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BIOAUMENTAÇÃO FÚNGICA E BIOESTIMULAÇÃO À BASE DE PLANTAS PARA A DEGRADAÇÃO DE HIDROCARBONETOS DE PETRÓLEO EM SEDIMENTOS DE MANGUEZAL

FUNGAL BIOAUGMENTATION AND PLANT-BASED BIOSTIMULATION FOR PETROLEUM HYDROCARBON DEGRADATION IN MANGROVE SEDIMENTS

BIOAUMENTACIÓN FÚNGICA Y BIOESTIMULACIÓN DE ORIGEN VEGETAL PARA LA DEGRADACIÓN DE HIDROCARBUROS DE PETRÓLEO EN SEDIMENTOS DE MANGLAR

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Resumo

Ecossistemas de manguezal são altamente vulneráveis à contaminação por petróleo, apresentando consequências ambientais duradouras devido à persistência de frações complexas de hidrocarbonetos. Embora estratégias de biorremediação, como bioaumento e bioestimulação, sejam amplamente estudadas, sua eficácia comparativa em sedimentos de manguezal permanece pouco compreendida. Este estudo avalia a degradação de hidrocarbonetos totais de petróleo (HTP) em sedimentos de manguezal contaminados com petróleo bruto da Bacia do Recôncavo (1% p/p), utilizando bioaumento fúngico (*Aspergillus* sp.), bioestimulação à base de plantas e a combinação de ambos. Um experimento controlado em mesocosmos simulou a dinâmica das marés ao longo de 45 dias, utilizando 80 biorreatores. Foram observadas reduções significativas nas concentrações da Mistura Complexa Não Resolvida (UCM) e de HTP em todos os tratamentos, com reduções de UCM

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atingindo até 78,4% no tratamento de bioestimulação. No entanto, não foram encontradas diferenças estatisticamente significativas entre os tratamentos ao final do experimento (p > 0,05). Isso sugere um papel predominante dos processos de biodegradação intrínseca, indicando que a atenuação natural pode ser tão eficaz quanto estratégias de engenharia em ambientes específicos de manguezal a longo prazo. Alterações nas razões diagnósticas (pristano/fitano e n-alcanos) confirmaram a degradação preferencial de hidrocarbonetos lineares. Esses resultados destacam o potencial da remediação de baixo custo com soluções baseada na natureza (SbN) e sugerem que processos intrínsecos podem ser suficientes para a recuperação a longo prazo, ressaltando a necessidade de estudos de maior duração para avaliar plenamente as vantagens dos tratamentos de engenharia.

Palavras-chave: Biorremediação; Soluções baseadas na Natureza (SbN); Derramamento de petróleo; Aspergillus; Petróleo.

Abstract

Mangrove ecosystems are highly vulnerable to petroleum contamination, with long-lasting environmental consequences due to the persistence of complex hydrocarbon fractions. While bioremediation strategies such as bioaugmentation and biostimulation are widely studied, their comparative effectiveness in mangrove sediments remains poorly understood. This study evaluates the degradation of total petroleum hydrocarbons (TPH) in mangrove sediments contaminated with Recôncavo Basin crude oil (1% w/w) using fungal bioaugmentation (*Aspergillus* sp.), plant-based biostimulation, and their combination. A controlled mesocosm experiment simulated tidal dynamics over 45 days using 80 bioreactors. Significant reductions in unresolved complex mixture (UCM) and TPH concentrations were observed across all treatments, with UCM reductions reaching up to 78.4% in the biostimulation treatment. However, no statistically significant differences were found among treatments at the end of the experiment (p > 0.05). This suggests a dominant role of intrinsic biodegradation processes, indicating that natural attenuation can be as effective as engineered strategies in specific mangrove environments over the long term. Changes in diagnostic ratios (pristane/phytane and n-alkanes) confirmed the preferential degradation of linear hydrocarbons. These findings highlight the potential of low-cost, nature-based remediation and suggest that intrinsic processes may suffice for long-term recovery, emphasizing the need for extended studies to fully evaluate engineered treatment advantages.

Keywords: Bioremediation; Nature-based Solutions (NbS); Oil spill; Aspergillus; Petroleum.

Resumen

Los ecosistemas de manglares son altamente vulnerables a la contaminación por petróleo, lo que genera consecuencias ambientales a largo plazo debido a la persistencia de fracciones complejas de hidrocarburos. Si bien las estrategias de biorremediación, como la bioaumentación y la bioestimulación, se estudian ampliamente, su eficacia comparativa en sedimentos de manglares aún no se comprende completamente. Este estudio evalúa la degradación de hidrocarburos totales de petróleo (TPH) en sedimentos de manglares contaminados con petróleo crudo de la Cuenca Recôncavo (1% p/p), utilizando bioaumentación fúngica (*Aspergillus* sp.), bioestimulación vegetal y una combinación de ambas. Un experimento controlado en mesocosmos simuló la dinámica de las mareas durante 45 días utilizando 80 biorreactores. Se observaron reducciones significativas en las concentraciones de Mezcla Compleja No Resuelta (UCM) y TPH en todos los tratamientos, alcanzando las reducciones de UCM hasta un 78,4% en el tratamiento de bioestimulación. Sin embargo, no se encontraron diferencias estadísticamente significativas entre los tratamientos al final del experimento (p > 0,05). Esto sugiere un papel predominante de los procesos de biodegradación intrínseca, lo que indica que la atenuación natural puede ser tan efectiva como las estrategias de ingeniería en entornos de manglares específicos a largo plazo. Los cambios en las proporciones de diagnóstico (pristano/fitano y n-alcanos) confirmaron la degradación preferencial de hidrocarburos lineales. Estos resultados resaltan el potencial de la remediación de bajo costo con soluciones

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basadas en la naturaleza (SBN) y sugieren que los procesos intrínsecos pueden ser suficientes para la recuperación a largo plazo, enfatizando la necesidad de estudios a más largo plazo para evaluar completamente las ventajas de los tratamientos de ingeniería.

Palabras clave: Biorremediación; Soluciones basadas en la Naturaleza (SBN); Derrame de petróleo; Aspergillus; Petróleo.

1. Introduction

Accidents involving extraction platforms and distribution pipelines, leading to oil spills, have become a growing global concern. These incidents are primarily caused by structural deficiencies in equipment, human errors during operation, and the high pressures exerted on the ocean floor, which can result in fractures or failures in subsea infra-structure, allowing oil to escape and contaminate fauna, flora, and human health (CARDOSO *et al*., 2025a; MIRANDA *et al*., 2016; MOREIRA *et al*., 2021). Although there is a history of such accidents, their occurrence has decreased with advancements in monitoring and control technologies. However, the risk of leaks still persists, as evidenced by the oil spill that occurred along the northeastern coast of Brazil in 2019, affecting an area of approximately 2,880 km (OLIVEIRA *et al*., 2020). This spilled oil represents a major environmental concern, particularly in sensitive coastal ecosystems such as mangroves, where the persistence of petroleum hydrocarbons can lead to long-term ecological dam-age. Mangrove sediments are characterized by low oxygen availability, high organic matter content, and complex microbial communities, conditions that directly influence hydrocarbon degradation processes (SILVA *et al*., 2019; SIQUEIRA *et al*., 2026; VERÂNE *et al*., 2020).

Bioremediation has emerged as a sustainable and cost-effective approach for mitigating petroleum contamination, relying on the metabolic capabilities of microorganisms to transform or mineralize hydrocarbons. This natural process employs organisms and their by-products to reduce or eliminate the adverse effects of pollutants in the environment and can be applied either in situ or ex situ, including in controlled systems such as bioreactors (MOREIRA *et al*., 2013a). Its attractiveness lies in its environmental compatibility and economic feasibility,

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particularly when applied in situ. In this context, bioremediation has become an important tool for reducing environmental contamination, especially in areas impacted by industrial activities. The structured application of these strategies is essential to improve efficiency and achieve more robust outcomes (MOREIRA, 2011).

Among these strategies, bioaugmentation and biostimulation have been widely investigated. However, their effectiveness in complex mangrove environments remains variable, often limited by environmental constraints and the low bioavailability of hydrocarbons. To overcome these limitations, fungal-mediated bioremediation has emerged as a highly promising alternative. Unlike many bacteria, filamentous fungi possess robust extracellular enzymatic systems, such as laccases and peroxidases, which enable them to degrade highly recalcitrant hydrocarbons even under adverse conditions. Among these organisms, species of the genus *Aspergillus* have demonstrated significant potential for hydrocarbon degradation, particularly under adverse environmental conditions. Mycoremediation has been recognized as an effective strategy for the recovery of environmental matrices contaminated with petroleum hydrocarbons (RODRIGUES *et al*., 2017).

