Chapter

Mangrove Ecosystem Restoration after Oil Spill: Bioremediation, Phytoremediation, Biofibers and Phycoremediation

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Abstract

Environmental accidents involving spills of oil and its derivatives in mangroves present themselves as difficult problems to be solved in the short term, as for example in the construction of emergency strategies to combat the arrival of oil stains and fragments. Petroleum its derivatives and the residues generated in this chain, have a complex mixture of hydrocarbons and are considered dangerous substances. This mixture is difficult to degrade and can cause multiple problems in the ecosystem. Our developed biofiber barrier removes oil more than five times in relation to its mass in a simple way and in a short time. However when the spilled oil reaches the mangroves, other biotechnologies were developed and applied such as phytoremediation (87% efficiency), the use of microalgae (94% efficiency) and the use of fungi and bacteria (70% efficiency). This chapter will present biotechnologies developed, patented and applied in cases of oil spills in tropical mangrove of Brazil. These generated biotechnologies have been applied together with civil society in tropical ecosystems that were hit by the Venezuelan oil spill in 2019. The use of advanced molecular biology (studies of genomics, transcriptome, proteomics and metabolomics) in the biotechnologies presented has shown a promising path to faster, viable economically and ecologically correct mangrove restoration.

Keywords: petroleum, bioeconomy, biodegradation, recovery of degraded areas, urban wastewater treatment

1. Introduction

The control of losses during the production, transport and disposal of oil is fundamental in preventing environmental accidents, in guaranteeing the safety

of the community and in the preservation of natural resources [1]. Process losses, such as leaks and ruptures, generate a double cost for companies and environmental pollution [2]. First, the cost of repairing the image, as a damaged image can trigger the closing of contracts with other companies and a reduction in sales. The second cost is to repair the environmental damage caused to people and environmental resources. It is important to note that if the company does not meet the legal criteria, it may still have additional costs with the request for licenses not previously requested. Hence, the need to establish an action plan for the prevention and containment of environmental accidents, that is, to think of all strategies from the containment of the problem to the repair or recovery of the affected area.

The first action to be considered before the arrival of the spot is what is the measure or set of containment measures that will precede the arrival of the spot on the coast. Especially because at first the objective is to prevent the stain from spreading and reaching the coastline, and to carry out this action, the support of the government, fire department, NGOs, scientists, companies and other professionals is necessary, depending on the severity of the problem [1]. Recently, the coast of northeastern Brazil was the victim of a natural disaster, during this period (2019–2020) some scholars sought answers, such as the origin of oil, how to contain and treat it [3].

Biosorbents are efficient in combating environmental accidents, since the bioproducts generated can act as adsorbents and co-products of added value and can adsorb contaminants [4, 5]. They thus allow the reduction of the arrival of the oil slick in the mangroves, in addition to assisting in phytoremediation through the adsorption of hydrocarbons. Coconut and sisal fibers, thermally and chemically pretreated [6–8], have high adsorption efficiency of aromatic hydrocarbons, such as anthracene and methylene blue [9, 10].

In biotechnological processes, it is common to use microorganisms to generate bioproducts of interest in the industry, such as drugs, beverages and fertilizers, as well as in the treatment of contaminated sediments and soils [11]. Rhizospheric microorganisms, also present in plants, can be applied in the bioremediation of areas contaminated by oil spills [12]. It is important to note that plants have abilities such as phytoextraction, phytostabilization, photodegradation and phytostabilization, which assist in the action of rhizospheric microorganisms. In phytostimulation, for example, the plant releases enzymes, metabolites and nutrients, which influence rhizodegradation and plant growth, given that there are bacteria that promote plant growth [12].

Phytoremediation also has the advantage of acting in the control of carbon dioxide emissions, which is a product of the biodegradation of hydrocarbons generated in the bioprocess [13]. It is also important to highlight the application of microalgae (phytoremediation) in the biofixation of carbon dioxide generated during the photosynthesis process [14]. In this way, phytoremediation is aligned with the Paris Protocol, the 17 UN SDGs and also with the circular economy, since the biomass generated can be reused directly in industrial processes.

2. Biotechnologies developed and applied in the restoration of oil-impacted mangroves

2.1 Phytoremediation with mangrove plants

The mangrove is strongly impacted by environmental accidents, due to the richness of biodiversity and abundance of resources, which are used by riverside communities as a source of subsistence [15]. Nowadays, one of the main problems

associated with the mangrove ecosystem is environmental accidents, resulting from failures in the process, during the transportation, production, storage and disposal of oil [16].

The presence of heavy metals in petroleum-contaminated areas also interferes in the rehabilitation of the mangrove ecosystem [17–20]. It is important to point out that certain metals, such as Al, Fe, Pb, Cr, Cu, Zn and Ni influence the biodegradation process of petroleum, which may make it impossible or reduce the efficiency of the implemented remediation strategy. [19] performed an integrated evaluation of the metals Al, Fe, Pb, Cr, Cu, Zn and Ni with the physical–chemical parameters (pH, temperature, dissolved oxygen and salinities), in order to assess how these factors contributed to the response variable, that is, in the percentage of removal of total hydrocarbons from oil, by phytoremediation and bioremediation. Pearson's correlation indicated the strength and direction of the linear relationship between the variables, and the principal component analysis (PCA) was applied to explain the events that were not fully clarified by Pearson's correlation.

