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Soil Corrosivity Under Natural Attenuation

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This study investigated natural attenuation conducted in soil artificially contaminated with low-sulfur diesel oil, and to evaluate the corrosion of ASTM A36 carbon steel (A36) during the bioremediation process. Microbiological quantification of hydrocarbonoclastic bactéria (HCB) and heterotrophic bacteria (Aerobic and Anaerobic) was carried out at 7, 14, 28, 42 and 56 days. Corrosion rate was quantified according to the NACE-SP-07-75 standard using the gravimetric method. Morphological analysis was conducted through Scanning Electron Microscopy. Results indicated a significant reduction of 24.03% in oils and greases in the soil, along with a respirometric degradation of 11.10%. Soil contamination with diesel led to microbial growth, mainly of HCB, during diesel bioremediation; however, there was no impact on the corrosion rate of A36. Soil corrosivity with diesel was classified as low after 56 days of experiment. These findings show that natural attenuation is a method capable of bioremediating soil with diesel without impacting nearby steel structures.

Keywords: *Corrosion; bioremediation; natural attenuation; diesel oil, ASTM A36 steel.*

1. Introduction

The extensive network of buried pipelines, such as oil pipelines, gas pipelines, and water mains, has been the subject of much research due to the corrosive behavior of soil[1-3]. Soil contamination, in addition to causing environmental impact and affecting human health, can alter the physical, chemical, and biological parameters and accelerate the corrosive process of soil on metallic materials.

The oil industry is significantly impacted by corrosion, resulting in repair costs on the order of billions of dollars (USD)[4,5]. The use of metal alloys throughout the oil supply chain, including extraction, transportation, and storage, exposes the industry to significant challenges related to corrosive processes[2]. In particular, many types of carbon steel are widely used in the manufacturing of pipelines and various equipment (such as reactors, cooling towers, and containers) due to their low manufacturing cost, good mechanical properties, plasticity, toughness, and welding characteristics[6]. However, the corrosion resistance property of carbon steel is poor, making it susceptible to corrosion in various types of environments[7], such as in acidic aqueous solution[8], marine atmosphere[9], saline water[10], dried sandy soil[6], soil containing NaCl[11], and silt soil[12].

Corrosion of pipelines and tanks leads to material loss, with the potential for structural failure and subsequent soil contamination, as well as groundwater contamination[13]. There is growing concern regarding the corrosion of structures buried in or in contact with soil, driven by increasingly stringent environmental regulations aimed at preserving ecosystems[14].

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The exploration of petroleum and its derivatives can result in air, water, and soil contamination. The release of contaminants, such as sulfur, volatile organic compounds, polycyclic aromatic hydrocarbons, heavy hydrocarbons among others, into the environment disrupts the existing balance in ecosystems and can have negative impacts on human health due to the carcinogenic and/or mutagenic properties found in various petroleum derivatives[15,16].

Bioremediation proves to be promising in managing the decontamination of aquatic and terrestrial environments, as it is an economically viable and relatively straightforward strategy. Natural attenuation is the simplest form of bioremediation, as it is a process that occurs naturally in contaminated areas. The efficiency of remediation can vary considerably depending on the concentration of the contaminant and type of contaminant, as well as soil characteristics such as moisture and the presence of indigenous microorganisms capable of degrading the contaminant[17,18]. The indigenous microorganisms can eliminate or transform toxic substances into less environmentally aggressive components[16].

The microorganisms, including various species of bacteria, algae, and fungi, have been recognized for their biodegradation potential in soil environments. In this context, numerous bacterial species, such as *Sphingomonas sp*., *Bacillus sp*., and other species, have demonstrated specialized metabolisms capable of playing a crucial role in the degradation of soils contaminated with hydrocarbons. In addition to these bacteria, certain species of fungi and algae, such as *PenicilLm sp*., *Aspergillus sp*., *Spirulina platensis*, and *Chlorella vulgaris*, have been effectively utilized in

initiatives for bioremediating soils polluted by petroleum and its derivatives[19].

The adaptation of microorganisms to different environments is attributed to their intrinsic ability to adjust the composition of their cell membranes, a direct response to the physicochemical changes imposed by the surrounding habitat[20]. In the scope of hydrocarbonoclastic bacteria, recognized as 'oil-eating'[21,22], their capacity for biosurfactant production stands out. This characteristic enhances their affinity for hydrophobic compounds, facilitating the binding to petroleum-derived substances. This adaptation not only allows efficient access to hydrophobic environments, but also enables the metabolization of petroleum and its recalcitrant derivatives as a source of carbon and energy[23].

In natural attenuation, several natural processes contribute to the remediation of a contaminated area, such as the volatilization of contaminants, which occurs through dispersion by natural environmental factors, such as leaching, dilution, and adsorption of the contaminant. Among these natural processes, only biodegradation performed by microorganisms present in the environment chemically destroys the contaminant, often recalcitrant compounds, which in the chemical industry would require much energy for their deterioration. The other processes, such as volatilization and dispersion, only transfer the contaminant to another area, resulting in degradation through abiotic factors[24,25].

There are many studies focused on the bioremediation of petroleum and its derivatives through natural attenuation, often employing bio-stimulants and in proximity to buried metallic structures. However, to date, there is no evidence of any paper investigating the effect of bioremediation on the corrosion process in low-carbon carbon steels close to areas of natural attenuation.

In this sense, this research aimed to investigate natural attenuation conducted in soil artificially contaminated with low-sulfur diesel oil, and to evaluate the influence of variations in chemical, physical, and biological factors during the bioremediation process on the corrosion of ASTM A36 carbon steel. To achieve these objectives, the biodegradation of diesel oil was assessed by quantifying the content of oils and greases (O&G) at the beginning and end of the experiment, conducted on the day 84. Furthermore, respirometric biodegradation was monitored during the same period.

