Research Article

Bioremediation of soils contaminated with petroleum solid wastes and drill cuttings by *Pleurotus* sp. under different treatment scales

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Abstract

Wastes from the oil industry represent one source of soil pollution with a great environmental impact. Both drill cuttings and crude residues are delivered to the soil and produce toxic effects, mainly due to the polycyclic aromatic hydrocarbons. Various bioremediation technologies have been implemented to restore the soil quality and the natural auto depuration capabilities, amongst them: composting, bioaugmentation and biostimulation. These bioremediation techniques promise to be eco-friendlier and cheaper alternatives than other approaches. In this work we have evaluated several strains of *Pleurotus* sp. for their effect on the bioremediation of oil-contaminated wastes and drill cuttings disposed in storage tanks or in open-air soil lots for many years. Our results support that combined natural attenuation mechanisms and directed fungal biodegradation activities, could be promising strategies to remediate heavily petroleum polluted soils and drilling wastes both at the laboratory and in field conditions. Furthermore, we present data that supports the *Pleurotus* genus as able to degrade asphaltenes, the most recalcitrant fraction of petroleum. In addition, the annotation of the genome representative of *Pleurotus ostreatus* revealed clues about the possible enzymatic factors related to the mobilization of carbon from both aromatics and aliphatic derivatives from petroleum hydrocarbons in the genus. This study proposes an approach that at the same time can treat soils contaminated with waste from drill cuttings and bottoms of crude storage tanks.

Keywords Bioremediation · *Pleurotus* · Drilling cuttings · Petroleum wastes · Natural attenuation

1 Introduction

The exploitation of petroleum resources is one of the anthropogenic activities with the greatest environmental impact on aquatic and terrestrial ecosystems. Cleaning of crude oil storage tanks and drilling operations, among other activities, produces petroleum wastes that are accumulated in open-air lots, negatively impacting soil quality. Petroleum-contaminated drill cuttings regularly contain a mix of water, oil or synthetic-based fluids with significant amounts of petroleum hydrocarbons. The hydrocarbon content includes a wide range of saturated and high-molecular-weight aromatic compounds [1]. Crude oil and drill cuttings are toxic, mainly due to polycyclic aromatic hydrocarbons (PAH) present in the muds (5–10%) [2]; and at least sixteen different PAH are alleged cancer-causing agent's [3]. There

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are several physicochemical methods for drill cuttings treatment: cuttings re-injection, microwave drying, and thermal desorption. Unfortunately, these methods have serious technical and environmental challenges, such as expensive implementation, high energy demand, risk of accidental releases to the environment and a high level of pollutant residues. Many biological processes have been investigated to overcome these drawbacks, e.g. composting [4], bioaugmentation [5], or biostimulation [6]. These bioremediation techniques promise to be better alternatives, eco-friendly and money-wise, than physicochemical approaches. Although bioaugmentation (the practice of adding cultured microorganisms for biodegrading soil or water contaminants), has been applied successfully in several studies of petroleum hydrocarbons biodegradation by bacteria, there are few reports of the use of processes that combine degradative activity of indigenous bacteria and the bioaugmentation with fungal specimens in soils contaminated with oil-based drill cuttings. Furthermore, fungi gather valuable metabolic characteristics as they are natural xenobiotic degraders and, many species have been isolated from oil-polluted sources [7, 8], although most species of the genus *Pleurotus* have cosmopolitan origin [9]. They contribute to overall geomicrobial activities that play key roles in the cycling of organic matter and diagenesis [10]. Their ability to be massively cultured on industrial wastes with conventional microbiological techniques makes fungi attractive candidates for soil bioremediation processes.

This paper presents a comprehensive analysis to assess the applicability for large-scale bioremediation processes of several fungal strains, as part of the environmental management and final disposal of pollutants from the Cuban oil industry. The effect of four strains of *Pleurotus* sp. has been evaluated on the bioremediation of oil-contaminated soils and drill cuttings wastes disposed in storage tanks or in open-air soil lots for years, and three scales were implemented for their study at laboratory, microcosm and field levels. Our results show that several isolates of *Pleurotus* sp. are able to reduce asphaltenes and resins content in the samples tested, a task that only a few microorganisms can do. Also, we annotated the representative genome of *Pleurotus ostreatus* in the KEGG database [11], and observed that some sequences of the aromatic compound metabolism showed signatures of functional redundancy within the genome. In addition, these results extend the scope of a prior prepublication posted on bioRxiv server describing our early experimental research findings [12].