Fungi stand out among hydrocarbon-degrading microorganisms due to their tolerance to high concentrations of toxic compounds, their ability to grow under environmental stress, and their adaptability to nutrient-limited conditions (MAAMAR *et al*., 2020). These characteristics enable them to colonize and persist in contaminated environments where other microorganisms may be inhibited. In particular, *Aspergillus* spp. have shown high efficiency in degrading petroleum hydrocarbons in contaminated soils, driven by their enzymatic capacity to break down complex organic compounds (KADRI *et al*., 2018; LI *et al*., 2020).

More broadly, studies have demonstrated that both fungi and bacteria play key roles in the degradation of recalcitrant compounds, highlighting their potential in environ-mental remediation processes (ALAO; ADEBAYO, 2022; MEIRELLES *et al*., 2022). However, the efficiency of microbial degradation depends on factors such as the molecular structure of hydrocarbons and the presence of specific

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enzymatic pathways, which often exhibit substrate selectivity (HAMOUDA *et al*., 2022). In parallel, plant-derived extracts have been explored as biostimulants due to their potential to enhance microbial activity and increase contaminant bioavailability. Despite these advances, few studies have comparatively evaluated fungal bioaugmentation and plant-based biostimulation in mangrove sediments, particularly with respect to complex hydrocarbon fractions such as the Unresolved Complex Mixture (UCM). Therefore, this study aims to evaluate the effectiveness of different bioremediation strategies (bioaugmentation, biostimulation, and their combination) on the degradation of total petroleum hydrocarbons in mangrove sediments under controlled mesocosm conditions.

2. Materials and Methods

2.1. Experimental Site and Experimental Design

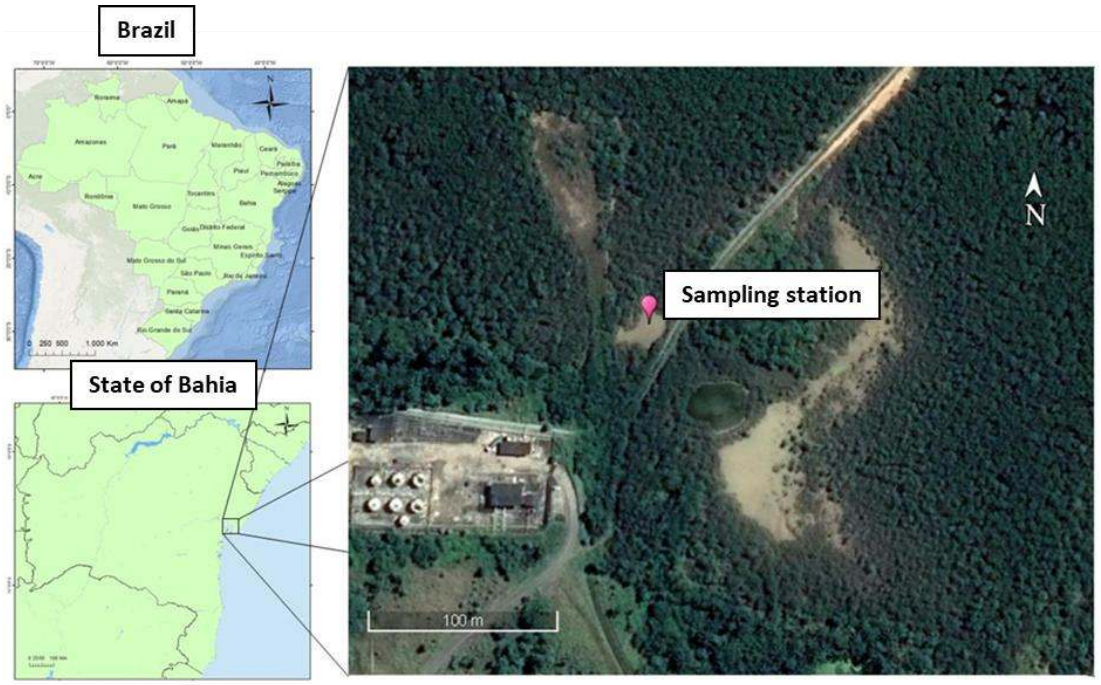
For the bioremediation experiment, coastal hydrodynamic conditions were simulated using bioreactors containing sediments collected from a mangrove area of the São Paulo River, located in the municipality of São Francisco do Conde, Bahia, Brazil, at UTM coordinates 12º43'04.9"S 38º32'44.9"W (Figure 1). The São Paulo River discharges into the northern region of the Todos os Santos Bay (BTS), which covers an area of 1,233 km² and hosts ten large port terminals. The BTS has a coastal perimeter of approximately 300 km, and along its margins lies one of the largest onshore petroleum reserves in Brazil (MOREIRA, 2011).

The BTS stands out due to the numerous activities related to the petroleum industry, particularly following the establishment of Petrobras in the 1950s. During the second half of the 20th century, several environmental accidents involving oil spills occurred in the northern region of the bay, reaching the mangroves of the São Paulo River. More recent incidents were recorded in June 2018, when approximately 3 m³ of oily water dis-charged directly impacted the mangroves of the São Paulo River (MOREIRA *et al*., 2011; MOREIRA *et al*., 2013b; MOREIRA *et al*., 2016; RIOS *et al*., 2017).

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Figure 1 - Map showing the mangrove sediment sampling station for the bioremediation experiment, located at São Paulo River, Bahia, Brazil.



Source: Survey data. (2026)

The bioremediation experiment was conducted at the Laboratory of the Center of Excellence in Petroleum Geochemistry (LEPETRO), at the Institute of Geosciences of UFBA, where bioreactors were assembled using sediments and water collected from the mangrove. Initially, the physicochemical characteristics of both the sediments and the water were analyzed to determine the experimental conditions. Subsequently, a plant extract was prepared and added to the bioreactors along with the mangrove sediments and water. The experiment also included inoculation with fungal strains isolated and prepared specifically to evaluate their effectiveness in the biodegradation of contaminants. The experimental setup was carried out according to the different conditions applied, using the previously described resources.

The sediment used in this study was collected using a stainless-steel shovel and subsequently stored in aluminum containers previously calcined at 400 °C for 4 h to eliminate any potential contamination. The water used in the bioreactors was collected from the margins of the São Paulo River at a sampling station

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located near the sediment collection site. Sampling was conducted to ensure that the experimental conditions closely represented the natural characteristics of the mangrove environment. The materials were then transported to the laboratory, where the physicochemical properties of sediment and water samples were determined prior to the experiment to establish baseline conditions.

The bioremediation experiment was designed to simulate mangrove tidal conditions, with “tide” cycles occurring every 6 h. The experimental system consisted of 80 glass bioreactors (3.5 L capacity; 23 cm height and 11.5 cm width) connected via hoses to an additional set of 16 smaller containers (1.3 L capacity; 16.5 cm height and 11.5 cm width) (Figure 2a). Each reactor contained approximately 0.850 g of sediment and 1.650 L of estuarine water. In the larger containers, the sediment was placed inside a sleeve made of 100% cotton fabric to prevent suspension during water circulation associated with the simulated rising tide (Figure 2b). The containers were interconnected using two polyvinyl chloride (PVC) hoses (½ × 1.5 mm and 1 × 2 mm). Prior to assembly, all containers were decontaminated with 10% Extran detergent and dichloromethane, while the hoses were cleaned using 10% Extran detergent followed by 5% hydrochloric acid.