The experiment was carried out for three months in a greenhouse near the mangrove, where samples of sediment and residual oil were collected. The tidal regime was simulated daily in each unit, in order to provide models for the remediation conditions closest to a marshy ecosystem. Altogether, 72 seedlings were selected and cultivated, 36 of which were *Avicennia schaueriana* and 36 were *Rizophora mangle* [18, 19].

Seedlings of mangrove plants were collected at low tide, taking into account their height (average of 3 months), defining a standard sample. [19] found after fifteen days of application, that intrinsic bioremediation showed greater efficiency in relation to phytoremediation, which required a longer period to reach maximum efficiency. As [20] this result was expected, since the plants have a longer response time to the pollutant, which varies according to the species and the conditions of the natural system studied or simulated in the laboratory. It is also important to highlight the peculiarity of the mangrove sediment, which has low or no oxygen availability, medium salinity and low diversity of microorganisms in relation to other environmental compartments. These factors can provide greater or lesser biodegradation of toxic compounds. However, the impact of petroleum on mangroves also depends on the types of pollutants, concentration, toxicity, distribution and the retention time [18]. In many cases, the sediment can behave as a reservoir of pollutants such as heavy metals from the marine or terrestrial environment and, thus, the toxicity of the contamination is greater and the degree of difficulty in removing the organic compounds derived from oil becomes more complex [15].

After three months, [19] proved that phytoremediation showed a removal of compounds in the sediments from 33.0 to 4.0 μg / g initially, while the intrinsic bioremediation decreased from 33.0 to 9.0 μg/g (**Figure 1**). Thus, phytoremediation was able to remove the sediment about 20% more than intrinsic bioremediation.

A higher efficiency of phytoremediation, in relation to the removal of the intrinsic bioremediation of petroleum hydrocarbons was observed. The presence of metals did not influence directly on Bioremediation, except for Cu, which may have moderately inhibited greater efficiency in the process (**Table 1**). However, Ni and Al seem to have been absorbed by mangrove plants, while they were removed from the hydrocarbons, which may have favored more the growth of microorganisms in the rhizosphere, besides the stimulation by the allelopathic compounds. Finally, it was emphasized that the implementation of the Phytoremediation model in areas impacted by oil activities can be very important, since it is an inexpensive, environmentally friendly and socially correct technique. Moreover, this process may also contribute to reduce global warming through carbon.

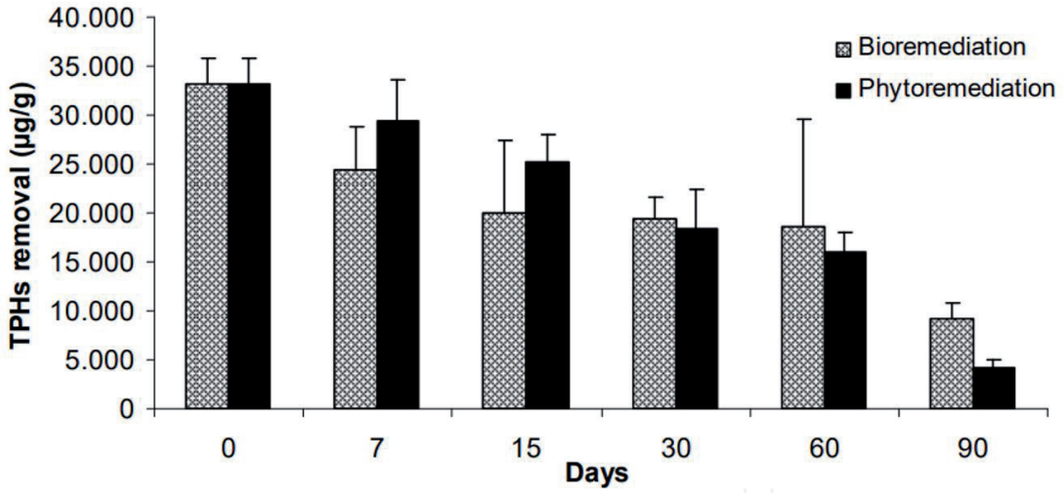

Figure 1.
Temporal removal of total petroleum hydrocarbons (TPHs) during the application of intrinsic bioremediation and phytoremediation in mangrove sediments.

2.2 Bioreactors

The mangrove is one of the coastal ecosystems impacted by socioeconomic and environmental activities, which directly or indirectly affect the distribution of hydrocarbons derived from oil [21]. Marine sediment is one of the main matrices for the deposition of organic contaminants in mangroves, as the biogeochemical characteristics contribute to the accumulation of these compounds and associated with environmental legislation limit the local application of biotechnologies [22]. Thus, studies in bioreactors are great strategies for studying bioprocesses for treating sediments contaminated by oil, without inflicting the requirements established by Brazilian environmental legislation [19–24].

To address this issue, [24] evaluated the efficiency of the bioreactor in the biodegradation kinetics of Total Petroleum Hydrocarbons (TPHs) in the Campos basin present in mangrove sediment. To this end, [24] monitored over 187 days the variation of the physical–chemical and biological characteristics in the sediment and in the circulation water in the bioreactor, used in the simulation of high and low tides.