2 Materials and methods

2.1 Fungi isolation, culture and identification

Four isolations belong to *Pleurotus* sp. were obtained from representative basidiomata collected on different decaying woods pieces collected in May 2010, from a Zoological park in Habana, Cuba. The location coordinates were 23°06′40.6″N 82°23′48.8″W. In order to obtain axenic cultures, pieces of the fruiting body were inoculated in Petri dishes with Sabouraud Dextrose Agar (BioCen Cat. 4018) and 25 μg. μL^{-1} of streptomycin, at room temperature (26 ± 2 °C) for 7 days. Agar plugs with pure mycelium were passed to new dishes and grown for another 7 days. Besides the evidence offered by the fructification bodies, the isolated strains showed morphological features (mycelia growth pattern and conidial morphology) consistent with the genus *Pleurotus*. The strains were coded as B-1, B-7, B-10, B-15 and pure cultures were conserved in the Chemistry and Biotechnology Laboratory of the Petroleum Research Center, Cuba.

2.2 Solid state fermentation (SSF): laboratory scale assay

Three grams of sugarcane bagasse (particle size < 1.6 mm) and 17 grams of soil contaminated with petroleum solid wastes were added to conical flasks of 100 mL capacity. We sterilized them for 15 min at 121 °C. A solution of ammonium sulfate (0.25%) sterilized independently was used to moisten each flask (32 mL). As inoculant, we used two agar slants (5 mm in diameter) extracted from the active edge of colony growth of the four *Pleurotus* strains. The fermentation lasted 15 days in static condition at room temperature (26 ± 2 °C). Five replicate were used for each treatment, including an abiotic control (without fungal treatment).

2.3 Hydrocarbon degradation in microcosms

The microcosm assay was carried out in composters (diameter 35 cm, depth 18 cm) with sterilized petroleum solid wastes (1 kg), sugarcane bagasse (16 g) and then brought up to 60% of moisture with ammonium sulfate solution (0.25%) sterilized independently. The composters were inoculated with circular sections of mycelia (~ 20 cm^2) from the four *Pleurotus* strains already described, with 4 fragments for each composter. The incubation period lasted 30 days at room temperature (26 ± 2 °C) on static regime, with period (6 h) of sunlight in order to simulate field conditions. The microcosms

were covered with aluminum foil and the soil was moistened by the addition of 250 mL sterile distilled water every week until the end of the experiment.

2.4 Bioaugmentation of parcels contaminated with petroleum solid wastes and drill cuttings using a *Pleurotus* sp. strain

Two parcels (15 × 15 × 0.30 m) from a long-time contaminated (~ 10 years) region with petroleum solid wastes and drill cuttings, in La Cantera Birama, located on Matanzas province, Cuba; were selected for a field-based bioremediation experiment. First, the soil was manually conditioned by removing the surface layer. The field contained aged hydrocarbon residues and a significant rock content. Then, the parcels were biostimulated by adding sawdust according to [13] and moistened with a natural surfactant solution obtained as a byproduct of the processing of henequen fibers (*Agave fourcroydes*). Subsequently, one parcel was bioaugmented with a mixture of spores and mycelium in Sabouraud liquid medium, from *Pleurotus* sp. B-7 strain (10E±06 spores mL^{-1}); and the other parcel was left intact, as a natural attenuation control. No physical barrier was established between the parcels. The specifications for transportation and release of the inoculum were authorized by the Biosecurity Licenses CH 17-P (78) 10 and CH17-P (81) 10 granted by the National Center for Biological Safety of Cuba (CSB). The analytical monitoring of the bioremediation process was assessed by extracting a subset of field topsoil samples (~ 10 grams of soil) for chemical and microbiological analysis. The following variables were recorded at 0, 30 and 70 days: total petroleum hydrocarbons (TPH) [14], Oils and grease [15], saturated, aromatic, resins and asphaltenic components [16, 17], total nitrogen [18], total phosphorus [14], total microorganism count, hydrocarbon-degrading microorganisms [19].