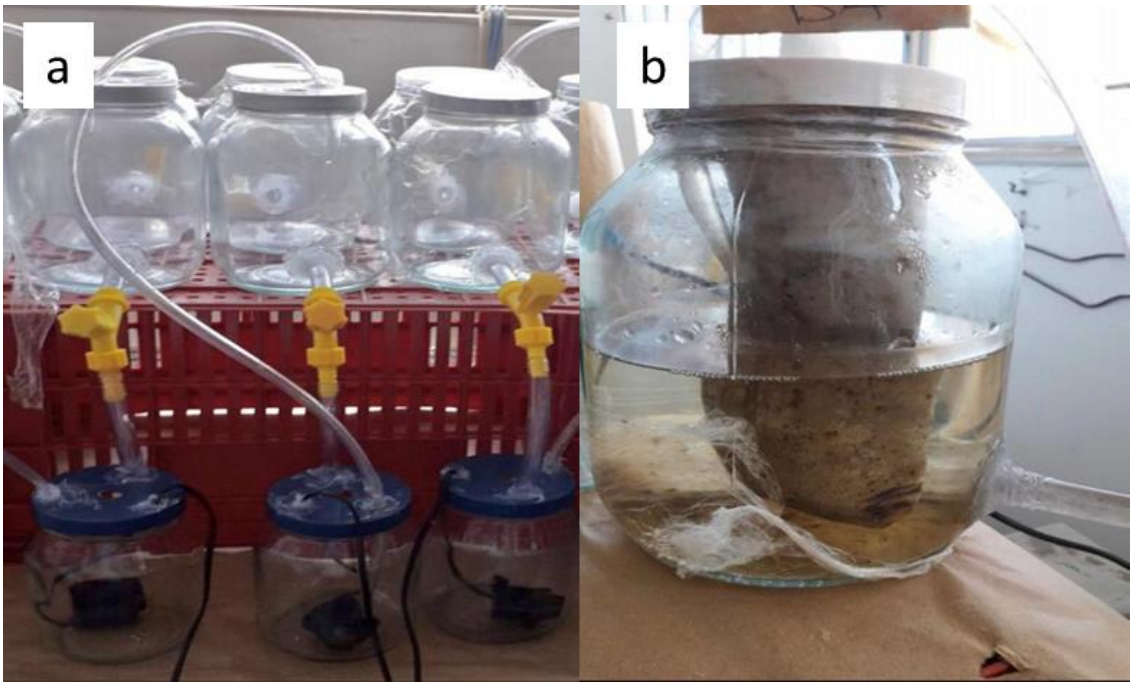
The plant extract used for biostimulation was obtained from Giant Elephant Ear, the common name given to *Alocasia macrorrhizos* (L.) G. Don, as all parts of the plant (leaves, stem, and roots) contain high concentrations of calcium oxalate (CaC_2O_4). Botanical identification was confirmed at the Institute of Biology of UFBA, Department of Botany. *Alocasia macrorrhizos* was selected because previous studies and the patented protocol demonstrated its potential to stimulate hydrocarbon biodegradation by supplying readily available organic compounds and naturally occurring calcium oxalate, which may enhance microbial activity and increase hydrocarbon bioavailability. The amount of plant extract (8 g per reactor) followed the protocol previously standardized and was maintained constant throughout all treatments to ensure experimental comparability. The extraction was carried out following the protocol described in patent (QUINTELLA, ALMEIDA and GONÇALVES, 2018). The fungal strain (*Aspergillus* sp.) used in this study was isolated from a petroleum-contaminated soil sample and cultivated in

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Bushnell–Haas broth supplemented with agar and 1% petroleum, in order to assess its growth using petroleum as the main carbon source. The fungal isolate was identified at the genus level (Aspergillus sp.) based on morphological characteristics routinely adopted in laboratory isolation procedures. Molecular identification was beyond the scope of the present study. It was subsequently cultured on Sabouraud agar (4%) supplemented with 0.1 g L^{-1} chloramphenicol dissolved in 10 mL of 95% ethanol, and later grown on plant residue from the extract leaves prior to inoculation into the bioreactor.

Figure 2 - Experimental Design: a) Bioreactor model; b) Bioreactor in operation.



Source: Survey data. (2026)

A culture of *Aspergillus* sp. that was 20 days old was used in this study. The cultivation procedure was carried out by subculturing the fungus onto Sabouraud Dextrose Agar plates containing 2.0 g of dehydrated Giant Elephant Ear (GE) leaf and 2 mL of 1% saline solution. The fungi were incubated at 30 °C for 20 days, and the results after incubation are shown in Figure 4. An abiotic control without fungal inoculation was conducted in parallel.

The amount of fungal inoculum (4 g per reactor) was selected based on

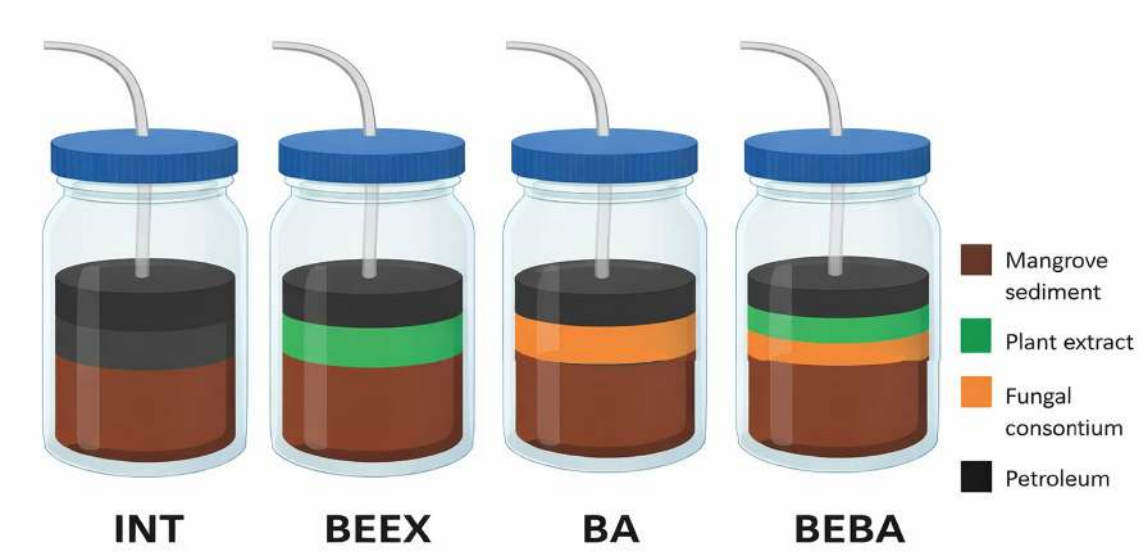
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preliminary laboratory standardization tests and previous studies conducted under similar experimental conditions, ensuring visible fungal colonization and homogeneous distribution throughout the sediment matrix. The inoculum consisted of colonized plant residue supporting actively growing fungal mycelium, which was transferred directly to the sediment to simulate practical bioaugmentation conditions rather than inoculation with purified spore suspensions.

The experiment was conducted under four distinct conditions to evaluate different bioremediation strategies (Figure 3). The experimental setups are detailed in Table 1.

Figure 3 - Experimental conditions in the reactors.



Source: Survey data. (2026)

Table 1 - Experimental conditions applied in the bioreactors.

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Treatment Code	Strategy	Sediment Condition	Additives
INT	Intrinsic Bioremediation	Non-sterilized	1% crude oil
BEEX	Biostimulation	Non-sterilized	1% crude oil + 8 g plant extract
BA	Bioaugmentation	Sterilized	1% crude oil + 4 g fungal inoculum (leaf residue)
BEBA	Biostimulation + Bioaugmentation	Sterilized	1% crude oil + 8 g plant extract + 4 g fungal inoculum

Source: Survey data. (2026)

Sediment sterilization was adopted exclusively in the bioaugmentation treatments to minimize interference from the indigenous microbial community, allowing the degradation potential of the inoculated Aspergillus strain to be evaluated under controlled conditions. The authors recognize that sterilization may modify some physicochemical and microbiological properties of the sediment; therefore, comparisons involving bioaugmentation treatments should be interpreted considering this experimental limitation.

Water circulation was driven by a Sarlo Better Mini A-60 A pump (flow rate: 170 L h^{-1}) controlled by a timer programmed to operate every 6 h. When activated, water was pumped into the bioreactor, and upon deactivation, it returned to the smaller container by gravity, reproducing tidal fluctuations.

The experiment followed a completely randomized factorial design comprising four bioremediation treatments and five sampling times (0, 7, 15, 29, and 45 days). Four independent bioreactors were prepared for each treatment × sampling-time combination, resulting in 20 bioreactors per treatment and 80 experimental units in total. Each bioreactor constituted an independent experimental unit and was destructively sampled only once at its designated sampling time.

An abiotic control was included to verify whether non-biological processes (e.g., volatilization, adsorption, or physicochemical losses) contributed substantially to TPH removal during the experimental period.

For the initial sampling (day 0), four independently prepared bioreactors per

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treatment were sacrificed immediately after assembly, following the same destructive-sampling procedure adopted for the subsequent sampling times.

For contamination, 3.6 kg of sediment were weighed in an aluminum container and mixed with 36 g of crude oil from the Recôncavo Basin (1% w/w). The mixture was homogenized using aluminum spatulas and subsequently divided into four fractions of 0.850 g, which were placed into cotton sleeves and inserted into the bioreactors. Regarding treatment conditions, 4 g of fungal inoculum were added to the sediment in the BA and BEBA systems, while 8 g of plant extract were incorporated into the BEEX and BEBA systems. Estuarine water was then added using a graduated cylinder, with 1.150 L introduced into the lower container and 0.450 L into the upper container.