The microbiological quantification of hydrocarbonoclastic bacteria and heterotrophic bacteria (Aerobic and Anaerobic) was carried out at five moments (7, 14, 28, 42 and 56 days) to evaluate the influence of microorganisms capable of biodegrading diesel oil and inducing microbiological corrosion on ASTM A36 carbon steel. Additionally, the assessment of soil corrosivity during bioremediation included the measurement of pH in the studied bioreactors at the same time points. The thermodynamic spontaneity of the corrosion reactions on the carbon steel in the soil was initially evaluated by the open circuit potential (OCP). This experiment was conducted in two scenarios, involving two bioreactors: (i) soil with moisture adjustment, control bioreactor, and (ii) soil with moisture adjustment and artificially contaminated with 5% ultra-low sulfur diesel oil, diesel bioreactor. The assessment of soil corrosivity in both bioreactors during bioremediation was conducted through the corrosion rates of ASTM A36 carbon steel at five time periods: 7, 14, 28, 42, and 56 days. In addition, the morphological analysis of the ASTM A36 carbon steel was conducted through Scanning Electron Microscopy (SEM), providing results of surface changes of the interaction between soil and metallic material.

Thus, this study sought to provide insight into the interconnection between diesel oil bioremediation and the corrosion process of ASTM A36 carbon steel, contributing to the advancement of knowledge in this specific field.

2. Experimental

2.1. Materials

The soil was collected at the Suape Port, Engenho Salgado Road, Ipojuca city (Brazil/Pernambuco), at a depth between 15 and 20 cm, following ABNT-NBR-14283:1999 standard[26]. ASTM A36 carbon steel was purchased from Maquidema Metais Ltd. (Brazil). The diesel oil was purchased from the BR Petrobras gas station network. Isopropyl alcohol and Hydrochloric acid were purchased from Anidrol Produtos para Laboratório Ltd. (Brazil). Acetone was obtained from Química Moderna Indústria e Comércio Ltd. (Brazil). Potassium hydroxide was purchased from Neon. Potassium carbonate was obtained from Vetec. Bartha Respirometer was purchased from Amitel vidros para laboratório Ltda. Bushnell Hass Agar mineral medium to hydrocarbonoclastic bacteria was obtained from the Himedia. The components for aerobic heterotrophic bacteria medium, meat peptone, meat extract and sucrose were obtained from Merck. Anaerobic heterotrophic bacteria medium was obtained from Merck. The distilled water was prepared in the laboratory by a distillation system.

2.2. Soil preparation

The soil sample was composed of various sampling points from the collection area. Therefore, all samples were placed in a single container and manually homogenized to obtain a composite sample. In the laboratory, the soil sample was spread across the bench at a temperature of 30 °C for 4 days, until it was suitable for sieving through a 2.0 mm mesh.

The determination of granulometry was carried out by Department of Geology, located at the Federal University of Pernambuco (Brazil), according to Embrapa methodology[27]. The real and apparent density were determined as described by Teixeira et al.[27]. Analyzes of soil pH, moisture and retention capacity were carried out according to Luchese et al.[28]. The total organic carbon was quantified using the Walkley-Black method, as described in Gatto et al.[29].

2.3. Preparation of bioreactors

Polypropylene bioreactors, with dimensions of 28 cm × 18.2 cm × 8.4 cm, were employed to assess soil bioremediation through natural attenuation. Two bioreactors were developed: control soil (soil with moisture adjustment, B_{Ct}) and soil with moisture adjustment artificially contaminated with 5% ultra-low sulfur diesel oil (B_{Di}). The soil moisture adjustments in both bioreactors followed the criteria established in the ABNT-NBR-14283:1999 standard[26], which recommends

maintaining the moisture at 50% of the soil's water holding capacity.

The configuration of each bioreactor is as follows. For B_{Ct}, 2745.00 g of soil and 255.00 g of water were added, with no diesel. For B_{Di}, 2595.00 g of soil, 255.00 g of water, and 150.00 g of diesel were added.

ASTMS A36 Carbon steel metal coupons were used, with dimensions of 10 mm × 20 mm × 6 mm. The coupons were polished using a grinder with #400, #600 and #1000 water sandpapers grits, cleaned with isopropyl alcohol and acetone. Subsequently, the coupons were dried using a hot air blower and weighed to the tenth of a milligram.

The coupons were attached to nylon threads, pre-identified by numbers, and positioned in the soil at a distance of 4 cm from each other, at a height of 0.5 cm from the base of the bioreactor, as shown in Figure 1. The bioreactors were kept covered with plastic to preserve soil moisture, maintained at room temperature, approximately 30°C.

2.4. Corrosion rate and weight loss

The ASTM A36 carbon steel coupons underwent pickling cycles according to ASTM G1-03 (2017)[30]. After complete removal of the corrosion product, the coupons were weighed to calculate the mass loss. The corrosion rates were calculated according to Equation 1:

$$CR = \frac{8,76*10^4*W}{A*t*D} \quad (1)$$

where, CR represents the corrosion rate in millimeters per year (mm/year), W is mass loss in grams, t is exposure time in hours, and D is steel density in g/cm^3. The corrosivity classification of each environment was assigned according to NACE Standard SP 0775-2013[31].

2.5. Open circuit potential monitoring in soil

The surfaces of ASTMS A36 carbon steel, with dimensions of 20 mm × 10 mm, were subjected to the redox potential test in the B_{Ct} and B_{Di} soil. The coupons were embedded in Bakelite resin. A hole was made in the bakelite resin until it reached the steel. A copper wire (cross section of 2.5 mm^2) was introduced by interference until it reached the substrate, in order to establish an electrical connection between the coupon and the potentiostat. Subsequently, the coupons were polished on a grinder with #400, #600 and #1000 sandpaper, and cleaned with isopropyl alcohol and acetone. Figure 2A illustrates the steel coupon prepared for open circuit potential (OCP) test.