2.5 Biodegradation analysis

The rates of biodegradation expressed as a percentage were determined by monitoring the reduction of petroleum components for all samples at the initial and final stages of each experiment. Uninoculated controls supplemented with contaminated soils samples were always considered. The following equation was used for calculations:

$$Biodegradation\ rate = \frac{(Ci - Cf)}{Ci} \times 100$$

where *Ci* indicates initial concentration; *Cf* indicates final concentration.

2.6 Statistical analysis

For each experiment, we carried out a completely randomized design. We used Kolmogorov–Smirnov test to check the normality premise and Cochran–Hartley and Bartlett test to check the homogeneity of variance. We used the Duncan test for "a posteriori" means comparison. The ANOVA and two-way joining clustering analysis were assessed using the statistical package Statistica 7.0 StatSoft, Inc. (2004). We give additional data in Online Resource 1.

2.7 Annotation and analysis of *Pleurotus ostreatus* representative genome

The genome representative of *Pleurotus ostreatus* PC15 (GenBank assembly accession GCA_000697685.1) was selected as a predictive model of the metabolic capabilities of the genus to degrade petroleum hydrocarbons. We downloaded the protein sequences in FASTA format from GenBank and submitted to KEGG's internal annotation tool for KO (KEGG Orthology) number assignment [20]. The resulting file with orthologous assignments was used to map molecular functions and reconstruct metabolic pathways within the KEGG mapper portal. The enrichment of molecular functions belonging to aromatic-hydrocarbons degradation was analyzed by a binomial test, where the abundance of target enzymes in *Pleurotus* genome was set as *k* parameter, the total KEGG enzymes needed to complete the corresponding pathway was set as *n* parameter, and p was estimated as 1/n for the target enzymes represented by a unique group of orthologs (as with: Phenol 2-monooxygenase (K03380), Amidase (K01426), Salicylate monooxigenase (K00480)). When the target enzymes were represented by several groups of orthologs, we estimated p parameter as: Σ KO/n. A group of orthologs on KEGG Database is represented by a KO identifier and corresponds to groups of highly similar experimentally characterized genes and proteins.

3 Results and discussion

3.1 Bioremediation of soils contaminated with petroleum solid wastes (lab scale)

In order to characterize the petroleum-based hydrocarbon content in the soil at the beginning of the experiment, the samples were subjected to an initial analysis by standard laboratory methods for the fractionation of petroleum (Table 1). The soil samples were rich in compounds that primarily come from crude oil, being the resin and the oils and grease, the major fractions with a content

Table 1 Initial characterization of soils contaminated with petroleum solid wastes in laboratory scale experiment

Treatments	Oils and grease (mg kg^{-1})	TPH (mg kg^{-1})	Saturateds (mg kg^{-1})	Aromatics (mg kg^{-1})	Resins (mg kg^{-1})	Asphaltenic components (mg kg^{-1})
Abiotic control	3.62E+04	8.74E+03	4.81E+03	4.19E+03	2.69E+04	5.75E+03
Pleurotus sp. B-1	3.92E+05	6.55E+04	1.00E+04	5.40E+05	1.09E+05	8.87E+04
Pleurotus sp. B-7	2.34E+05	3.70E+04	9.53E+03	4.72E+04	7.91E+04	1.81E+04
Pleurotus sp. B-10	2.15E+05	5.19E+04	5.17E+03	5.07E+05	4.63E+04	4.20E+04
Pleurotus sp. B-15	9.16E+04	1.11E+05	3.99E+03	6.91E+05	2.11E+05	1.14E+05

