedure described by Ferreira (2005).

Approximately 500 g of soil sample was transferred to a plastic tray, then this sample was manually disaggregated and left to dry in ambient air for 5 days. With the help of tweezers any leaves, branches and roots present were removed from the sample, then it was sieved through a 2.5 mm mesh sieve, the passing material was macerated in an agate mortar/pistil.

Finally, the soil aqueous extract was prepared to determine the concentration of soluble species that could interfere with the corrosive process of the soil. Among these, the concentrations of sulfates, chlorides, potassium, sodium and calcium were of interest.

The preparation of the aqueous extract basically consisted of a solution composed of soil and water in the proportion of 1:100, which was kept under agitation for 24 hours, enough time for the dissolution of the ions to occur. A 1 mL aliquot of the sample was inoculated into a nutrient medium composed of tryptose, lactose, bile salts, monopotassium phosphate, dipotassium phosphate, sodium chloride and distilled water, followed by incubation for a period of 48 hours in a bacteriological oven at 35 °C.

This temperature was maintained for the selection of mesophilic bacteria (most bacteria prefer growth temperatures of 37 °C). After this period, the cultures of this sample were prepared in an acid medium (), being 30 mL of sulfuric acid at 0.5 mol L⁻¹, 10 mL of nutrient medium, 60 mL of distilled water, which was incubated for 24 h at 35 °C.

The final identification was made using a specific broth for the bacilli. *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*, respectively, in which the two media were distributed in test tubes and inoculated with 1 mL of the concentrate obtained from the nutrient medium and incubated in a bacteriological oven at 35 °C for 3 to 5 days. For a positive result, the solution should be cloudy, indicating the presence of microorganisms.

After this period, the cultures of this sample were prepared in an acid medium () and incubated for 36 hours at 35 °C. For the first identification of bacteria, the manual by Bergey (1994) was used, initially pre-selecting two possible organisms, *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*, both of which are Gram-negative and that could grow in these parameters and temperature where the experiments were carried out.

For the samples collected in December 2018 (Samples A3 and A4), the procedure adapted from Videla (2002) was used. Then, they were transferred to Petri dishes and distributed in bacteriological ovens, at temperatures of 25, 30 and 35 °C, for an average period of 3 to 5 days. After this period, noticing the appearance of formed colonies, these bacteria were isolated, in the respective culture medium and, in order to preserve and favor the growth of a single type of bacteria, being able to identify it. Identification was performed as described by Holt (1994), where Gram staining and chemical tests, temperature, motility, oxidase, catalase and nitrate reduction were performed.

Mass Loss Rate

The determination of the mass loss (difference between the initial and final masses) allows calculating the corrosion rate and, therefore, evaluating the intensity of the corrosive process and estimating the wear of the metallic material in a given environment (Castro, 2013; Gentil, 2022).

The specimens were treated, and the masses were measured before the beginning of the experiments. The treatment consisted of sanding in an Arotec polishing machine, with sandpaper n° 80, 120, 200, 400, 600, 800 and 1200, for a better visualization of the corrosion. Mass measurements were taken daily on an analytical scale with 0.0001g precision, during a period of 30 days to calculate the corrosion rate, which were subjected to situations that simulate a steel tube or a pipeline

buried in the ground. The three forms of evaluation and treatment of steel in soil samples were carried out in accordance with ASTM G1-90 (2003):

- Experiment 1 with fresh soil + 1020 steel;
- Experiment 2 containing aqueous soil extract + 1020 steel (cleaned with isopropyl alcohol);
- Experiment 3 with aqueous soil extract + 1020 steel.

The estimate of the corrosion rate was made with the results of the mass losses, from Equation 1:

$$TC = \frac{K \times \Delta m}{A \times t \times \rho} \quad (1)$$

where K is the constant of proportionality tabulated (mm year^{-1}) equal to $8,76 \times 10^4$; Δm is the difference between the masses, before and after exposure, in g; A is the exposed area of the metallic sample, cm^2 ; t is the exposure time, in hours, and ρ is the density, g cm^{-3} (carbon steel, 7.86 g cm^{-3}).