Two systems of soil were assembled in a polypropylene container, with a volume of 350 mL. A first system was set up according to proportionality conditions of B_{Ct}: 320.25 g of soil and 29.75 g of water. The second was set up according to the B_{Di} proportion conditions: 302.75 g of soil, 29.75 g of water, and 17.5 g of diesel oil.

The experiments were conducted using a computer-controlled electrochemical workstation (AUTOLAB PGSTAT 302N - interface NOVA 2.1) at room temperature in both soil

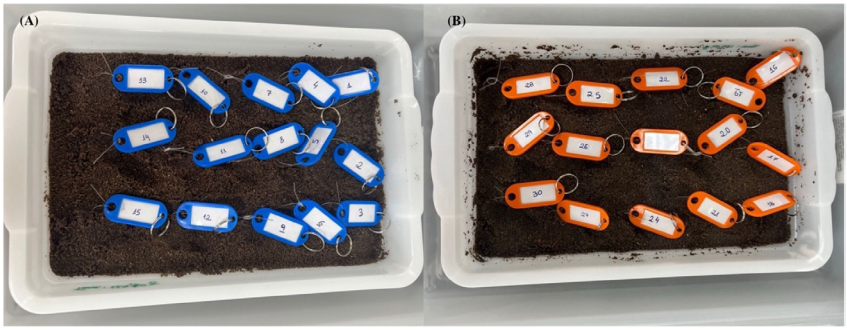

Figure 1. Coupons buried in bioreactors: (A) B_{Ct} and (B) B_{Di}.

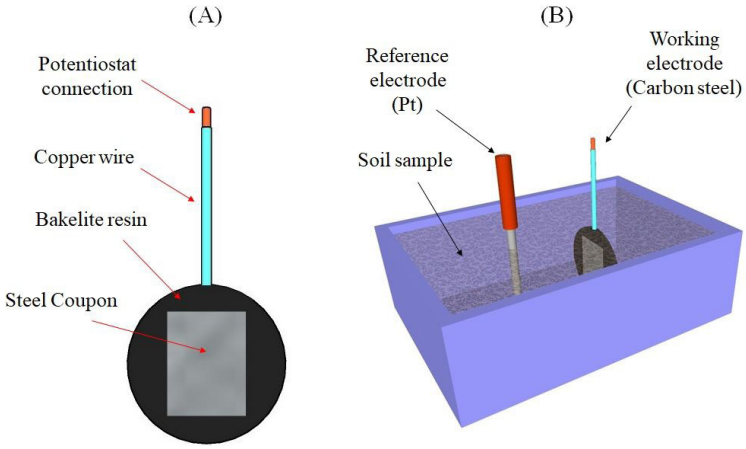

Figure 2. OCP test in soil samples (A) specimens, and (B) electrochemical cell.

systems. The experiment was performed using platinum as reference electrode and steel coupons as the working electrode, as illustrated in Figure 2B. The electrodes remained with a distance of 3.0 cm between them[11].

2.6. Respirometric biodegradation of diesel oil

The assessment of diesel oil biodegradation in the soil was conducted through respirometry, with an adaptation of the methodology proposed by Bartha and Pramer[32]. Carbon dioxide (CO_2) was employed as an indirect measure to estimate the amount of degraded carbon. Soil basal respiration is intrinsically related to the indigenous microbiota present in the soil[29].

Bartha respirometers were used to prepare triplicates of experiments for the control (B_{Ct}) and diesel (B_{Di}) systems, along with triplicates of blank respirometers containing only the alkaline solution (0.2 N KOH). Bartha respirometers consist of a closed system composed of two interconnected compartments by a lateral loop. The contaminated soil to be degraded is placed in the Erlenmeyer flask, while the KOH solution is placed in the lateral loop. Figure 3 illustrates the schematic respirometer diagram. Before adding to the Bartha respirometer, soil samples were adjusted to a moisture content equivalent to 50% of the water holding capacity, following ABNT-NBR-14283:1999 standard[26]. An alkaline solution (10 mL of 0.2 N KOH) was added to react with the CO_2 generated during biodegradation. Microbial CO_2 production was monitored for 84 days, initially at 24-hour intervals until 37 days, transitioning to 48-hour intervals until 78 days, and subsequently at 72-hour intervals until the remaining 84 days.

The quantification of CO_2 reacted with the KOH solution was performed using the electrochemical method, through the measurement of the conductivity of the purged solutions from the respirometers, according to Mazzeo et al.[33] and Strotmann et al.[34]. The electrochemical method consisted in the proportionate relationship between the KOH consumed and the K_2CO_3 produced, following to Equation 2:

$$2KOH + CO_2 \rightarrow K_2CO_3 + H_2O \quad (2)$$

The biodegradation efficiency in terms of carbon mineralization was calculated by dividing the amount of biodegraded carbon in the sample (µmol) by the initial amount of organic carbon in the soil (µmol).

2.7. Determination of diesel oil biodegradation by oil and grease content

The quantification of diesel oil biodegradation was estimated by measuring the content of oils and greases. The analysis was carried out by the Department of Geology, located at the Federal University of Pernambuco (Brazil), according to the SMEWW – 5520D and 5520F methodologies, provided for by Standard Methods[35].

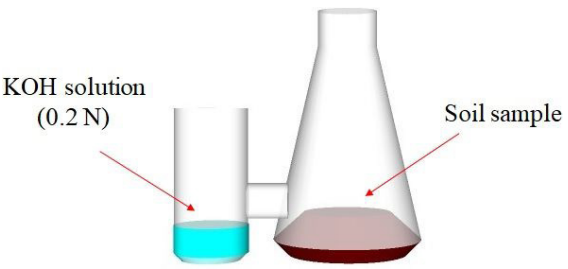

Figure 3. Respirometer diagram.