The numerical values represent the average of five replicates per treatment

of 104 mg kg^{-1} in average. The total petroleum hydrocarbons, saturated, aromatics and asphaltenic components oscillated in the 103 mg kg^{-1} order. This confirmed that complex mixtures of hydrocarbons derived from crude oil composed almost exclusively the soil samples. After 15 days of treatment with four strains of the basidiomycete *Pleurotus* sp. (as described in MM) we observed that all of the strains tested decreased the content of several of the oil fractions contained in the polluted soil, in contrast to the abiotic control for which no significant change was detected. The highest degradation rates were reached for saturated hydrocarbons (36%, 41.6%, 54.4% and 53.56% for the B-1; B-7; B-10 and B-15 strains, respectively) (Fig. 1). The resins (maximum biodegradation of 40.5% by B-10 strain), and oils and grease (maximum biodegradation of 46.34% by B-15 strain) fractions were degraded by all strains. However, the aromatic fraction was highly elusive to biodegradation, with just 9.2% achieved by B-7 strain. Remarkably, we observed asphaltenes biodegradation in the experiment, being B-7 the strain with the higher biodegradation rate (46.45%) followed by B-10 strain with 21.6%. There is very little evidence of fungi that can degrade asphalts and resins; to date, some examples have been described in detail e.g., the ascomycetes *Neosartorya fischeri* (15.5% asphaltene biodegradation in several weeks) [21], *Pestalotiopsis* sp. (77% in 30 days) [22] and recently the basidiomycete *Daedaleopsis* sp. (88% in 30 days) [23]. Asphaltenes biodegradation rates achieved by *Pleurotus* sp. B-7 and B-10 are superior compared with *Neosartorya fischeri*, and they are capable to reach five times more resins degradation compared to *Daedaleopsis* sp. in significantly lesser time. *Pleurotus* sp. B-7 was the only strain capable to modify all fractions in the polluted soil, a remarkable fact considering the petroleum hydrocarbons chemical complexity. For example, the resins influence the structural stability of petroleum, asphaltenes contain the highest molecular weight constituents in the crude [24], and aromatic compounds are very stable and resistant to biological degradation; these factors play a major role preventing biodegradation processes by microorganisms therefore, finding microbes capable to degradation of these fractions is valuable for the development of effective bioremediation strategies. These findings are not uncommon, for several studies support the role of *Pleurotus* for bioremediation of petroleum hydrocarbon contaminants in soil [25–27]. The degradative activity is often associated with the ability of fungi to produce extracellular hydrolases and oxidoreductases [28]. We conclude that *Pleurotus* sp. B-7 is a good candidate for deeper studies in bioremediation process.

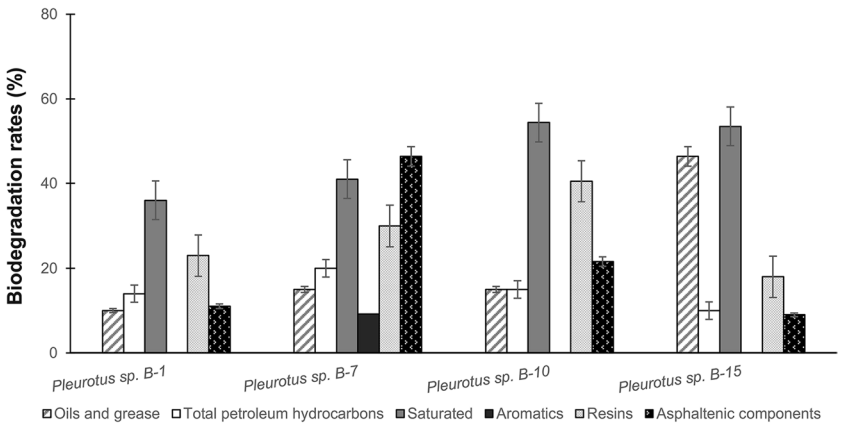

Fig. 1 Biodegradation rates (%) for soil samples contaminated with petroleum solid wastes during 15 days of treatment with four strains of *Pleurotus* sp. (n = 5)

3.2 Microcosm bioremediation of soils contaminated with petroleum solid wastes

To scale up the laboratory soil biodegradation experiment, we carried out a microcosm soil biodegradation study for 30 days. The treatments assessed were: a non-sterile natural attenuation control (to supervise natural ability for intrinsic soil remediation in biostimulation conditions); *Pleurotus* sp. strains in contaminated soil as described in MM; and appropriate sterilized controls to assess abiotic degradation. The natural attenuation treatment achieved significant biodegradation for all fractions in the polluted soil (maximum biodegradation for saturated hydrocarbons 30.6%), except for asphaltenes for which no biodegradation was detected, reinforcing the fact that native microorganisms are rarely capable of asphaltenes degradation (Fig. 2). Biodegradation by natural attenuation is not unusual, due to indigenous degrader microorganisms in soil samples with potential effect in intrinsic bioremediation [29, 30]. However, an effect of biostimulation should not be discarded since in real field conditions the nutritional status can be that of oligotrophy.