3. Results and Discussion

Microbiological Analysis

Microbiological analyzes were performed in the following steps:

- Step 1: Enrichment that consisted of sowing each sample in Petri dishes, with agar and salts, with the objective of verifying the bacterial colonies that grew in this medium, under pre-established conditions of temperature.
- Step 2: Isolation on new Petri dishes, each colony identified in Step 1 was streaked.
- Step 3 - Identification was performed according to the parameters of the Bergey manual (1994), using Gram stain and chemical tests, , temperature, motility, nitrate reduction, oxidase and catalase.

Table 1 - Identification parameters of microorganisms adapted from Holt (1994).

Parameter	<i>A. Thiooxidans</i>	<i>A. Ferrooxidans</i>
motility	+	+
<i>pH</i>	2 to 4	2 to 4
nitrate reduction	-	-
ideal temperature	25 to 30	30 to 35
Oxidase	+	+
catalase	No reaction	No reaction

Source: Authors.

Temperature and *pH*

Microbiological analyzes were performed in the following steps:

- Step 1: Enrichment that consisted of sowing each sample in Petri dishes, with agar and salts, with the objective of verifying the bacterial colonies that grew in this medium, under pre-established conditions of temperature.
- Step 2: Isolation on new Petri dishes, each colony identified in Step 1 was streaked.
- Step 3 - Identification was performed according to the parameters of the Bergey manual (1994), using Gram stain and chemical tests, temperature, motility, nitrate reduction, oxidase and catalase.

Table 2 - Identification parameters of microorganisms adapted from Holt (1994).

<i>pH</i>	Temperature / °C		
	25	30	35
two	No growth observed within 15 days	No growth observed within 15 days	No growth observed within 15 days
3	No growth observed within 15 days	No growth observed within 15 days	No growth observed within 15 days
4	Slow growth (± 13 days)	Slow growth (± 10 days)	Moderate growth (3-5 days)
5	Slow growth (± 15 days)	Slow growth (± 10 days)	Moderate growth (3-5 days)

Source: Authors.

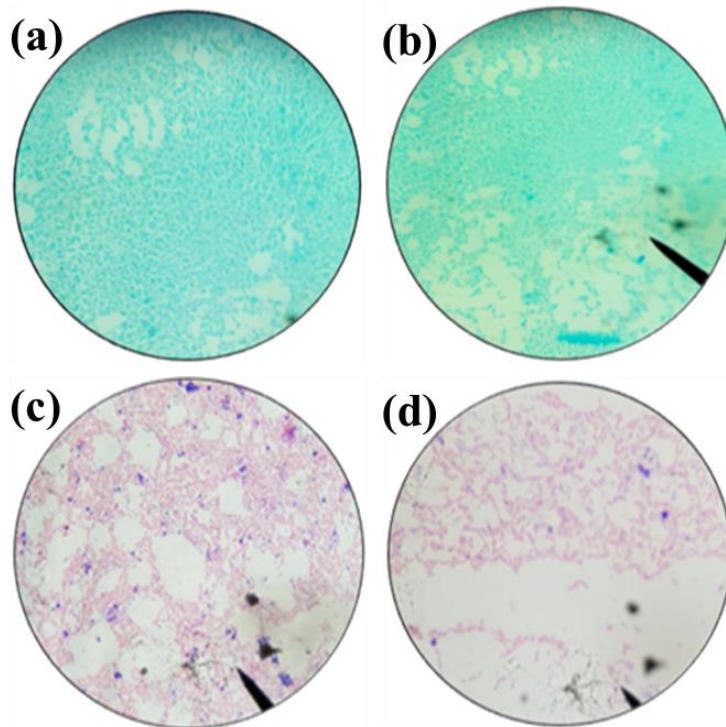
Starosvetsky et al. (2013) observed the first signs of formation of bacterial colonies between 3 and 4 days, at a temperature of 31 ± 0.5 °C, using bromine-cresol green and phenol red, for a total period of 10 days.