The methodological steps were carried out in two phases. In the first phase, only bioremediation was tested in the bioreactor, in phase 2, bioremediation plus phytoremediation was tested. The kinetics of oil hydrocarbon biodegradation was evaluated using the [25] model (Eq. (1)), previously selected by [24] in a systematic review. The experimental design was composed of two treatments, the control (sediment without oil) and the contaminated (sediment with oil), both in triplicate, totaling six simulation units, with 6 mini reservoirs/simulation unit.

$$\text{Substrate consumption speed} = \mu\text{máx.S}\left(\text{S0} - \text{X0} - \text{S}\right)/\left(\text{Ks} + \text{S}\right) \qquad (1)$$

In Eq. (1), KS represents the concentration of half saturation, the S was the concentration in time t (final bioprocess time), μmáx was maximum growth rate and the S0 the concentration in time t0 (initial bioprocess time).

The method 3051A (extraction in microwaves) was used for the determination of HTP and HPA in sediment [26]. Subsequently, the extracts were injected in a gas-operated chromatograph (VARIAN brand, model CP3800), with DB 5 capillary column and flame ionization detector (CG / FID) for the determination of total petroleum hydrocarbons. HPAs were detected by gas chromatography (Mark

VARIAN) coupled to a mass spectrometer (GC / MS). In the water samples the extraction method applied was that of U.S. EPA 3510C (liquid–liquid extraction) [27]. Subsequently, the extract was concentrated with the aid of a rotovaporator, model R-215 and, finally, transferred to vials and sent for reading in the gaseous chromatographs. HTP reading was performed on the CP3800 gas chromatograph, with a DB 5 capillary column and flame ionization detector (CG/FID), and HPA determination on the gas chromatograph (VARIAN) coupled to a mass spectrometer (GC/MS). The colony forming units were counted through the micro droplet technique [28] and the culture medium used was Nutrient Agar.

The maximum and specific growth rates of microorganisms were similar in the simulation units in the contaminated treatment, but the speeds obtained were different between the units. In the contaminated treatment the value of the maximum rate in the simulation units ranged from 0.10 day −1 to 0.14 day −1, the temperature ranged from 25° C to 30° C and the pH ranged from 6.5 to 8,5 in the simulation units, a suitable range for biodegradation of petroleum hydrocarbons [29].

The mangrove ecosystem is influenced by the variation of the tides, and to simulate this process in the bioreactor, water from the São Paulo river estuary (class 1 saline water) was used [30].

The catabolism and anabolism reactions that occur during the biodegradation of compounds are influenced by abiotic and biotic factors that interact in biological systems, both natural and simulated. Understanding how these factors influence the response of microorganisms is of fundamental importance in phytoremediation and bioremediation studies.

The uses of predictive models bring satisfactory results for understanding interactions during phytoremediation and bioremediation processes. Therefore, to understand how these variables influence or contributed to the response of microorganisms, [24] generated a generalized linear model, which did not evaluate the contribution of independent variables in the variable response, and also did a Pearson correlation analysis to verify how variables correlated with each other.

In all contaminated treatment simulation units, estuary water significantly influenced the growth of microorganism cells in the sediment. Water pH and salinity had a positive contribution to the number of colony forming units, the number of microbial cells increased with increasing pH and salinity of water used in the bioreactor ($p < 0.05$). Total nitrogen, as well as total organic carbon and total hydrocarbon concentration of petroleum significantly decreased in the sediment with the increase of colony forming units (p value <0.05). We also observed a reduction in the number of colonies with increasing temperature (**Table 2**). Therefore, we believe that microbial cell growth is associated with total nitrogen consumption and total oil hydrocarbon catabolism. Probably, this total nitrogen reduction in the mangrove sediment is available to detect fixed bacteria as rhizospheric roots, such as those that were probably stimulated with the activation of enzymes and metabolites by plant roots.

Correlation analysis of the variables indicated that there was a negative correlation between the number of colony forming units and the dissolved oxygen concentration[1] (**Tables 1** and **3**). This correlation is expected since the mangrove sediment is anoxic, so we believe that the bacteria present in this sediment are facultative anaerobic) [32].

The temperature had a positive correlation with dissolved oxygen concentration and salinity (**Table 2**). Therefore, we believe that increasing the temperature in

[1] The correlation test evaluated the interaction of the independent variables with the response variable, and the pvalue was used to assess the degree of significance of this interaction, so the p <0.05 indicates that the null hypothesis was rejected, and the result was significant [31].