2.8. Microbial quantification

The microbiological quantification of the native soil from Suape Port and the control (B_{Ct}) and diesel (B_{Di}) bioreactors was conducted at 7, 14, 28, 42, and 56 days. Aerobic heterotrophic bacteria (AHB) and anaerobic heterotrofic bacteria (HAnB) groups were monitored using the Most Probable Number (MPN) method, following to Silva et al.[36]. The hydrocarbonoclastic bacteria (HCB) were quantified through colony-forming units (CFU) counting, methodology adapted from Ali et al.[37].

Initially, 10g of each sample was mixed in 90 mL of sterile distilled water. 1 mL of the solution was transferred to sterile test tubes containing 9 mL of aerobic heterotrophic bacteria medium, and incubation was carried out at 30 ± 1°C for 48 hours. For HCB quantification, 1 mL of solution was inoculated onto sterile Petri dishes containing the hydrocarbonoclastic bacteria medium and 0,16 mL of diesel oil (1% v/v), as the sole source of carbon[18]. The incubation was at 30 ± 1°C for 7 days. The diesel oil used was sterilized using UV radiation for 30 minutes in a biosafety cabinet. For HAnB quantification, 1g of soil sample was mixed with 9 mL of reducing solution. After manual homogenization, 1 mL of the reduction solution was transferred to sealed penicillin tubes containing 9 mL of heterotrophic anaerobic bacteria medium. The incubation was at 30 ± 1°C for 28 days[37].

The entire inoculation procedures were carried out in an ESCO Class II BSC a biosafety cabinet.

3. Results and Discussion

3.1. Soil characterization

The soil characterization analyses revealed a pH of 7.8, residual moisture of 1.5%, water holding capacity of 20%, total organic carbon of 0.150%, and oil and grease content of 0.250%. These initial soil parameters are critical for the success of the bioremediation experiment, influencing fundamental soil characteristics that play a crucial role in experiment design. Particularly, pH has a direct influence on microbial metabolism, and research suggests that the optimal range is between 5 and 8[16]. The detection of residual concentrations of oils and greases suggests that the soil collection area was susceptible to some form of spill or leakage. This result, combined with the microbiological analyses, confirmed the presence of microorganisms adaptable to petroleum derivatives.

The soil classification was conducted through particle size analysis, as presented in Table 1.

The particle size analysis indicated that the soil is predominantly composed of sand, followed by clay and silt, respectively. Therefore, in terms of texture, the soil is classified as sandy. Sandy soil, due to the lack of particle aggregation, promotes the volatilization of pollutants and their movement through the soil. The higher porosity also

Table 1. Soil granulometry.

Granulometry	Result
Sand (g / kg)	957.0
Silt (g / kg)	12.0
Clay (g / kg)	31.0
Actual Density (kg / dm^3)	2.83
Bulk Density (kg / dm^3)	1.52
Texture Classification	Sandy

increases the oxygenation of the environment, benefiting microbial activities essential for contaminant degradation[38].

3.2. Oil and grease biodegradation efficiency

The quantification of oils and greases (O&G) was conducted it the initial time and after 84 days. The initial residual concentration of oils and greases of 0.250% represents the baseline concentration for the control bioreactor (B_{Ct}). The diesel-contaminated bioreactor (B_{Di}) received a 5% (m/m) introduction of diesel oil, resulting in an initial oil and grease concentration of 5.70%. After 84 days of incubation, the oil and grease removal efficiencies for B_{Ct} and B_{Di} bioreactors were 4.00% and 24.03%, respectively.

The O&G content in the B_{Ct} bioreactor remained practically the same as initially found (4%). However, the B_{Di} bioreactor showed a reduction in O&G content consistent with the 84-day experimental period (24.03%). This reduction is probably related to the natural soil phenomena, including volatilization and the natural attenuation process exercised by the indigenous microbiota present in the soil[39]. According to Silva et al.[39], the natural attenuation of light crude oil over 180 days can achieve an oil and grease removal efficiency of 42.86%.

In the literature, various distinct responses have been found in bioremediation experiments. In the present study, it was observed that the removal of O&G through natural attenuation showed similar results when compared to other studies[39-41]. Reginatto et al.[41] investigated the bioremediation of a blend of diesel oil and biodiesel in clayey soil, achieving a notable 63.83% removal of oils and greases (O&G). This outcome was accomplished through the implementation of the bioventing technique during natural attenuation over a 60-day period. Bioventing, which involves the introduction of oxygen to the soil microbiota, serves as a stimulus for the growth and activation of metabolic pathways capable of degrading the contaminant[16].

Namkoong et al.[42] reported that the volatilization of n-alkane compounds from diesel oil varied according to the input of organic matter and nutrients into the soil. Therefore, depending on the carbon and nutrient content available in the soil, the contaminant tends to undergo sorption more or less, thus varying the volatilization of the compounds.

3.3. Respirometric assessment

Figure 4 displays the analytical curve of conductivity (y-axis) versus n moles (x-axis) of CO_2 according to Mazzeo's methodology[33].

The line generated from the scatter plot using the least squares method was: y = -139.27x + 1922.4. The linear

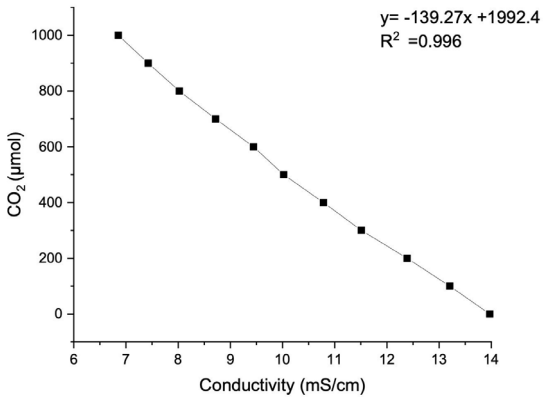

Figure 4. Calibration line of CO_2 production as a function of conductivity in KOH solution.