The monitoring of the bioremediation experiment was carried out at 0, 7, 15, 29, and 45 days. The bioreactor system demonstrated acclimation and stability for microbial growth, uniform hydration across all experiments, homogeneous distribution of microorganisms, and consistent addition of 1% petroleum from the Baía de Todos os Santos (BTS).

At each sampling time, four independent bioreactors from each treatment were terminated, and the sediment from each reactor was collected and analyzed separately. Therefore, the four analytical results obtained for each treatment × time combination represented four independent experimental replicates.

2.2. Laboratory analyses

Sediment analyses included total organic carbon (TOC) and total nitrogen (TN) (determined by combustion using an elemental analyzer, LECO, based on USEPA (2015), as well as total phosphorus (USEPA, 2007) followed by ICP-OES), inorganic phosphorus (acid extraction followed by colorimetric determination), assailable phosphorus and organic matter (SILVA, 2009), and total sulfur (adapted of USEPA, 2016). Water samples were analyzed for pH, dissolved oxygen, and salinity (multiparameter), while ammoniacal nitrogen, nitrate, and phosphorus concentrations were determined using standard analytical

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methods (FERREIRA *et al*., 2011; SILVA *et al*., 2015).

TPH analysis was performed using Gas Chromatography coupled with Mass Spectrometry (GC–MS) on an Agilent Technologies 7890B system. Initially, 2 g of each sample were placed in 40 mL extraction tubes previously decontaminated with dichloromethane, and 25 mL of a dichloromethane:hexane solution (1:1, v/v) was added. The samples were then spiked with 20 µL of the surrogate standard p-terphenyl-d_{14} (1000 µg L^{-1}).

Extraction was carried out in an ultrasonic bath (ELMA Ultrasonic TI-H-10) at 40 °C and a frequency of 35 kHz. Each sample underwent three consecutive extraction cycles of 15 minutes, totaling 75 mL of DCM:HEX solvent mixture per sample. After each extraction, the supernatant was transferred using a glass Pasteur pipette into a 250 mL round-bottom flask through a funnel containing clean cotton (previously decontaminated with dichloromethane) and sodium sulfate calcined at 400 °C for 4 hours, in order to remove residual moisture. Activated copper was added to the flask to eliminate elemental sulfur from the samples. Following extraction and concentration, sample extracts were diluted whenever necessary to ensure that analyte concentrations remained within the linear calibration range (100–2000 µg L^{-1}) prior to GC-MS analysis. UCM abundance was estimated by integrating the unresolved chromatographic envelope between the selected retention time markers after subtraction of the chromatographic baseline. The analytical procedures adopted in this study were based on previously validated EPA protocols routinely employed for petroleum hydrocarbon determination in environmental samples. Quality assurance and quality control (QA/QC) procedures, including instrument calibration, blank analyses, surrogate standard monitoring, and routine verification of analytical performance, were performed throughout the analyses according to the laboratory's established protocols.

Subsequently, the extracts were concentrated using a rotary evaporator (Büchi Rotavapor R-210), transferred to 1.5 mL vials, and adjusted to volume with dichloromethane. All samples were fortified with the surrogate standard (p-terphenyl-d_{14}), with 20 µL added at a concentration of 1000 µg L^{-1} to evaluate

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recovery throughout the analytical procedure. The recovery ranged from 62% to 95%. TPH quantification was performed using a gas chromatograph (Agilent 7890B GC system) coupled to a mass spectrometer (Agilent 5977A GC/MS) operating in Selected Ion Monitoring (SIM) mode. The initial oven temperature was set at 100 °C, increasing stepwise up to 300 °C. The ion source temperature was 230 °C, and the quadrupole temperature was 150 °C.

High-purity helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. Internal calibration was performed using five calibration points with TPH standard mixtures at concentrations ranging from 100 to 200 µg L^{-1}. The accepted correlation coefficient was 0.99. The capillary column used was HP-5MS (60 m × 250 µm × 0.25 µm film thickness).

2.3. Statistical analysis

Statistical analyses were performed using the open-source software R, version 3.6.0 (R Development Core Team, 2019), with the RStudio interface, version 1.1.463 (RStudio Team, 2015). Data were evaluated using the Shapiro–Wilk test ($p < 0.05$) to assess normality. However, the results did not follow a normal distribution, requiring the use of non-parametric tests. To compare the different treatment conditions, the Kruskal–Wallis test was applied, which is used to compare two or more independent samples. The null hypothesis (H_0) assumes that the samples originate from the same population and have the same distribution shape.

Statistical analyses were performed exclusively using the measurements obtained at the final sampling time (45 days), which corresponded to the experimental endpoint established for comparing the effectiveness of the different bioremediation strategies. Data from intermediate sampling times (7, 15, and 29 days) were used only to describe temporal degradation trends and were not pooled with the day-45 dataset for hypothesis testing. Therefore, the Kruskal–Wallis test compared four independent treatment groups at a single sampling time (45 days), each represented by four independent experimental replicates. The

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Kruskal–Wallis test was selected because the response variable did not satisfy the assumptions required for parametric analysis, while preserving the comparison among independent treatment groups at the experimental endpoint.

3. Results and Discussion

The chemical characterization of the mangrove sediment (Table 2) showed a total organic carbon (TOC) content of 1.6%. The total nitrogen (TN) concentration was below the quantification limit (<0.10%). The phosphorus concentration was approximately 302 mg kg^{-1}. Based on these values, an imbalance in the C:N:P ratio was observed. Additionally, the sediment presented low nitrogen availability and measurable phosphorus levels un-der the analyzed conditions.

Table 2 - Characterization of mangrove sediment used in the bioremediation experiment.

Parameters	Analytical results
Total Organic Carbon (TOC)	1.6 %
Total Phosphorus	302.5 mg kg^{-1}
Inorganic Phosphorus	1577 mg kg^{-1}
Total Nitrogen (TN)	< 0.10%

Source: Survey data. (2026)

Additionally, the chemical characterization of the water (Table 3) showed a pH of 7.5, dissolved oxygen at 90% saturation, and salinity of 29 PSU. Ammoniacal nitrogen and nitrate were detected in the aqueous phase. Phosphorus concentrations were low, with assailable phosphorus values below 0.005 mg L^{-1}. Based on these values, a variation in nutrient availability between nitrogen and phosphorus was observed under the analyzed conditions.

The chemical characteristics of the mangrove sediment indicate conditions that may directly influence hydrocarbon biodegradation. The total organic carbon

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(TOC) content (1.6%) was relatively low for mangrove environments, which are typically enriched in organic matter (PEREIRA *et al*., 2016), and the total nitrogen (TN) concentration was below the quantification limit (<0.10%), indicating strong nitrogen limitation. Although phosphorus was present (~302 mg kg^{-1}), suggesting it was not limiting, the imbalance in the C:N:P ratio relative to the optimal proportion (~100:10:1) indicates suboptimal conditions for microbial degradation (SIQUEIRA *et* al., 2026; WU *et* al., 2017). Since nitrogen is essential for microbial metabolism, its low availability, combined with reduced organic matter, may have restricted nutrient cycling and microbial activity, ultimately influencing hydrocarbon biodegradation (JULIO *et al*., 2018).

Table 3 - Characterization of water used in the bioremediation experiment.

Parameters	Analytical results
pH	7.5
Dissolved Oxygen (DO)	90%
Salinity (PSU)	29
Ammoniacal Nitrogen	0.9 mg L^{-1}
Nitrate	19.6 mg L^{-1}
Total Phosphorus	0.11 mg L^{-1}
Assimilable Phosphorus	< 0.005 mg L^{-1}

Source: Survey data. (2026)

The chemical characterization of the water indicates conditions consistent with an estuarine environment, with pH (7.5), dissolved oxygen (90% saturation), and salinity (29 PSU) within typical ranges for mangrove systems. The presence of ammoniacal nitrogen and nitrate indicates nitrogen availability in the aqueous phase, while phosphorus concentrations, particularly assimilable phosphorus (<0.005 mg L^{-1}), were low, suggesting potential phosphorus limitation. This is relevant because nitrogen and phosphorus are essential for hydrocarbon biodegradation, as microorganisms require these nutrients to metabolize carbon compounds efficiently (GALINATO *et* al., 2025). Thus, despite favorable

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physicochemical conditions, the imbalance in nutrient availability, with relatively higher nitrogen and limited phosphorus, may have influenced microbial activity and biodegradation processes in the aqueous phase.