	pH	Eh	T	Sal	DO	Cu	Zn	Pb	Cr	Ni	Fe	Al	TOM	TOC	TN	P	TPH
pH	1																
Eh	−0.92	1															
T	-0.23	0.18	1														
Sal	-0.8	0.58	0.31	1													
DO	-0.31	-0.07	0.1	0.74	1												
Cu	-0.21	-0.09	0.04	0.73	0.9	1											
Zn	-0.04	0.03	0.93	0.27	0.09	0.17	1										
Pb	-0.39	0.28	0.76	0.71	0.46	0.57	0.85	1									
Cr	-0.17	0.23	0.88	0.32	-0.02	0.11	0.97	0.86	1								
Ni	-0.01	0.08	0.64	0.29	0.03	0.3	0.87	0.84	0.92	1							
Fe	-0.46	0.32	0.79	0.65	0.5	0.39	0.72	0.84	0.7	0.54	1						
Al	0.48	-0.34	0.43	-0.3	-0.22	-0.14	0.59	0.33	0.57	0.6	0.4	1					
TOM	-0.47	0.22	0.05	0.85	0.86	0.89	0.13	0.6	0.14	0.27	0.57	-0.05	1				
TOC	0.5	-0.23	-0.71	-0.82	-0.73	-0.68	-0.67	-0.87	-0.6	-0.5	0-0.82	0	-0.65	1			
TN	0.67	-0.38	-0.5	-0.91	-0.83	-0.68	-0.39	-0.72	-0.35	-0.23	-0.83	0.13	-0.79	0.92	1		
P	-0.52	0.24	0.43	0.91	0.86	0.87	0.46	0.83	0.44	0.46	0.76	-0.02	0.9	-0.91	-0.93	1	
TPH	0,52	-0.16	-0.24	-0.85	0.91	-0.83	-0.18	-0.54	-0.09	-0.07	-0.49	0.44	-0.74	0.84	0.87	0.86	1

Table 1.
Pearson correlation in intrinsic bioremediation.

Variables	Estimate	Std. Error	z value	Pr (>|z)
Intercept	72987.51	727.12	100.38	<2e-16
TOC (sediment)	-757.15	22.96	-32.97	<2e-16
TPH (sediment)	-0.01	0.00	-370.31	<2e-16
NT (sediment)	-107861.61	1138.39	-94.75	<2e-16
DO (Water)	6855.73	31.41	218.26	<2e-16
P (Water)	-31438.05	126.42	-248.69	<2e-16
P (sediment)	631.38	3.11	202.94	<2e-16
pH (water)	11954.19	63.08	189.50	<2e-16
Salinity (water)	440.45	2.58	170.48	<2e-16
Temperature (water)	-6374.67	30.03	-212.24	<2e-16

Table 2.
Contribution of water and sediment biogeochemical variables in the growth of colony forming units in simulation units.

bioreactors generates higher evaporation rate in the system, increased salinity and reduced number of colonies forming units. The reduction of colony forming units causes increased availability of dissolved oxygen in the water used to simulate tidal variations.

One of the factors that may indicate the degradation of petroleum hydrocarbons is the increase in the number of microorganism cells. [22] identified that a number of *Bacillus subtilis* bacteria cells were using hydrocarbon biodegradation from the petroleum bioremediation experiment. Analyzing a growth curve of unit 1, it was possible to observe the microorganisms reached in the stationary phase in the final stage of the experiment, from the 153 days of experiment.

In unit 2, the stationary phase of growth of microorganisms was not observed. However, just as in unit 1 there was variation of exponential death phase in the ranges from 42 to 62 days (death phase), 62 to 99 days (exponential), 99 to 118 days (death), 118 to 153 days (exponential) and 153 to 187 days (death). The same was observed for unit 3, which was also not detected in the stationary phase, but there was exponential phase variation in the 42 to 99 days (death), 99 to 118 days (exponential), and 118 to 153 (death) intervals. And 153 to 187 (exponential) (**Table 4**).

The unit 2 in the second phase showed an efficiency of 85.93% in the biodegradation of low molecular weight Aromatic Polycyclic Hydrocarbons compared to the first phase of the bioprocess experiment (49.18%) (**Table 4**) [11].

The three simulation units presented greater removal of hydrocarbon from the sediment in phase 2 of the bioprocess experiment. Thus, we believe that oil contaminated sediment remediation was efficient with bioprocess joining. And, In relation to biodegradation of total petroleum hydrocarbons, unit 2 stood out with the percentage of biodegradation of petroleum from 81,25% and GCR from 0,04 mg.kg-1.day-1 (**Table 5**).

It is important to note that in the first phase of the unit 3 of bioprocess experiment the concentration of low molecular weight hydrocarbons increased in the sediment (**Table 4**). Probably, the increase of low molecular weight HPAs was the result of catabolism of high molecular weight HPAs.

The substrate consumption velocity differed significantly between units, with a confidence level of 5%. There was no interaction of velocity with time and unit. However, at certain time intervals (153 until 187 days and 62 until 99 days) there was similarity in velocities.

Variables	Intercept (CFU)	TOC	TPH	NT	DO	P (water)	P (sediment)	pH	Salinity	Temperature
Intercept (CFU)	1.00									
TOC	0.57	1.00								
TPH	-0.42	-0.02	1.00							
NT	-0.07	-0.36	0.15	1.00						
OD	-0.54	-0.26	0.48	-0.12	1.00					
$P_{(water)}$	0.12	0.36	0.64	0.08	0.26	1.00				
P (sediment)	-0.18	-0.40	-0.49	-0.44	-0.02	-0.53	1.00			
pH	-0.65	-0.26	0.06	0.20	-0.06	-0.35	-0.15	1.00		
Salinity	0.24	0.03	-0.19	-0.31	0.06	0.07	0.39	-0.58	1.00	
Temperature	-0.06	-0.19	0.29	-0.24	0.54	0.33	0.40	-0.71	0.54	1.00

[1] *pvalor < 0.05.*

Table 3.
Correlation between the variables of the generalized linear model and the CFU response variable.