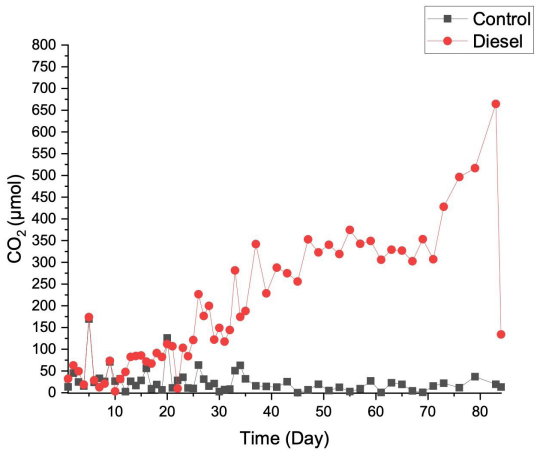

Figure 5. Daily CO_2 production in the respirometry assays over 84 days.

regression coefficient of 0.996 indicates that the model is well-fitted. The mathematical model was used to determine CO_2 production.

The CO_2 production standard (as shown in Figure 5) differs between the two investigated bioreactors due to the contaminant load introduced into the diesel bioreactor (B_{Di}). The control bioreactor (B_{Ct}) exhibited reduced CO_2 production values, which remained constant, indicating potentially natural metabolic functions of indigenous soil microorganisms. On the other hand, in the diesel bioreactor (B_{Di}), a greater production of released CO_2 was observed, suggesting a potential increase in microbial growth and metabolic activity[43].

Initially, a lower CO_2 production was observed in the diesel bioreactor (B_{Di}). According to Maletić et al.[44], the toxicity of recently contaminated soils may impose a period of adaptation on indigenous microorganisms to the new physical, chemical, and biological conditions introduced by contamination. After 7 days of the experiment, an increase in CO_2 production was observed, indicating a more intense period of microbial activity in the biodegradation of the contaminant, with basal respiration growing over the analyzed days. Polyak et al.[45], also observed a moderate increase in CO_2

production during the implementation of natural attenuation in soil contaminated with crude oil.

In bioremediation treatments, in which the strategy involves natural attenuation or the application of stimulation to the microbiota (biostimulation), and/or bioaugmentation with microorganisms whose metabolism of a specific contaminant is known, there is an observed initial adaptive phase, as demonstrated in studies by Napp et al.[46], occurring between 1 and 10 days. Respirometric assessment can provide data on diesel oil biodegradation using various strategies, including natural attenuation[47-49]. The evaluation of diesel oil biodegradation efficiency under attenuation is shown in Figure 6.

Following the same basal respiration standard, both control (B_{Ct}) and diesel (B_{Di}) bioreactors showed increasing biodegradation efficiencies over the 84-day period. The control bioreactor exhibited higher degradation efficiency until the 69th day, reversing with an increase in the diesel bioreactor's biodegradation efficiency. At the end of the 84-day incubation period, the biodegradation efficiency of the diesel bioreactor (B_{Di}) was relatively higher than that of the control bioreactor (B_{Ct}), as expected due to the low carbon concentration from the diesel oil load.

The efficiency of respirometric biodegradation under natural attenuation demonstrates that the indigenous soil microbiota can metabolize diesel oil. However, the absence of stimuli, such as nutrient balancing or surfactant addition, led to a slower degradation rate.

3.4. Microbiological analyses

The microbiological behavior in the soil was assessed by quantification of aerobic heterotrophic bacteria, anaerobic bacteria, and hydrocarbonoclastic bacteria at the temporal points 0, 7, 14, 28, 35, 42, and 56 days, shown Figure 7.

The microbial concentration of aerobic and anaerobic heterotrophic bacteria decreased in the same proportion after 7 days of incubation in both studied bioreactors. This suggests that the toxicity resulting from the addition of diesel oil was not the main reason for the reduction of these microorganisms. The reduction in heterotrophic bacteria may be related to stress caused by changes in soil conditioning conditions and nutrient depletion. This result was also observed by Bidja et al.[50], who found a reduction in this group of bacteria, including in the control system, where there was no contamination. The decrease in quantification of heterotrophic bacteria in the following days, mainly in the B_{Di} soil, shows that these microorganisms were able to adapt to the new conditions imposed by the soil. The highest concentration of aerobic heterotrophic bacteria in the diesel bioreactor (B_{Di}), occurred at 28 days, with a value of 7.60×10^8 MPN/g, while for the control bioreactor (B_{Ct}), the bacterial concentration was 5.8×10^6 MPN/g, this demonstrates that diesel oil was used as a source of carbon and energy[51].

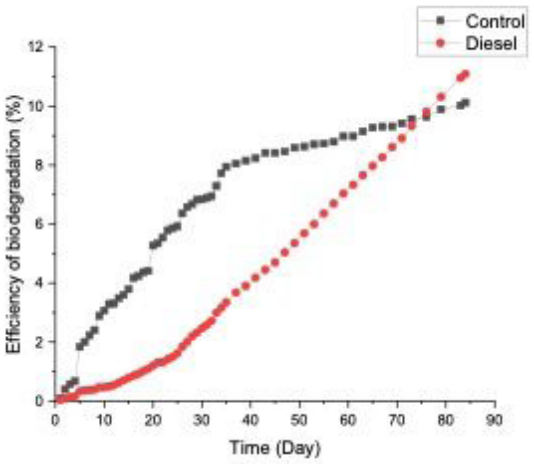

Figure 6. Diesel oil biodegradation efficiency under natural attenuation, calculated from respirometric evaluation.