The statistical analysis of TPH degradation was performed to evaluate differences among the bioremediation strategies after 45 days of experimentation. Descriptive statistical parameters, including mean, median, minimum, maximum, and standard deviation, are presented in Table 4.

Table 4 - Statistical analysis of TPH degradation after 45 days of experimentation.

Treatment	Mean	Median	Minimum value	Maximum value	Standard deviation
BA	2047.14	2261.62	906.99	2977.28	644.53
BEBA	1729.78	1596.38	1103.94	2747.72	531.37
BEEX	1964.39	1839.75	659.25	3406.43	687.03
INT	1807.60	1668.04	828.61	3681.49	807.10

Source: Survey data. (2026)

As shown in Table 4, the treatments exhibited variability in TPH concentrations after the experimental period. The BA condition presented the highest mean value (2047.14 mg kg^{-1}), followed by BEEX (1964.39 mg kg^{-1}), INT (1807.60 mg kg^{-1}), and BEBA (1729.78 mg kg^{-1}), which showed the lowest mean concentration among the evaluated treatments. The dispersion of the data, as indicated by the standard deviation, was higher for INT (807.10 mg kg^{-1}) and BEEX (687.03 mg kg^{-1}), suggesting greater variability in these conditions. In contrast, BEBA presented the lowest standard deviation (531.37 mg kg^{-1}), indicating more consistent results. The median values also provide additional insight into data distribution. For BA, the median (2261.62 mg kg^{-1}) was higher than the mean, suggesting a distribution influenced by higher values. In contrast, BEBA showed a median (1596.38 mg kg^{-1}) lower than the mean, indicating a different distribution pattern.

The abiotic control exhibited only minor hydrocarbon losses throughout the

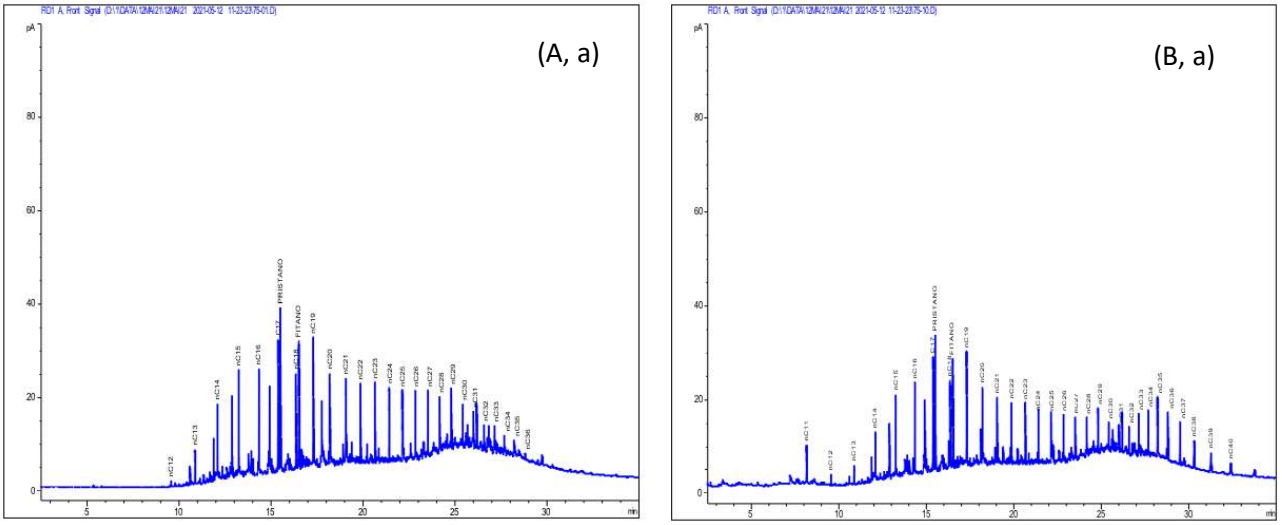
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experimental period (8%), indicating that physicochemical removal processes contributed negligibly to the overall decrease in TPH concentrations. Therefore, the reductions observed in the experimental treatments were predominantly associated with biological processes. The minimal losses observed in the abiotic control support the interpretation that the reductions in TPH concentrations were primarily associated with biological degradation rather than abiotic dissipation mechanisms.

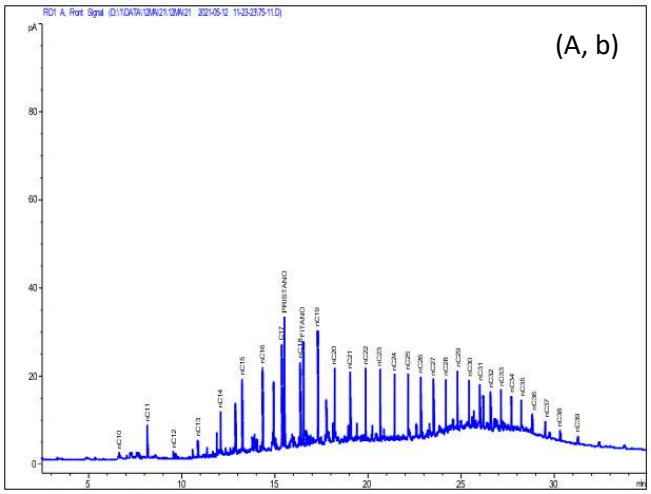
The analysis of chromatograms corresponding to times 0 and 45, under the influence of different bioremediation strategies, revealed significant changes in the evaluated parameters, particularly in UCM and in the specific com-pounds detected (Figure 4). These results reflect the positive impact of the biological approaches applied to the degradation of hydrocarbons present in contaminated residues.

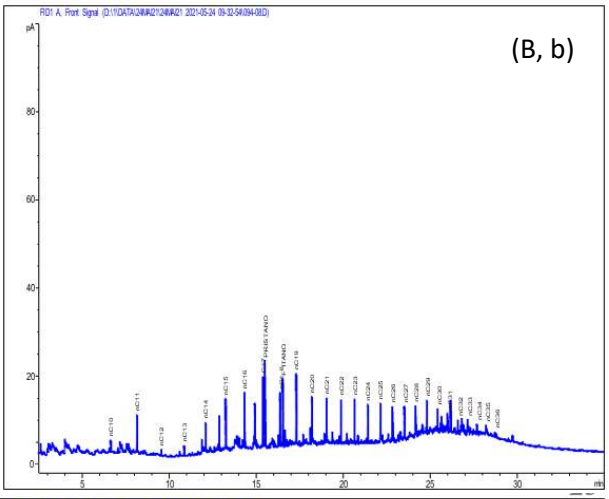
Figure 4 - Chromatographic analysis at times 0 (A) and 45 (B) under the influence of different bioremediation strategies on the degradation of petroleum hydrocarbons (TPH): (a) BA, bioaugmentation consisting of leaf residue and fungal inoculum; (b) BEBA, biostimulation associated with bioaugmentation; (c) BEEX, biostimulation with plant extract; (d) INT, intrinsic bioremediation.