UNIT	% of biodegradation of PHA of low molecular weight	
	Step 1- Bioremediation	Step 2- Bioremediation + Phytoremediation
1	76,38	82,16
2	49,18	85,93
3	55,28 of increase	83,63

Table 4.
Percentage of low molecular weight aromatic polycyclic hydrocarbon (PHA) removal from mangrove sediment.

SIMULATION UNITS	GCR (ppb.day-1)	GCR (mg.kg-1. day-1)	% BIOD.
UNIT 1	10,82	0,01	46,05
NIT 2	40,14	0,04	81,25
UNIT 3	22,14	0,02	64,95
	24,37 ± 14,79	0,02 ± 0,01	64,08 ± 17,61

Table 5.
GCR in the simulation units.

2.3 Phycoremediation with marine microalgae

The produced water is one of the effluents generated after oil extraction. It is estimated that the production of water produced/oil can reach approximately 3: 1 (v/v) of barrels/day [31]. Organic compounds from produced water such as polycyclic aromatic hydrocarbons (PAHs) are classified as highly hazardous pollutants mangrove ecosystems because they are compounds recalcitrant, carcinogenic, teratogenic and mutagenic. When PAHs come into contact with plants and animals, toxic effects are alarming by oxidative stresses, genetic mutations and biomagnification.

Mangrove swamps are coastal ecosystems of great ecological importance to tropical countries. These environments return biomass and nutrients to the sea and act as ecologically nurseries of marine organisms. However, according to the Environmental Sensitivity Index for Coastal Areas published by NOAA, the mangrove habitat is classified as a tropical habitat sensitive to oil spills due to the difficulties of implementing a contingency plan [33].

Therefore, it is necessary to use biotechnologies that increase the efficiency in removing PAHs before being released into water bodies. The use of microalgae-based biotechnologies is characterized as an self-sustaining treatment, since these microscopic beings are photosynthesizes and can be mixotrophic, that is, they perform photosynthesis using sunlight as an energy source and biofixation of CO2 for their own development, but are also able to absorb organic carbons from polycyclic aromatic hydrocarbons as a potential source of energy [34, 35]. Thus, the objective of this research is to evaluate the potential of the removal of polycyclic aromatic hydrocarbons (PAH's) for the treatment of produced water using a photobioreactor system with the marine microalgae species *Nannochloropsis oculata*.

To assess the potential, the removal of polycyclic aromatic hydrocarbons (PAHs) by the photobioreactor system with marine microalgae an experiment was carried out with gradients of different concentrations, for 28 days in the laboratory. The samples produced water were provided by the company Petróleo Brasileiro S.A. (Petrobras). The saline water samples, for dilution of concentrations, were collected at the pier of Porto da Barra whose bounding coordinates were: 13°00′14.12"S and

38°32′01.81”O. The marine microalgae species *Nannochloropsis oculata* was acquired from the Culture Collection of Algae at The University of Texas at Austin (UTEX).

A set of photobioreactors with different gradients of produced water concentration diluted in saline water was assembled, establishing five gradients was called: Photobioreactor with 25%, 50%, 75% 100% (v/v) of produced water and photobioreactor with Conway medium (control). The monitoring of the removal of PAHs chosen in this research was planned in five intervals of different times: consider the 1st day, 7th day, 14th day, 21st day, 28th day. The evaluation of the removal of PHAs was made from the Liquid–liquid extraction was performed in the laboratory [36]. All samples extracted were transferred for injection into the gas chromatograph coupled with mass spectrometer (GC–MS).

The monitoring of microalgae growth was determined through cellular density (cell number ml^{-1}) from the correlation curve (Eq. (2)) between cell count using a Neubauer camera [37] and spectrophotometer absorbance (Agilent Cary 60 UV–Vis) using 680 nm wavelength. The experiment was carried out in triplicate and one-way analysis of variance (ANOVA) with a significance level of $p < 0.05$ was used for statistical analysis. The analyses were conducted using BioEstat 5.3 software.

$$n° \frac{Cel}{ml} = (3E+07)\, x\, OD680nm + (8E+06) \quad R^2 = 0{,}9231 \tag{2}$$

The first five days corresponded to the microalgae acclimatization process to the new cultivation medium (**Figure 2**). After these days of experiment, the growth of microalgae *Nannochloropsis oculata* increased by 25% in all photobioreactors, presenting resistance to organic contaminants until reaching cell stabilization from the 15th day. The results show that its higher growth was presented on the 19th day and in Conway cultivation medium with a concentration of 6.50 x 10^7 cells ml^{-1}, followed by cultivation in water produced at 25% presenting final result 5.24 x 10^7 cells ml^{-1} and the cultivation in water produced at 50% with final concentration of 4.09 x 10^7 cells ml^{-1}.

Considering 75% produced water its highest growth was presented on the 17th day with about 2.77 x 10^7 cells ml^{-1} subsequently begins its decay. 100% produced water presented lower growth of the marine microalgae where it remained constant during the experiment, with about 1.17 10^7 cells ml^{-1}. Constant growth may have been influenced by ions present in the desemulsifiers inserted in the produced water, where they are introduced directly before the water was collected in the well.

The ions of the demulsifying, when at high concentrations, have the ability to neutralize the cellular surface by inhibiting the multiplication of them, being reported the same results in crops above 50% of water produced in experiments developed by [34].