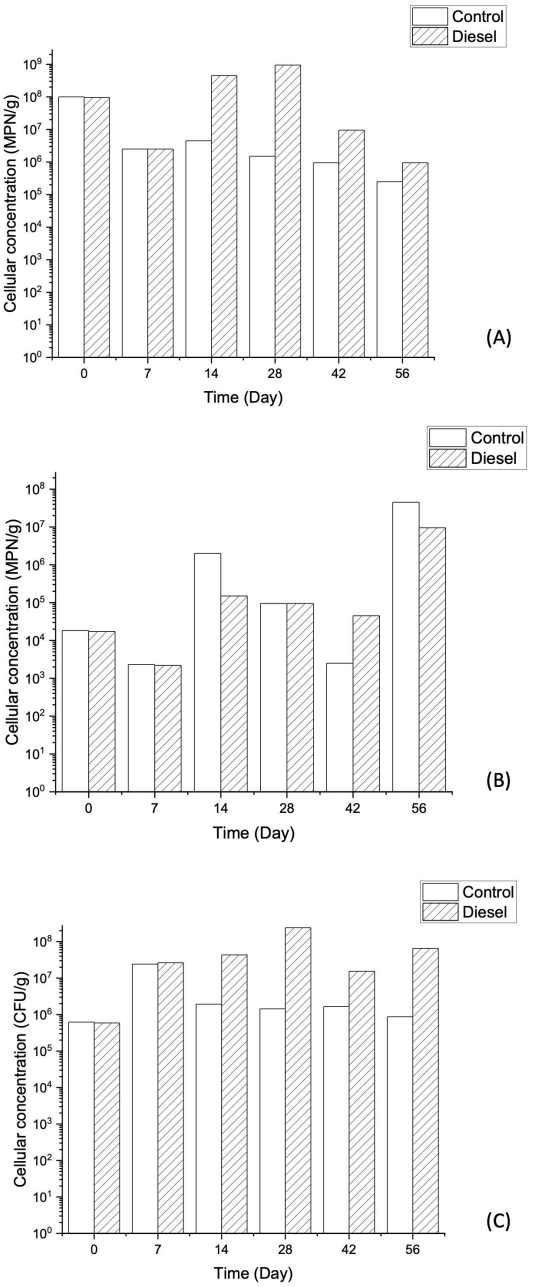

Figure 7. Microbiological quantification. (A) Aerobic heterotrophic bacteria; (B) Anaerobic heterotrophic bacteria; (C) Hydrocarbonoclastic bacteria.

Due to the conditions imposed on the bioreactors, characterized by a predominance of aerobic conditions, the highest concentration of anaerobic heterotrophic bacteria was observed at 56 days, with values of 2.70×10^7 MPN/g and 7.6×10^6 MPN/g in the control (B_{Ct}) and diesel (B_{Di}) bioreactors, respectively. Aerobic biodegradation of hydrocarbons over anaerobic predominates over anaerobic degradation[52]. However, there are anaerobic metabolic pathways capable of oxidizing hydrocarbons into methane and carbon dioxide[16,53]. From Figure 6 it is possible to observe that the concentrations of anaerobic individuals were low while the number of aerobic individuals was high. As the number of aerobic heterotrophic individuals increases, resources, including oxygen, become scarcer. Under these conditions, the system becomes conducive to the growth of anaerobic heterotrophic bacteria[54,55].

The concentration of hydrocarbonoclastic bacteria increased in both bioreactors after 7 days of incubation resulting from the degradation of traces of organic matter in each soil. However, in subsequent analyses, the concentration of hydrocarbonoclastic bacteria in the control bioreactor decreased and remained constant, while it continued to rise in the diesel bioreactor, reaching its peak concentration at 28 days, with 2.4 x 10^8 CFU/g. This result reinforces the high microbial activity of hydrocarbonoclastic bacteria in media containing petroleum derivatives. The HCB were the only microorganisms that showed quantification greater than 10^7 CFU/g on all days of analysis in the B_{Di} bioreactor, highlighting the capacity to utilize carbon from diesel oil as a source of carbon and energy.

3.5. pH assessment of bioreactors

The pH assessment in the bioreactors (B_{Ct}) and (B_{Di}) is presented in Figure 8, with constant values throughout the analyzed period, except in the diesel bioreactor (B_{Di}), which recorded a pH reduction after 28 days of incubation. The decrease in pH in diesel-contaminated soil during bioremediation is attributed to the production of organic acids as byproducts of bacterial degradation, indicating an increase in the production of acidic metabolites by acid-degrading microorganisms[54,56]. Furthermore, the pH reduction is linked to the solubility of certain macronutrients in the soil, promoting microbial activity and, consequently, enhancing biodegradation efficiency[18]. The pH decrease in the diesel bioreactor over 28 days coincided with an increase in the concentration of aerobic heterotrophic bacteria and hydrocarbonoclastic bacteria, suggesting a correlation between these two events.

The soil pH has little dominance in soil corrosivity. According to Ismail et al.[57], soils typically fall within a pH range of 5 to 8, and within this range, it has minimal influence on the corrosive nature of the soil. Corrosive soils can induce metal corrosion, and this acidity can result from processes such as mineral leaching, decomposition of acidic plants, industrial waste, acid rain, and microbial metabolism, for example, for example, sulfate-reducing bacteria that produce acidic metabolites[13].