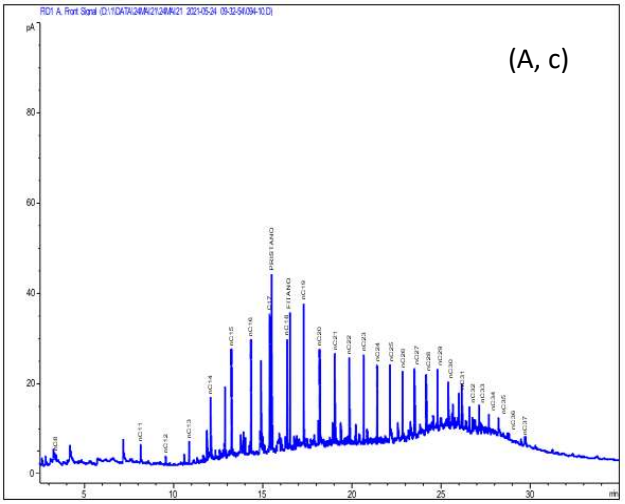


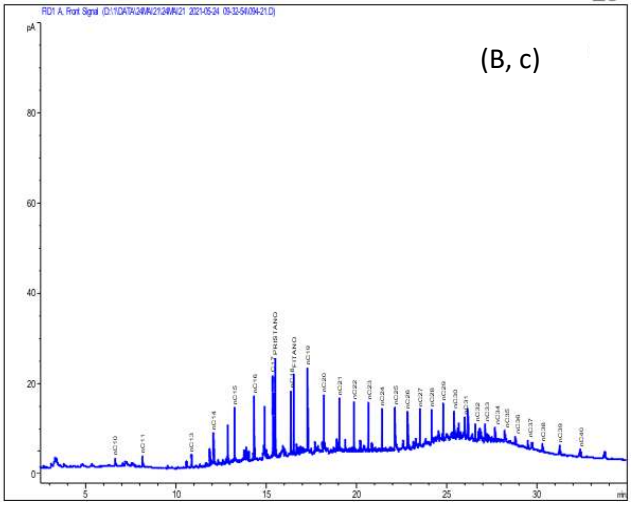
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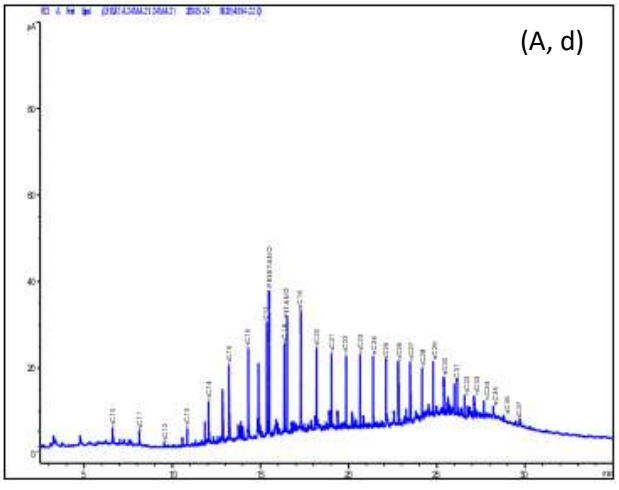
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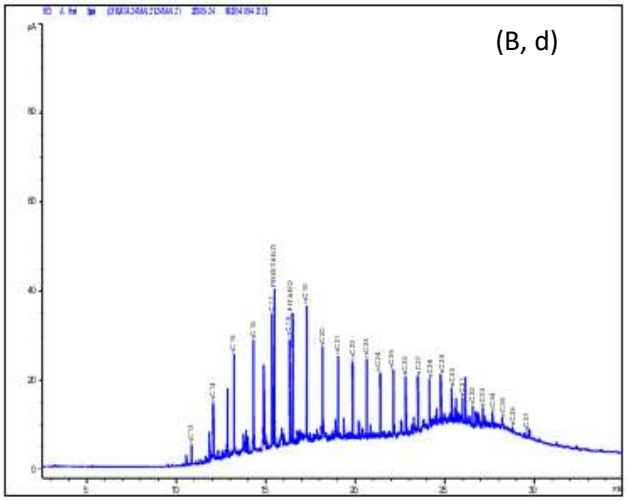












Source: Survey data. (2026)

Under BA conditions, the UCM concentration decreased from 1318.72

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mg/kg at time 0 to 803.71 mg/kg at time 45, corresponding to a reduction of approximately 39%. The chromatograms showed a decrease in peak intensity, particularly in regions associated with longer retention times. In addition, a reduction in the overall area corresponding to the unresolved region was observed. For BEBA, the UCM decreased from 1129.33 mg/kg to 671.20 mg/kg, representing a reduction of approximately 40.6%.

The chromatographic profile at time 0 exhibited a more continuous and elevated baseline signal, whereas at time 45 the unresolved region was visibly reduced, with lower signal intensity across the retention time range. Under BEEX conditions, the UCM decreased from 1411.82 mg/kg to 304.44 mg/kg after 45 days, corresponding to a reduction of approximately 78.44%, which represents the highest reduction among the evaluated treatments. The chromatograms showed a marked decrease in the unresolved region, with a more defined baseline and lower peak intensity at time 45. Under INT conditions, the UCM decreased from 1200.59 mg/kg to 414.28 mg/kg.

The chromatographic profiles also showed a reduction in signal intensity and changes in peak distribution between the initial and final times. Across all treatments, reductions in UCM were observed together with changes in chromatographic peak distribution, including decreases in intensity and modifications in the unresolved region.

The reduction in UCM across all treatments, together with the decrease in TPH concentrations, chromatographic fingerprint evolution, diagnostic hydrocarbon ratios, and the negligible hydrocarbon losses observed in the abiotic control, provides strong geochemical evidence for the progressive biodegradation of complex petroleum hydrocarbons. Under BA conditions, the decrease in UCM from 1318.72 mg/kg to 803.71 mg/kg indicates that bioaugmentation contributed to the degradation of more complex compounds, reflecting the synergistic action of the microbial consortium, in which fungal microorganisms, supported by the organic matter from leaf residues, contributed to the breakdown of recalcitrant compounds (KARISHMA *et al*., 2024)

This reduction is visually evidenced by a decrease in peak intensity,

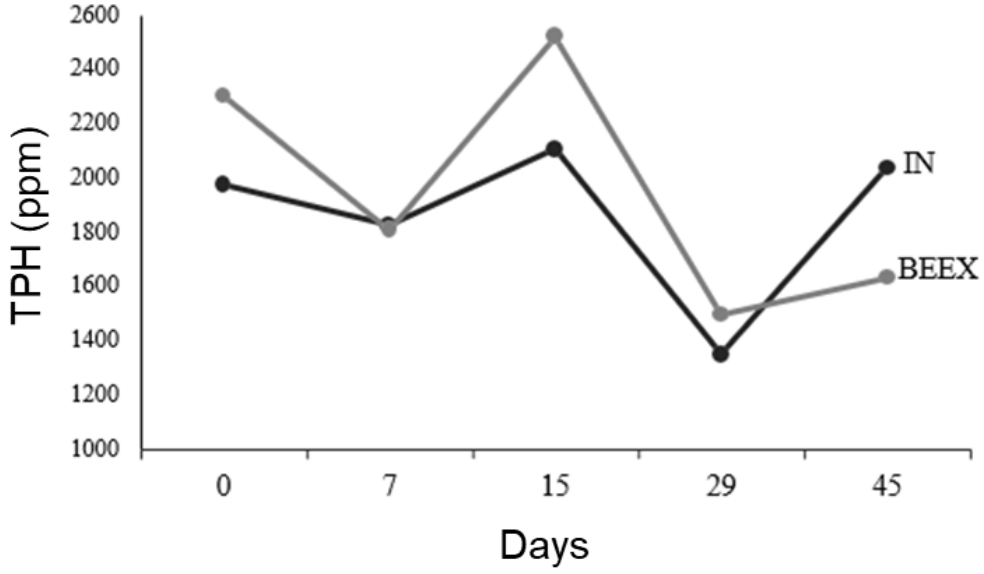
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particularly at higher retention times, where heavier hydrocarbons predominate. The activity of oxidative enzymes produced by fungi, such as laccases and peroxidases, plays a relevant role in the breakdown of complex aromatic and aliphatic compounds. This behavior is consistent with the reduction observed for compounds such as pristane and phytane, as well as with the increase in diagnostic ratios (pristane/phytane, pristane/n-C17, and phytane/n-C18), indicating progressive changes in hydrocarbon composition (CARDOSO *et al*, 2026b; MOREIRA, 2011; REYES *et al*., 2014).

The degradation of total petroleum hydrocarbons (TPH) exhibited a dynamic behavior over time across the different treatments, with varying levels of efficiency. Figure 5 shows the evolution of TPH degradation under intrinsic bioremediation (IN) and biostimulation with plant extract (BEEX) conditions.

Figure 5 - TPH degradation under intrinsic bioremediation (IN) and biostimulation with plant extract (BEEX).



Source: Survey data. (2026)

Under IN (intrinsic bioremediation) and BEEX (biostimulation with plant extract) conditions, at the initial time (day 0), TPH values were similar for both treatments, with BEEX showing a slightly higher concentration (Figure 5). By day 7, a reduction in TPH values was observed under both conditions, indicating an initial

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progression in the hydrocarbon degradation process. Under IN conditions, TPH decreased from 1815.59 mg/kg to 828.61 mg/kg (45.3%), highlighting the role of endogenous microorganisms in the degradation of complex organic compounds. At day 15, an increase in TPH values was observed, more pronounced under BEEX conditions. This behavior may be associated with the mobilization of hydrocarbons that were previously unavailable or adsorbed onto the sediment matrix, becoming detectable during analysis (MOREIRA *et al*., 2021). Subsequently, at day 29, a significant decrease in TPH values occurred, particularly under IN conditions, indicating effective degradation of contaminants. Under BEEX, a reduction was also observed, although to a lesser extent.