Although microalgae growth is reduced when the water concentrations produced in the medium increase, an increase in efficiency in the removal of total PAHs by microalgae was also increased when the water concentrations produced are also increased, there was a reduction of PAH in low molecular weight compounds and those of high molecular weight in all produced water concentrations, and the largest reduction was observed in 100% produced water, presenting about 3.016 μg L^{-1} reducing to 152.22 μg L^{-1} of light compounds. This reduction represents about 94% efficiently (**Table 6**).

Removal of PAHs in water using microalgae can occur through two pathways: by intracellular bioaccumulation or biodegradation [38]. Biodegradation of polycyclic aromatic compounds by marine microalgae occurs under mixotrophic conditions, with compounds oxidized by oxireductose enzymes, in addition, can

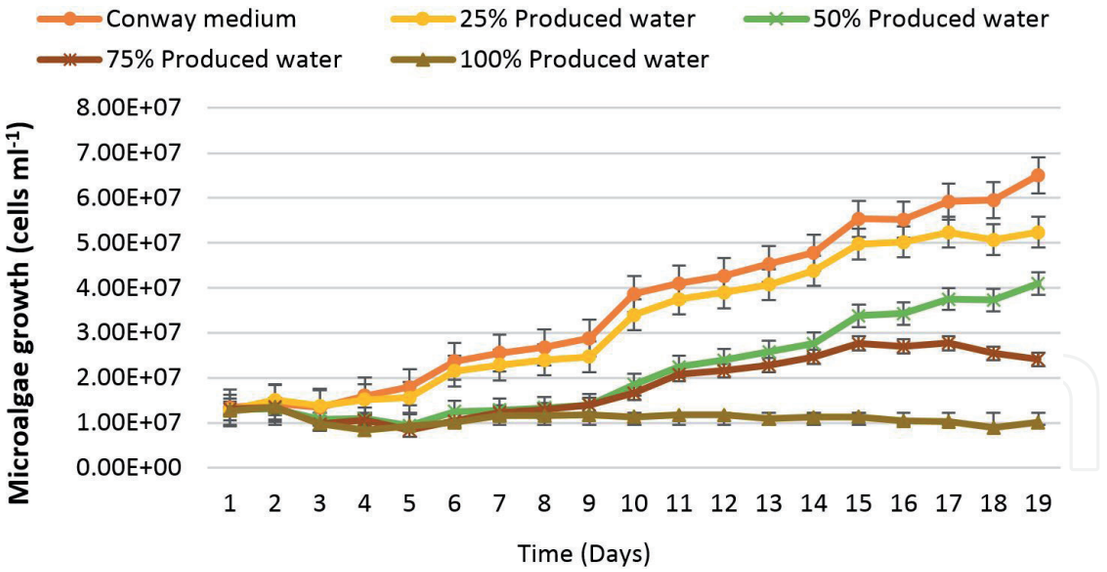

Figure 2.
Effect of different water concentrations produced in the growth of the Nannochloropsis oculata *microalgae measured in cells mL⁻¹. Concentrations: Conway medium, 25% produced water, 50% produced water, 75% produced water and 100% water produced. Data are presented mean value ± margin of error, n = 3.*

form hydroxylated intermediate fractions, justifying the increase in intermediate fractions in the medium [39].

Microalgae are presented as a profitable biotechnology for the efficient removal of PAHs, being exploited as a sustainable source in the treatment of different types of effluents and can be reused for the generation of biofuels, enabling its multi-restorative effects to prevent PAHs from reaching sensitive ecosystems such as mangroves.

2.4 Biofibers

Biotechnologies such as the use of biosorbents and phytoremediation can be applied in situ if there are specialists who know the technique and the ecosystems affected. Adsorbent containment barriers are alternative technology used as a response to spills to contain and recover oil, preventing stains from spreading affecting sensitive areas, as mangroves [40]. Currently the main sorbent materials used in the commercialized barriers are synthetic, however these materials are expensive, are not biodegradable, which makes it difficult to use [7]. In this scenario, some strategies are studied to make the use of sorbents economically viable, through the implementation of natural fibers. The main advantages of natural fibers are low cost, low abrasiveness, not toxic, low density, as well as ecological and social aspects, due to better recyclability and biodegradability [41, 42]. The coconut and sisal fibers, thermally and chemically pre-treated, have high efficiency of oil adsorption in marine and sweet waters, and can be applied from the construction of a containment bar [43–45]. These fibers can also be applied directly to coastal surfaces (beach sediments, mangrove sediments, exposed rocks) and with little contact time and in a simple way is able to remove oil. The use of biofibers should be encouraged, as it allows the reuse of oil adsorbed in the industry for various purposes. This biotechnology is directly aligned with the circular economy [40, 42].