Through the results in Table 2, it is clear that there was a moderate growth for pH 4.0 and 5.0 at a temperature of 35 °C, thus confirming the presence of these bacterial colonies.

Gram stain

After the isolation of the cultivated bacterial colonies, the Gram staining test was performed to verify the shape, cell arrangement and coloration, identified through the result in Gram positive and Gram negative, illustrated in Figure 2.

Figure 2 - Gram stain. (a) Sample A1, (b) Sample A2, (c) Sample A3, and (d) Sample A4.



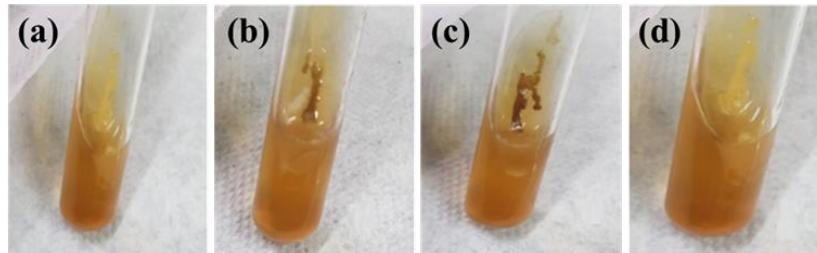
Source: Authors.

The results confirm what Bergey (1994) denotes, which shows that Gram-positive bacteria are classified by the color they acquire after applying a chemical process called Gram staining. Gram-positive bacteria stained blue, in the case of samples A1 and A2, while the other bacteria-stained red.

Motility

To verify the locomotion capacity of the microorganism, the samples were distributed in four test tubes and incubated in semisolid medium for 24 hours. It is also possible to identify motility when the culture medium becomes cloudy, as shown in Figure 3 (Houry et al., 2018).

Figure 3 - Motility test. (a) Sample A1, (b) Sample A2, (c) Sample A3 and (d) Sample A4.

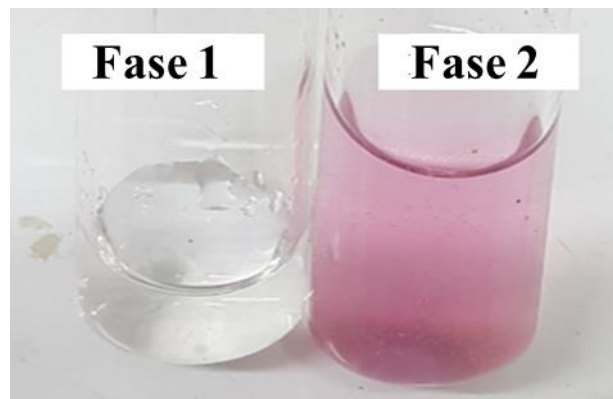


Source: Authors.

Nitrate Reduction

The bacteria were added to the nitrate medium (broth). Incubation was carried out for 5 days at 37°C. After this period, the tests were carried out in two steps: the first, adding 1 mL of solution A (sulfanilic acid) and the second, adding solution B (α -naphthyl -amine). The two-phase test is necessary because some bacteria convert nitrate to nitrite, and nitrite to nitrogen at high speed, as a result, there is no color change; and when powdered zinc was added, it reacted with the nitrate present.

Figure 4 - Nitrate reduction assay.



Source: Authors.

Catalase

To verify the presence of catalase in the selected bacteria, a slide was divided into four parts (Figure 5). A bacterial concentrate was placed in each part, adding 1 mL of hydrogen peroxide.

Figure 5 - Catalase test.