3.6. Biodegradation versus bacterial concentration

Based on the results obtained in the microbiological quantification of aerobic heterotrophic bacteria and hydrocarbonoclastic bacteria from the B_{Di} bioreactor, it was observed that the highest concentrations of these two microbial groups were reached at 28 days of incubation, coinciding with the increase in CO_2 production. Both the studies by Trejos-Delgado et al.[58] and Yalaoui-Guellal et al.[23] emphasize a correlation between the increase in aerobic heterotrophic bacteria and hydrocarbonoclastic bacteria and the increase in basal soil respiration. Furthermore, the decrease in pH in the diesel bioreactor corroborates this association between increased bacterial concentration and metabolic processes related to the biodegradation of diesel oil.

3.7. Corrosivity of the bioreactors studied

Figure 9 shows open circuit potential (OCP) behavior for the B_{Ct} and B_{Di} systems. By the potential value, it is possible to assess the thermodynamic spontaneity between the anodic and cathodic reactions in each system[59]. Equations 3 and 4 show the corrosion reactions of the steels in the soil. Qi et al.[60]

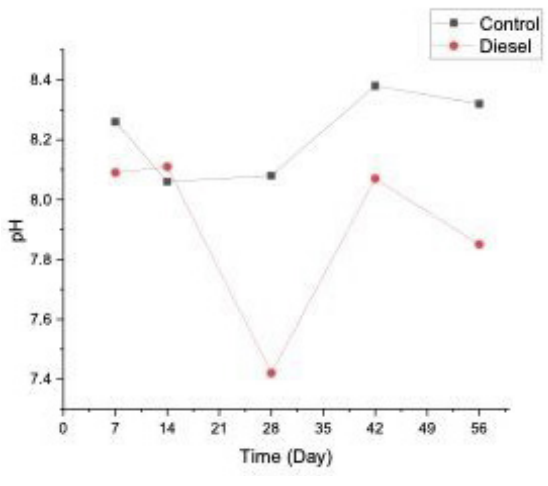

Figure 8. pH assessment.

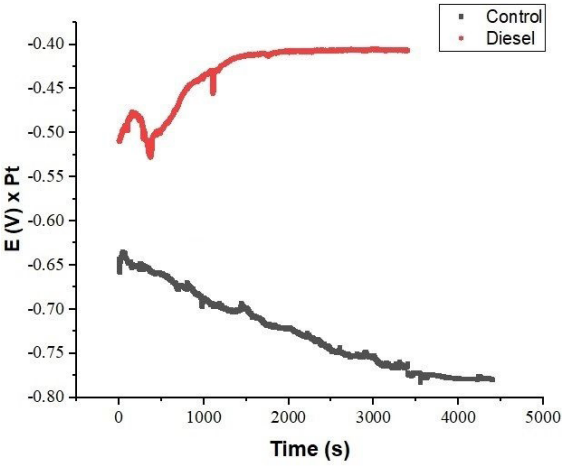

Figure 9. OCP values of the B_{Ct} and B_{Di} systems using platinum as a reference electrode.

reported that iron is dissolved at the anode (Equation 3), and oxygen is reduced at the cathode (Equation 4).

$$Fe \rightarrow Fe^{2+} + 2e- \quad (3)$$

$$O_2 \rightarrow 2H_2O + 4e- \rightarrow 4OH^- \quad (4)$$

The potential of the B_{Ct} bioreactor decreases over time and tends to stabilize at a value of -0.773 V after 3500 s. Nevertheless, the behavior of the potential in the B_{Di} bioreactor is increasing, and the potential clearly stabilizes at the value of -0.406 V after 2000 s.

Therefore, the OCP value was significantly increased with the addition of diesel, most probably because this compound exhibits low conductivity. The reduction in the mobility of charged particles in the system reduces the thermodynamic spontaneity of the redox reactions between the pairs. The higher amount of water present in the B_{Ct} bioreactor tends to increase the ionic mobility between the anodic and cathodic regions[61]. Furthermore, the possibility of oil film adsorption on the carbon steel surface in the B_{Di} system is another factor that contributes to making OCP values more positive due to the formation of a temporary protective layer against corrosion[62].

The kinetic parameter was assessed by calculating the corrosion rate (CR) in mm/year according to the NACE-SP-07-75[31] standard for each system. Figure 10 shows the CR of the B_{Ct} and B_{Di} bioreactors at 7, 14, 28, 42, and 56 days. The results indicate that the coupons buried in the B_{Ct} bioreactor showed a higher corrosion rate compared to those in the B_{Di} bioreactor on all analysis days. The coupons in B_{Ct} exhibited relatively similar CR values, around 0.0957±0.012 mm/year. On the other hand, the coupons in B_{Di} started with higher values, 0.0314±0.0034 mm/year, and at the end of the experiment, the CR value decreased to 0.0121±0.0025 mm/year. Therefore, the intense microbial activity, especially of AHB and HCB, in the B_{Di} system (Figure 7) did not influence the CR values of the steel coupons during the bioremediation period though natural attenuation.

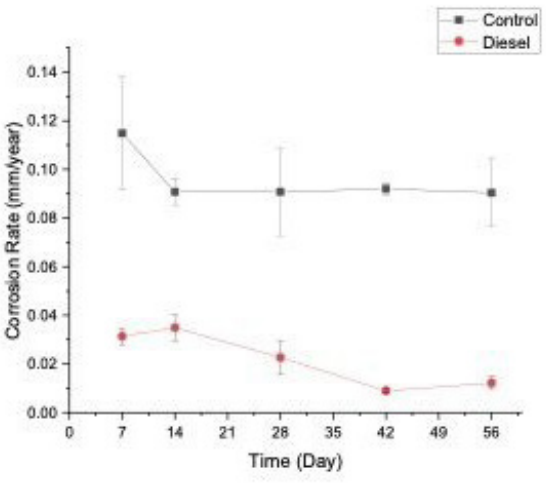

Figure 10. Corrosion rate of coupons buried in bioreactors B_{Ct} and B_{Di}.