The crushed and sieved coconut fibers were stirred in NaOH solution (5% w /v) at room temperature (approximately 25°C) for 1 hour for mercerization. Then, a wash was carried out with distilled water until a constant pH to ensure the removal of all residual solvent in the fiber [43]. This same procedure was used for acetylation

Concentration of polycyclic aromatic hydrocarbons (μg L $^{-1}$)									
	Initial concentration			Final concentration			Total composition of PAHs		
	LMW (2 - 4 rings)	**IMW (4 rings)**	**HMW (5 - 6 rings**	**LMW (2 - 4 rings)**	**IMW (4 rings)**	**LMW (5 - 6 rings**	**Initial**	**Final**	**Removal efficiency**
Conway medium	102.8 ±4.92	8.26± 0.35	5.84± 0.31	94± 4.91	9.99± 0.77	1.34± 0.02	116.9±4.78	105.33± 2.98	10%
25% produced water	687.84 ±19.23	14.09± 1.13	8.3± 0.98	92.44± 4.90	13.84± 1.02	2.32± 0.13	710.23± 4.88	108.6± 2.57	85%
50% produced water	1537.25± 41.48	13.52± 0.47	5.38± 0.71	127.38± 13.84	9.08± 0.71	1.87± 0.01	1556.15± 4.61	138.33± 2.06	91%
75% produced water	1823.72±166.45	13.29± 0.57	5.02± 0.12	131.79± 8.74	14.56± 0.91	5.43± 0.01	1842.03± 4.86	151.78± 1.05	92%
100% produced water	3016.43± 93.75	13.59± 0.89	7.38± 0.49	152.22± 1.91	35.73± 4.05	6.89± 0.02	3037.4± 2.40	194.84± 4.56	94%

Low Molecular Weight (LMW), Intermediate Molecular Weight (IMW) and Hight Molecular Weight (HMW). Data shown as the mean ± SD, n = 3.

Table 6.
Removal efficiency of polycyclic aromatic hydrocarbons in relation to the molecular compounds present in the five photobioreactors with different gradients concentrations of water produced diluted in saline water.

and treatment with Protic Ionic Liquids (PIL). In acetylation, mercerized fibers were immersed in a solution of acetic anhydride and glacial acetic acid (1.5:1.0 by mass) with 12 drops of sulfuric acid at 80°C for 3 hours. For the treatment with PIL, a sample of coconut fiber was added in 2-hydroxyidoethylammonium acetate [2-HEA] [Ac] at 80°C for 2 hours [44]. These fibers (in natura, pretreated by mercerization followed by acetylation and with PIL) were characterized from the morphology by the Scanning Electron Microscope (SEM).

After the pre-treatment procedures, the fibers were weighed (0.5 g) and conditioned in mini barriers made from TNT (non-woven fabric) to continue the sorption and kinetics tests. The tests were performed in a thermostatic bath, with reciprocal movements of approximately 126 cycles/minute and temperature of 25°C (average temperature of the marine environment). The kinetic experiment was conducted in beakers with 95 mL of saline water and 5 mL of oil from the Campos Basin with the mini-barriers in contact with the oil slick for 120 minutes, in which samples were taken, in triplicate, in the time intervals of 5, 20, 40, 60, 90 and 120 min [7]. In the sorption equilibrium experiment, the oil concentration was varied for the construction of the isotherms [45]. After testing, the samples were cold dried in the lyophilize and weighed. The sorption capacity of the fibers was determined through Eq. (3), where S is the adsorption capacity (sorbate g/g of sorbent), S_0 (g) is the initial mass of the fiber and S_f (g) is the final mass of the fiber after adsorption [6, 7]. The tests were performed by the barriers with in natura and pre-treated fibers.

$$S = \left(S_f - S_o\right) / S_o \tag{3}$$

Through SEM analysis, it was possible to observe a large irregularity and pores on the surface of fibers in natura (**Figure 3a**). After the treatments, the mercerized/acetylated fiber increased the rough area of the cross section, in comparison with the fiber in natura (**Figure 3b**). The fiber with PIL, on the other hand, had a higher number of pores (**Figure 3c**), resulting from the cleaning by treatment with this organic solvent. Thus, chemically treated fibers have more space available for adsorption through the pores and the roughened surface compared to fibers in natura.

The kinetic results of the adsorption of the barriers with coconut fibers are shown in **Figure 4**. In all fibers studied (in natura, mercerized/acetylated and with PIL) the kinetic behavior was very similar. There was a marked sorption up to 5 minutes and then the sorption remained practically constant. This happens because the initial number of pores and available surface in the fibers are occupied over time, reducing the availability and consequently the sorption capacity [6, 7, 42, 45–47]. From these results, it can be concluded that the time of 5 minutes has more significant efficiency in adsorption, requiring a minimum contact time between the

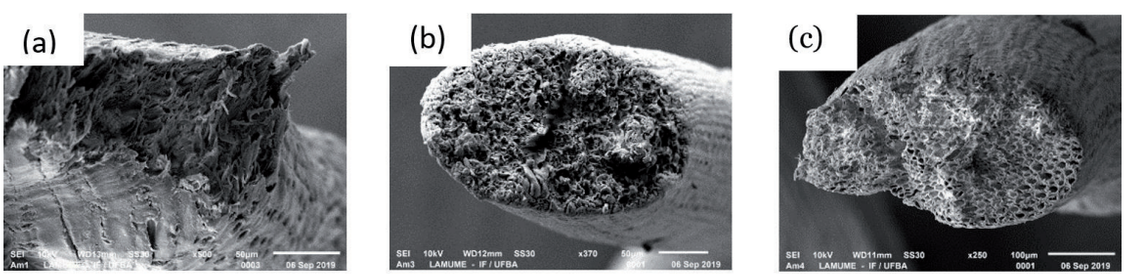

Figure 3.
SEM coconut fiber (a) in natura (b) treated with mercerization/acetylation (c) treated with PIL.