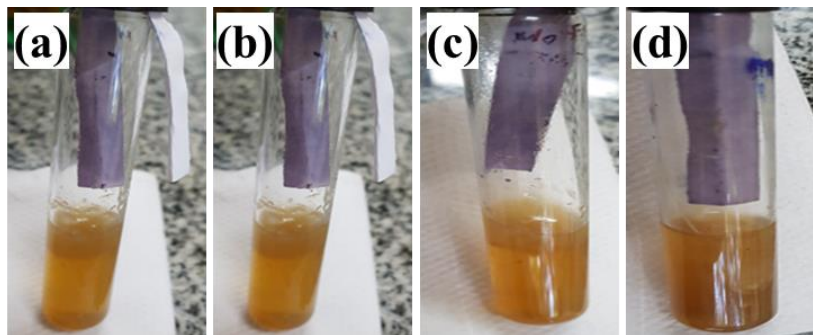


Source: Authors.

Oxidase

It is a qualitative procedure and serves to determine the presence or absence of cytochrome C oxidase activity in bacteria. Used as a test to differentiate between anaerobic and facultative aerobic Gram-negative bacteria. This activity depends on the presence of an intracellular cytochrome oxidase system that catalyzes the oxidation of cytochrome C through molecular oxygen, which, in turn, will function as a receptor in the body's electron transport system (Steel, 1961).

Figure 6 - Oxidase test. (a) Sample A1, (b) Sample A2, (c) Sample A3 and (d) Sample A4.

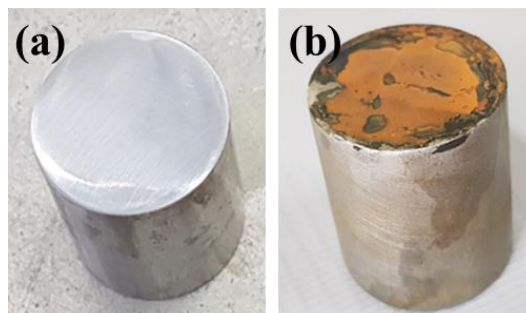


Source: Authors.

Scanning Electron Microscopy (SEM)

In order to characterize the occurrence of microbiological corrosion by indicating the presence of *Acidithiobacillus bacteria thiooxidans* and *Acidithiobacillus ferrooxidans*, the specimen that was submitted to experiment 1 was used. Figure 7 shows the images of the AISI 1020 steel sample before and after exposure to the aqueous extract formed with the clayey soil.

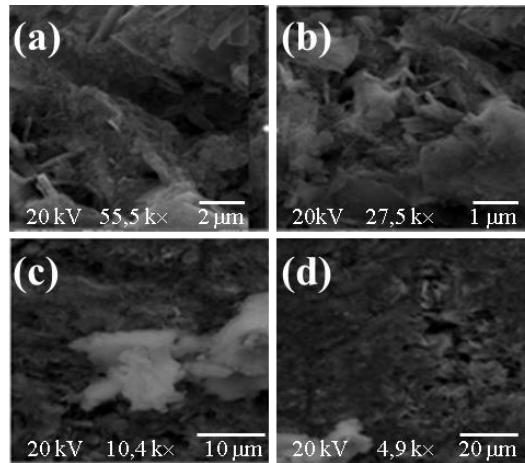
Figure 7 - AISI 1020 steel sample.



(a) Before exposure to experiment 1 and (b) After 30 days of exposure to experiment 1. Source: Authors.

The metallic specimens were previously weighed before the beginning of the experiments.
 The SEM images can be seen in Figure 8.

Figure 8 - SEM images of the steel sample. (a) Sample magnified 55,500× (2 μm), (b) Sample magnified 27,500× (1 μm), (c) Sample magnified 10,400× (10 μm) and (d) Sample magnified 4,900× (20 μm).



Source: Authors.

Figure 8 shows the SEM images of the specimen samples before and after exposure in experiment I.

Corrosion rate

The test was performed according to Ferreira's procedure (2005), the corrosion rate results are shown in Table 3.

Table 3 - Corrosion rate results with sterilized soil.