By the end of the experiment (day 45), TPH values increased again under IN conditions, while BEEX exhibited stabilization or a slight decrease. This behavior may be related to the volatilization of lighter compounds or the persistence of more recalcitrant fractions. Although plant extracts may enhance microbial activity and improve sediment structure, factors such as the presence of clay minerals and the low solubility of organic compounds may limit the efficiency of the process.

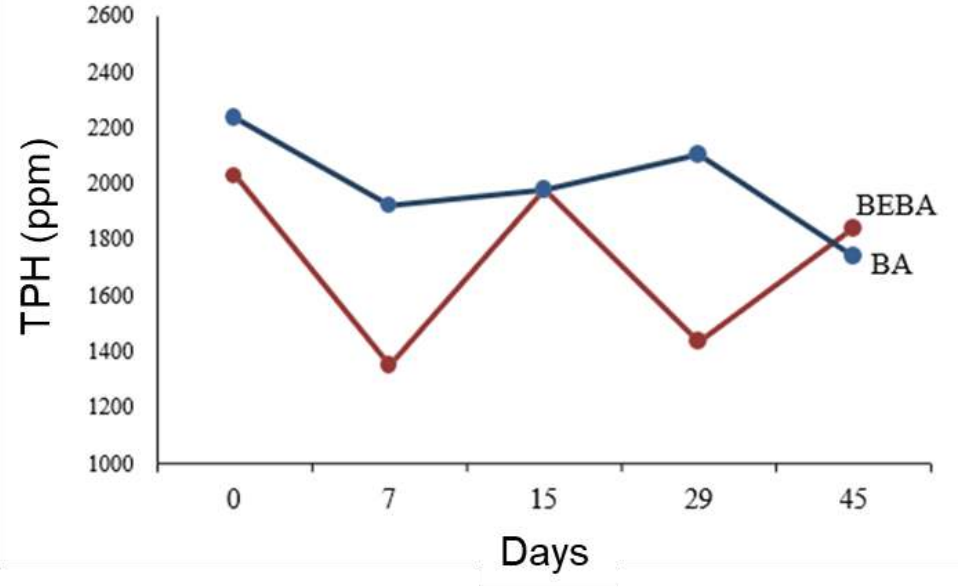
Considering these results, IN showed higher degradation efficiency up to day 29, followed by an increase at day 45, possibly associated with the mobilization of specific hydrocarbon fractions. In contrast, BEEX exhibited a more oscillatory behavior, with lower degradation intensity over time. These findings indicate that the plant extract influenced degradation dynamics but did not result in statistically greater hydrocarbon removal than intrinsic bioremediation under the evaluated conditions (MOREIRA *et al*., 2011).

Figure 6 presents the evolution of TPH degradation under bioaugmentation (BA) and bioaugmentation associated with biostimulation (BEBA) conditions. For these treatments, initial TPH values were similar, with BEBA presenting slightly higher concentrations, possibly due to the presence of biostimulatory compounds affecting hydrocarbon solubility (SU *et al*., 2022).

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Figure 6 - TPH degradation under bioaugmentation (BA) and combined biostimulation–bioaugmentation (BEBA) conditions.



Source: Survey data. (2026)

After 7 days, a sharp reduction was observed under BA conditions, indicating strong initial activity of the fungal inoculum. In contrast, BEBA showed a less pronounced response, likely due to competition between native and introduced microorganisms (PANDOLFO *et al*., 2023).

At day 15, BA exhibited an increase in TPH values, possibly associated with the mobilization of previously undegraded fractions. Meanwhile, BEBA maintained relatively stable values, indicating a slower degradation rate. At day 29, BA showed a new reduction, suggesting recovery of the degradation process, possibly related to microbial adaptation (MOREIRA *et al*., 2013a). BEBA also exhibited a gradual reduction, although less pronounced. By the end of the experiment, both treatments showed significant reductions, with BEBA approaching the values observed for BA. This behavior indicates that, despite a slower initial response, the combination of strategies may result in higher efficiency over time.

The BA condition exhibited an oscillatory pattern, with rapid initial degradation followed by an increase and subsequent progressive reduction. In contrast, BEBA showed a more stable and continuous pattern, becoming more effective in the later stages of the experiment. In summary, all treatments promoted

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TPH degradation over time, although with distinct dynamics. The overall analysis indicates that hydrocarbon degradation is governed by the interaction between microbial adaptation, nutrient availability, and changes in contaminant bioavailability, highlighting the complexity of bioremediation processes in impacted environments.

Variations in diagnostic ratios were observed across all treatments between times 0 and 45, indicating changes in hydrocarbon composition throughout the experiment. Un-der BA conditions, the pristane/phytane ratio increased from 1.20 at time 0 to 1.24 at time 45. The pristane/n-C17 and phytane/n-C18 ratios also showed increasing values over time. Under BEBA conditions, the pristane/phytane ratio increased from 1.25 at time 0 to 1.31 at time 45, with variations also observed in pristane/n-C17 and phytane/n-C18 ratios. Under INT conditions, the pristane/phytane ratio increased from 1.28 at time 0 to 1.34 at time 45, while pristane/n-C17 and phytane/n-C18 ratios showed slight variations over time. Across all treatments, changes in diagnostic ratios were consistently observed be-tween the initial and final sampling times. The variations observed in these diagnostic ratios throughout the experiment provide clear evidence of selective hydrocarbon degradation.

The fungal inoculum likely contributed to the transformation of complex compounds into smaller and less persistent metabolites, while the addition of leaf residues may have enhanced microbial activity by supplying nutrients and stimulating enzymatic processes. However, although fungi are capable of degrading hydrocarbons, this ability may be limited by environmental factors and compound complexity, often resulting in partial degradation (MOREIRA *et al*, 2016).

Under BEBA conditions, the reduction in UCM from 1129.33 mg/kg to 671.20 mg/kg indicates that the combination of bioaugmentation and biostimulation was effective in degrading both unresolved and resolved hydrocarbons. Compounds such as pristane and phytane also showed significant decreases, while the pristane/phytane ratio increased from 1.25 to 1.31, and other diagnostic ratios exhibited small but consistent variations. These results reinforce the

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interpretation that UCM represents one of the most recalcitrant fractions of weathered petroleum and that its reduction constitutes an important geochemical indicator of progressive biodegradation. Under BEEX conditions, the reduction in UCM from 1411.82 mg/kg to 304.44 mg/kg (78.44%) suggests that the plant extract enhanced microbial activity, promoting the degradation of recalcitrant compounds (MEKONNEN *et al*, 2024). However, some limitations were observed, including slower degradation of specific compounds, indicating the need for optimization. The use of plant extract in BEEX and BEBA treatments showed a moderate impact on hydrocarbon degradation, as observed in Figures 8 and 9, but did not necessarily result in higher efficiency compared to conditions without biostimulation. Cardoso Filho (2020), when investigating the bioremediation of polycyclic aromatic hydrocarbons (PAHs) in mangrove sediments, reported that biostimulation with plant extract led to a significant removal of PAHs, reaching 28.53%, whereas intrinsic bioremediation achieved only 6.61%. These findings reinforce that plant extract-based biostimulation can substantially enhance hydrocarbon degradation efficiency under certain environmental conditions.

Hydrocarbon degradation is primarily carried out by bacteria and fungi, due to their ability to adapt to hydrocarbon-rich environments and utilize these compounds as energy sources. Previous exposure to hydrocarbons promotes an increase in degradation rates through selective enrichment of microorganisms capable of metabolizing these compounds (PANDOLFO *et al*., 2023). This adaptation is also associated with the amplification of genes involved in hydrocarbon catabolism, further enhancing the efficiency of the biodegradation process (TONTTERI *et al*., 2023).

Under INT conditions, the decrease in UCM from 1200.59 mg/kg to 414.28 mg/kg demonstrates the capacity of autochthonous microorganisms to degrade hydrocarbons. Diagnostic ratios indicated selective degradation, with faster consumption of simpler compounds and greater persistence of branched hydrocarbons. The reduction in UCM, together with decreases in TPH, pristane, and phytane concentrations, supports the effectiveness of the process over 45 days, although some recalcitrant fractions persisted for longer periods (SU *et al*.,

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2022).

Although all treatments showed reductions in UCM, bioremediation efficiency may be limited by factors such as microbial availability, environmental conditions, and contaminant bioavailability. In addition, recalcitrant compounds such as polycyclic aromatic hydrocarbons (PAHs) may be degraded slowly or incompletely, while environmental parameters such as pH, temperature, nutrient availability, and redox conditions also influence microbial activity and degradation rates (MOREIRA *et al*, 2011).