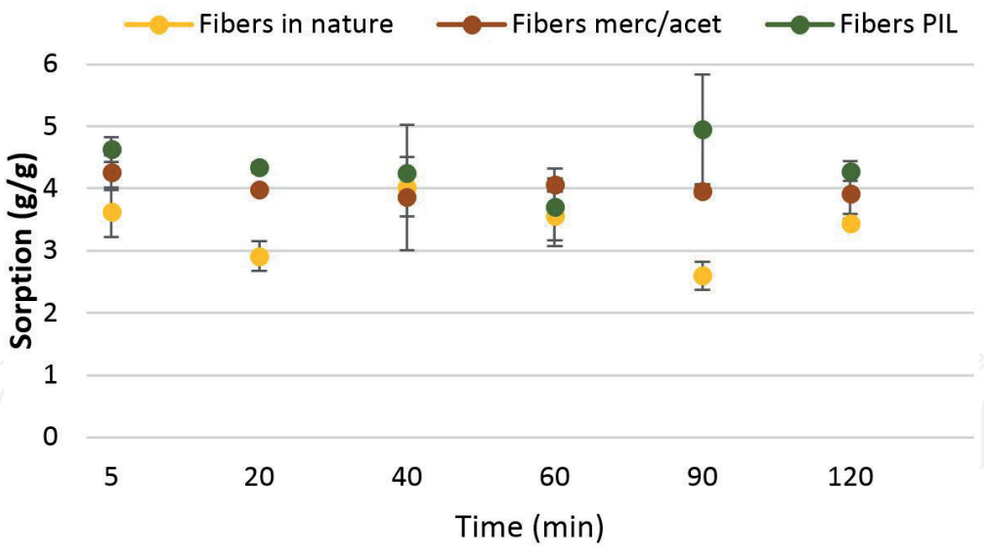

Figure 4.
Comparison of the kinetic behavior of sorption among all coconut fibers.

adsorbent material and the adsorbate to removal of crude oil in the marine environment, in addition to the contact technology operator time with toxic oil.

The equilibrium sorption was 4.00 g / g for fresh coconut fiber, 4.27 g / g for mercerized / acetylated fiber and 5.37 g/g for PIL fiber. Therefore, the fiber with PIL adsorbed 20.5% more than the mercerized/acetylated coconut fiber and 25.5% more than the fresh fiber. (b).

The result of the higher sorption of the treated coconut fibers can be explained by the chemical, structural and morphological modification presented in relation to the natural fibers through the characterizations. The greater quantity and density of pores resulting from the removal of chemical constituents, such as lignin and hemicellulose, waxes and impurities, made the pores of coconut fibers clear and consequently increased the surface area for interaction with oil.

3. Conclusions

Our study with phytoremediation in mangroves, showed that it is possible to accelerate the process of removing oil hydrocarbons in sediments when using the mechanisms of plants, their rhizosphere and the associated microorganisms. Phytoremediation is the most suitable technique for mangrove areas, since sediments have low oxygen solubility and have granulometric characteristics that increase the residence time of persistent organic pollutants such as PAHs. Based on the results found, it can be said that the barriers with chemically treated fibers are more efficient than in natura to be used in the containment and cleaning of oil spilled in marine environments so that it does not affect sensitive areas such as mangroves. The barrier composed of the fiber treated with PIL obtained greater oil sorption, followed by the fiber treated by mercerization-acetylation and finally the fiber in natura. These fiber barriers that were produced by our group can be used during emergency combat of oil stains in estuarine waters, preventing oil from reaching the sediment. They can also be used as sponges to clean oil already adhered to the surface of plants and mangrove sediments, preventing the infiltration of hydrocarbons. In this study it was identified that the *Nannochloropsis oculata* marine microalgae used for the removal of polycyclic aromatic hydrocarbons in produced water showed greater efficiency in the produced water with 94% removal, demonstrating that this marine microalgae is able to contribute to the degradation of

organic pollutants and to prevent PAHs from reaching sensitive ecosystems such as mangroves. Microalgae photobioreactors can be used in the treatment of effluents from the oil industry that are released into the mangrove. In addition, the use of microalgae biorefineries has already been used to remedy river waters, and may be an option during the emergency combat of oil spills in mangrove estuarine waters. A sequential application of bioremediation and adequacy contributed positively to the biodegradation of petroleum hydrocarbons. There were improvements in the quality of the sediment, due to the variation of the physical–chemical characteristics provided by the action of rhizosmic microorganisms, stimulated by enzymes released by the plants, during the oil hydrocarbon metabolism. This process was also noticeable in the growth curve of microorganisms and in the variation in the speed of consumption of petroleum hydrocarbons. The studies that our group has been carrying out for more than 10 years show that there is not a single biotechnology that can restore oil-impacted mangroves. Each biotechnology presented here has its particular contribution in removing pollutants in the various environmental matrices of the ecosystem. Recently our group has advanced in the studies of advanced molecular biology (studies of genomics, transcriptome, proteomics and metabolomics) for the improvement of bioprocesses in a faster restoration and with economic viability (bioeconomy).

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