Experiment	Corrosion rate (mm year ⁻¹)			
	TO 1	A2	A3	A4
1	0.001	0.001	0.002	0.001
two	0.017	0.018	0.020	0.020
3	0.006	0.005	0.007	0.006

Source: Authors.

For the evaluation criterion of corrosivity in gas pipelines, the NACE Standard RP-07-75 was adopted, according to Table 4.

Table 4 - NACE-RP-07-75 frame classification.

potential of corrosiveness	uniform rate (mm year ⁻¹)
Severe	> 0.125
Moderate	0.025 to 0.125
Low	< 0.025

Source: Authors.

As for the sample without sterilization, the results can be seen in Table 5.

Table 5 - Corrosion rate result for unsterilized soil.

Experiment	Corrosion rate (mm year ⁻¹)			
	TO 1	A2	A3	A4
1	2,622	2,631	2,657	2,599
two	8.022	7,989	8.033	7,987
3	0.587	0.569	0.574	0.580

Source: Authors.

Microbiological analysis

Temperature and pH

Bergey's manual (1994), it is stated that the *pH* ideal for the growth of both species is 2.0 to 4.0, with temperatures in the ranges of 25 to 30 °C and 30 to 35 °C, for *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*, respectively.

In this test, a significant growth of bacterial colonies was observed at *pH* = 4,0, at a temperature of 35 ± 2.0 °C, over a period of 5 days (120 h) as shown in Table 2.

Gram stain

Figure 2 shows the presence of Gram negative bacteria, in the form of rods (bacilli) and arranged in diplobacilli. Jensen and Webb (1994), however, report that *Acidithiobacillus* can also occur alone or as streptobacillus.

Motility

In this test, it was possible to visualize that the bacteria grew along the incubation line, indicating the ability to move, as shown in figure 3. It is also possible to identify motility when the culture medium becomes cloudy.

Nitrate reduction

The bacteria selected for the test in the samples were not able to reduce nitrate to nitrite *in vitro*, as shown in Figure 4, which represents the four samples, this result is in line with the Bergey manual (1994).

Catalase

Bergey (1994) was used, whose expected result for these types of bacteria was a negative result. As shown in Figure 5, no bubble formation or effervescence was observed.

Oxidase

Bergey's manual (1994).

Scanning Electron Microscopy (SEM)

By means of the SEM images, a continuity in the corrosion pattern can be observed, with the accumulation of biological material on the steel surface, which can be seen in Figure 8. These images show the surface of the partially polished AISI 1020 steel, with appearance grooves caused by polishing the metal part. Comparing it with the images from experiment 2, it is possible to visualize the surface with a spongy appearance formed by the deterioration promoted by microorganisms.

Corrosion rate

For the evaluation criterion of corrosivity in pipelines, the NACE RP-07-75 Standard was adopted, which defines the intensity of the corrosive process, according to Table 4. It was observed that the result for the sterilized sample was considered low, as since it was free of microbial activity and any other contaminating factor.

As for the sample without sterilization, the soil was classified as having a severe potential, which can be seen in Table 5. There was a significant difference in the corrosion rate in sample 1 in relation to samples 2 and 3, since it refers to a sample with clayey soil *in natura* and the others are samples with aqueous soil extract.

4. Conclusion

According to the identification methodology of the Bergey manual, two microorganisms were found, *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans*, responsible for the biocorrosion process. The presence of these bacteria does not generate a classification for the soil, however, it is known that they can accelerate the corrosion process when in contact with a metallic structure.

As a process of corrosion of buried pipes due to the presence of microorganisms, which would correspond to microbiological corrosion. Although, in many cases, there is suspicion of the accuracy of corrosion monitoring techniques, mass loss and microbiological identification techniques were used, which had a positive result in relation to microbiology, in addition to emphasizing the idea that this monitoring of the corrosion rate it must be done with complementary techniques, which was the case with the chemical and mineralogical characterization techniques of the samples used.

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