The degradation of TPH exhibited dynamic behavior over time across the different treatments. Under IN conditions, the decrease from 1815.59 mg/kg to 828.61 mg/kg high-lights the role of endogenous microorganisms in hydrocarbon degradation, a trend also reported by Leoncio *et al*. (2020), who observed an average TPH reduction of 98.65% under low nutrient conditions. The temporary increase in TPH concentrations may be associated with the remobilization of previously sorbed hydrocarbons promoted by sediment disturbance and tidal simulation. However, alternative explanations such as analytical variability and sediment heterogeneity cannot be completely excluded. At later stages, the decrease in TPH indicates effective degradation, whereas the increase observed at the final stage under IN conditions may be related to volatilization of lighter compounds or persistence of more recalcitrant fractions. Biostimulation strategies should therefore be carefully evaluated in terms of nutrient availability and dosage (ŁAWNICZAK *et al*., 2020).

Hydrocarbon degradation is mainly carried out by bacteria and fungi, whose adaptive capacity allows them to use these compounds as energy sources. Previous exposure to hydrocarbons promotes selective enrichment of degrading microorganisms, often associated with amplification of genes involved in hydrocarbon catabolism (RUIZ et al., 2020).

Specialized microbial communities develop mechanisms such as emulsification, hydrocarbon metabolism, activation of metabolic pathways, quorum sensing communication, and biofilm formation. In addition, Posada-Baquero *et al*. (2020) demonstrated that the rhizosphere of sunflower, in the presence of a

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biosurfactant such as rhamnolipid, enhances the desorption of PAHs in creosote-contaminated soils. Furthermore, they observed that the increase in the slow desorption of PAHs contributed to faster biodegradation in experiments conducted with contaminated sludge.

In the study by Conceição *et al*. (2022), bioremediation using microbial consortia composed of autochthonous bacteria and fungi encapsulated in carriers proved effective in degrading TPH in oil-contaminated mangrove sediments. *Pseudomonas aeruginosa* was able to degrade n-alkanes (C16 and C19) and polycyclic aromatic hydrocarbons (PAHs), such as fluorene, phenanthrene, and pyrene. *Delftia* sp. NL1, isolated from an oil field in Algeria, was able to degrade more than 66.76% of diesel within 7 days, producing glycolipids that acted as biosurfactants and improving the accessibility and bioavailability of the insoluble hydrocarbon fraction (MEDIĆ *et al*., 2020).

The variations observed in diagnostic ratios throughout the experiment provide clear evidence of selective hydrocarbon degradation. Changes in pristane/phytane, pristane/n-C17, and phytane/n-C18 ratios indicate preferential consumption of specific hydrocarbon classes under the different bioremediation strategies. The degradation process followed a typical pattern in which linear alkanes were more readily degraded than branched compounds, reflecting their greater bioaccessibility. In contrast, branched com-pounds such as pristane and phytane exhibited greater resistance to microbial degradation. Petroleum compounds are preferentially removed in the sequence: n-alkanes, alkyl-cyclohexanes, acyclic isoprenoids, bicyclic alkanes, steranes, and hopanes (MOREIRA *et al*., 2016).

The increase in pristane/phytane and pristane/n-C17 ratios over time reinforces that biodegradation promoted the preferential removal of simpler compounds, while more complex and recalcitrant fractions were degraded more slowly. Although branched com-pounds are generally more resistant, they may also be degraded under favorable conditions, as reported by (MOREIRA *et al*., 2021).

The combined reduction in UCM, TPH, and biomarkers such as pristane and phytane reinforces the effectiveness of the biodegradation process. Similar trends

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were reported by (MOREIRA *et al*., 2011), indicating progressive degradation of petroleum components. In a study conducted (MOREIRA *et al*., 2013a), it was observed that the ratio between TPH and UCM decreased over 90 days of experimentation, indicating the degradation of petroleum compounds. Another study evaluated the influence of nutrients on hydrocarbon biodegradation and found that, over time, there was a reduction in the concentration of medium to high molecular weight TPH, including linear alkanes and isoprenoids such as pristane and phytane, indicating the degradation of these compound

The statistical analysis supports these observations, indicating variability among treatments. Although BEBA exhibited lower average residual TPH concentrations and greater temporal consistency, these differences were not statistically significant after 45 days, indicating that the engineered treatments did not outperform intrinsic bioremediation under the evaluated experimental conditions (ŁAWNICZAK *et al*., 2020). Studies such as those by Bento *et al*. (2005) corroborate these findings, demonstrating that in systems where biostimulation and bioaugmentation are applied simultaneously, an initial delay in degradation may occur due to the time required for the establishment of optimal conditions for both approaches. However, over time, the combined strategy tends to achieve higher efficiency, particularly in environments with high contaminant heterogeneity. In the study conducted by Cardoso Filho (2020), combined strategies were also considered advantageous for accelerating degradation, especially in environments with challenging characteristics. Overall, hydrocarbon biodegradation is a complex and selective process controlled by microbial activity, environmental conditions, and contaminant bioavailability. The persistence of recalcitrant fractions highlights the importance of optimizing bioremediation strategies to enhance the removal of complex hydrocarbons.

The lack of statistically significant differences in final TPH concentrations among the treatments highlights a crucial aspect of mangrove remediation: the robust intrinsic biodegradation capacity of autochthonous microbial communities. While engineered strategies like BEBA showed consistency and lower average residual TPH, the natural attenuation (INT) proved highly competitive over the 45-

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day period. From a practical and economic standpoint, these findings suggest that in specific mangrove environments with active microbial populations, monitoring natural recovery might be a viable and cost-effective alternative to active interventions. However, engineered strategies may still be necessary for rapid initial response or in environments where intrinsic microbial activity is severely inhibited by extreme contamination levels.

The performance observed in the bioaugmentation treatments reflects the combined influence of fungal inoculation and the experimental suppression of the native microbiota through sediment sterilization. Consequently, the isolated contribution of *Aspergillus* cannot be completely separated from potential effects associated with sediment sterilization, representing an inherent limitation of the experimental design. Future studies should evaluate fungal bioaugmentation under non-sterile conditions, allowing direct interaction between inoculated fungi and indigenous microbial communities. Such an approach would improve the ecological realism of the experiments and better represent field applications.

5. Conclusion

This study demonstrated that different bioremediation strategies promoted the degradation of petroleum hydrocarbons in mangrove sediment, exhibiting distinct efficiencies and temporal behaviors. Reductions in TPH and UCM, together with chromatographic fingerprint evolution, diagnostic hydrocarbon ratios, and the negligible hydrocarbon losses observed in the abiotic control, provided robust geochemical evidence for hydrocarbon biodegradation and compositional changes throughout the experimental period.

Among the evaluated treatments, BEBA exhibited the lowest average residual TPH concentrations and a more gradual degradation pattern. However, no statistically significant differences were observed among treatments after 45 days ($p > 0.05$), indicating that engineered bioremediation strategies did not outperform intrinsic biodegradation under the experimental conditions evaluated. Intrinsic

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bioremediation (INT) also exhibited significant degradation capacity, particularly during the initial stages of the experiment, highlighting the role of autochthonous microorganisms in hydrocarbon removal. Longer-term experiments are recommended to determine whether the distinct degradation dynamics observed among treatments persist beyond the 45-day experimental period.

The temporal analysis revealed distinct degradation patterns among treatments. INT showed higher efficiency up to day 29, followed by an increase in TPH at day 45. BEEX exhibited lower efficiency and oscillatory behavior, while BA showed an initial reduction followed by fluctuations. In contrast, BEBA exhibited a more gradual degradation profile, whereas intrinsic bioremediation promoted faster initial hydrocarbon removal.

Hydrocarbon degradation followed a selective pattern, characterized by preferential removal of linear compounds and greater persistence of branched and more recalcitrant fractions. Variations in diagnostic ratios supported this behavior, indicating differences in degradation pathways depending on the applied treatment.

Despite the observed reductions, the persistence of complex hydrocarbon fractions indicates that complete degradation was not achieved within the evaluated period, reinforcing the need for optimization of bioremediation strategies. Overall, the results demonstrate that mangrove sediments possess a remarkable intrinsic capacity for petroleum hydrocarbon biodegradation. Under the experimental conditions evaluated, bioaugmentation and biostimulation modified degradation dynamics but did not significantly increase hydrocarbon removal relative to intrinsic biodegradation over the 45-day period. These findings emphasize the importance of considering natural attenuation as a technically viable remediation strategy in mangrove ecosystems while recognizing that engineered biological interventions may become advantageous under different contamination scenarios or longer treatment periods.

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