Procópio[2] studied the corrosive process of steel in the presence of petroleum. In their research, they assessed the corrosion of API 5L steel buried in saline soil under different experimental conditions: control reactor, reactor contaminated with crude oil, and reactor contaminated with crude oil with the addition of a surfactant. The corrosion rates of the steels found were 0.084 mm/year, 0.023 mm/year, and 0.064 mm/year, respectively. The researchers, as well as Rajasekar[63], also reported that residual oil (such as petroleum) can form a temporary protective barrier to the corrosion of carbon steel. The oily film on the metal substrate acts against the diffusion of oxygen and aggressive ions from the environment, as well as repel water molecules[64]. In the B_{Ct} system, in addition to a greater quantity of water, the sandy soil has greater porosity and facilitates the entry of oxygen that can come into contact with the metal.

In the long term, there are no studies confirming that diesel oil maintains its protective characteristics against the corrosion of steels. This is because there are studies associating diesel oil with the corrosion process of storage tanks and other structures, especially when in contact with biodiesel, due to its hygroscopic nature[65-67]. Since low sulfur diesel oil, at least in Brazil, contains 10% biodiesel[68], it becomes important to consider its potential impact on corrosion.

Future studies also intend to investigate the influence of introducing bio-stimulants on the corrosion of carbon steel during the bioremediation process of diesel. The incorporation of bio-stimulants can significantly alter physical and chemical soil factors, and potentially increase the presence of undesirable microbial metabolites that may accelerate the corrosive process.

According to the NACE-SP-07-75 classification[31], the average corrosion rate of steels in the control bioreactor was considered moderate. On the other hand, steel coupons buried in the diesel bioreactor exhibited corrosion classified as low, indicating that diesel low sulfur diesel oil played a protective role against soil corrosiveness. Therefore, through the methodology applied, it was possible to carry out the bioremediation of diesel in soil by natural attenuation with 24,03% efficiency in areas close to ASTM A36 steel samples, without increasing the corrosion rate.

3.8. Morphological analysis of coupons

Figure 11 presents SEM images of the ASTM A36 steel surfaces after acid pickling, which removed corrosion products. The steel subjected to the B_{Ct} bioreactor for 56 days showed localized attacks with the formation of plates containing excavations. On the other hand, the coupon subjected to the B_{Di} bioreactor exhibited some localized attacks but with relatively unaffected areas, in line with the results obtained in the corrosion rate analysis, indicating diesel as a corrosion inhibitor in the soil.

According to Gentil and Carvalho[8], temporary corrosion protection methods act as a barrier that prevents the penetration of moisture and substances aggressive to metals. In the specific context of ASTM A36 steel subjected to the conditions present in the B_{Di} bioreactor, diesel oil acted as a protective oil, reducing direct physical contact between the steel surface and the aggressive medium, in this case, the soil.

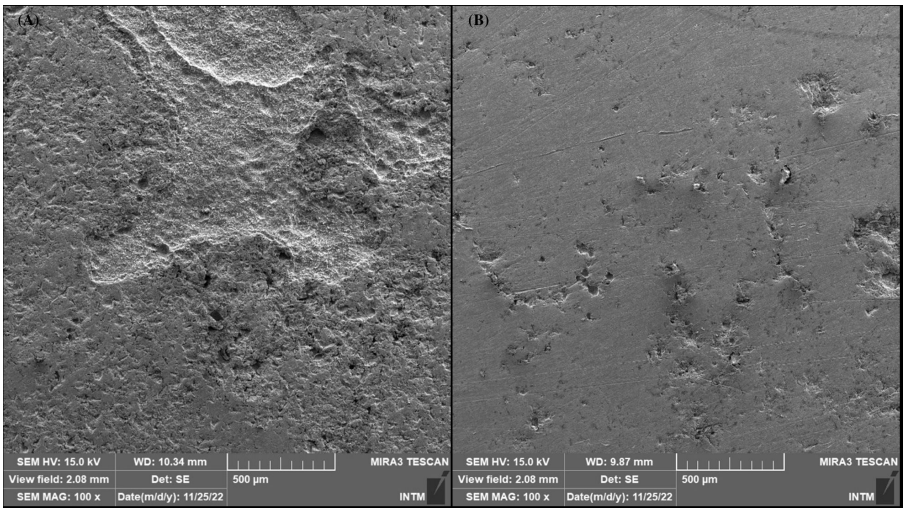

Figure 11. SEM micrograph of the bioreactor coupons: (A) B_{Ct} and (B) B_{Di}.

4. Conclusions

Taking in account all the results and discussions it is possible to conclude:

- Natural attenuation proved to be a viable technique for bioremediating diesel-contaminated soil with oil and grease removal efficiency of up to 24.04% during the 84 days of experiment, without the addition of any type of biostimulant or microorganism. Using the respirometric biodegradation technique, an 11% efficiency in the bioremediation process was quantified.
- The AHB and HCB bacteria exhibited higher quantification compared to AnHB, with HCB consistently surpassing 10^7 CFU/g in the diesel bioreactor. Despite intense microbial activity, especially from HCB during diesel bioremediation, the corrosion rate of ASTM A36 carbon steel was not influenced.
- The average corrosion rate for the control and diesel systems was 0.0957 mm/year and 0.0218 mm/year, respectively. Therefore, the control medium was classified as moderately corrosive and the diesel medium as low corrosive.
- It is suggested that the efficiency of biodegradation can be enhanced by imposing additional stimuli on the soil microbiota, and future investigations should seek a balance between improving bioremediation efficiency and minimizing risks associated with corrosion/biocorrosion in similar environments.

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