Pleurotus sp. B-1 could not degrade asphalts nor resins in the microcosm in contrast to our laboratory experiment, which showed a modest behavior at this respect (11–23%). *Pleurotus sp.* B-7 and B-15 degrade resins fraction considerably more than any other treatment, and all fungal strains degraded the aromatics compounds, contrasting with the laboratory experiment. These results suggest that resin degradation is a consistent process in the strains studied, and again the rates are higher than those for other basidiomycete [23]. *Pleurotus sp.* B-10 and B-15 degrade aromatic fractions in a similar extension ~ 53.4–59.9% and significantly more than any other treatment. Maybe the aromatics fractions become more biodegradable when longer degradation times apply to the process. *Pleurotus* sp. B-7 was the only one that degraded all fractions, even asphalts (40.6%), which are elusive to degradation by other treatments. The concentration of contaminants for the abiotic control by the end of the assessment showed no significant differences with the initial concentrations (in average 4.2E+04 mg kg^{-1} versus 3.9E+04 mg kg^{-1}; standard error ~ 0.06%), suggesting that abiotic mechanisms like volatilization or dilution may not significantly contribute to degradation in these assays [31]. These observations indicated that fungal treatment improved the biodegradation of recalcitrant fractions like aromatics, resins and asphaltenes compared to natural attenuation in the polluted soil. Furthermore, B-7 strain excels once again as a compelling candidate to perform escalated soil bioremediation studies in order to overcome natural attenuation shortcomings.

3.3 Bioaugmentation of a field soil contaminated with petroleum solid wastes and drill cuttings with *Pleurotus* sp. B-7 strain

Pleurotus sp. B-7 was selected as study model for a real field scale bioaugmentation experiment, since it was the strain with better biodegradation performance in both the laboratory and in the microcosm experiments. Before the bioaugmentation treatment, the soil was conditioned as described in MM; a total of 10 L of the fungal inoculum was spread on the polluted soil surface. The selected soils had relatively high concentrations of petroleum solid wastes. At the beginning of experiment, the contaminant levels and hydrocarbon degrading microorganism count were quite similar for control and treated parcel according to F test (P value > 0.05), suggesting a similar potential for natural attenuation in both sites (Fig. 3a). Regardless of the treatment, the main effects were observed after 70 days in terms of reducing pollutants. We confirmed this by the presence of only two statistically different homogeneous groups (days 0 and 70) (Fig. 4), clustered respect to the temporal dynamics of degradation [Oils and grease Duncan test P value: 0.00; TPH Duncan test P value: 0.01;

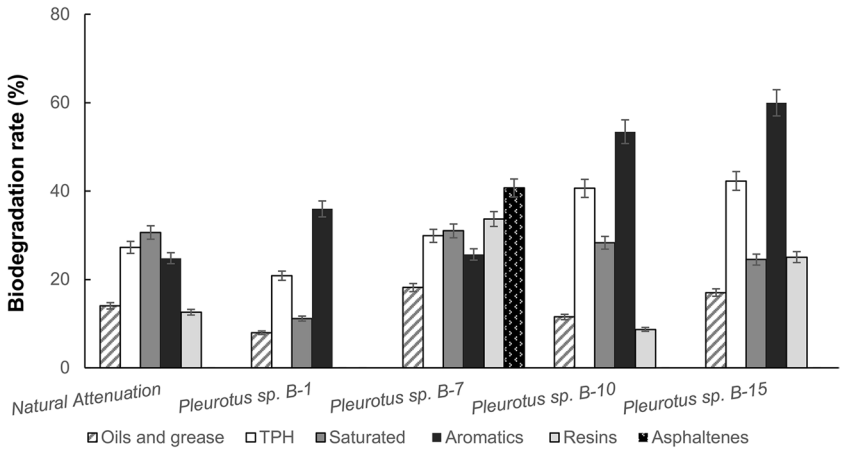

Fig. 2 Biodegradation rates (%) achieved by four *Pleurotus* sp. strains and a natural attenuation control in soil microcosm bioremediation assay for 30 days (n = 2)

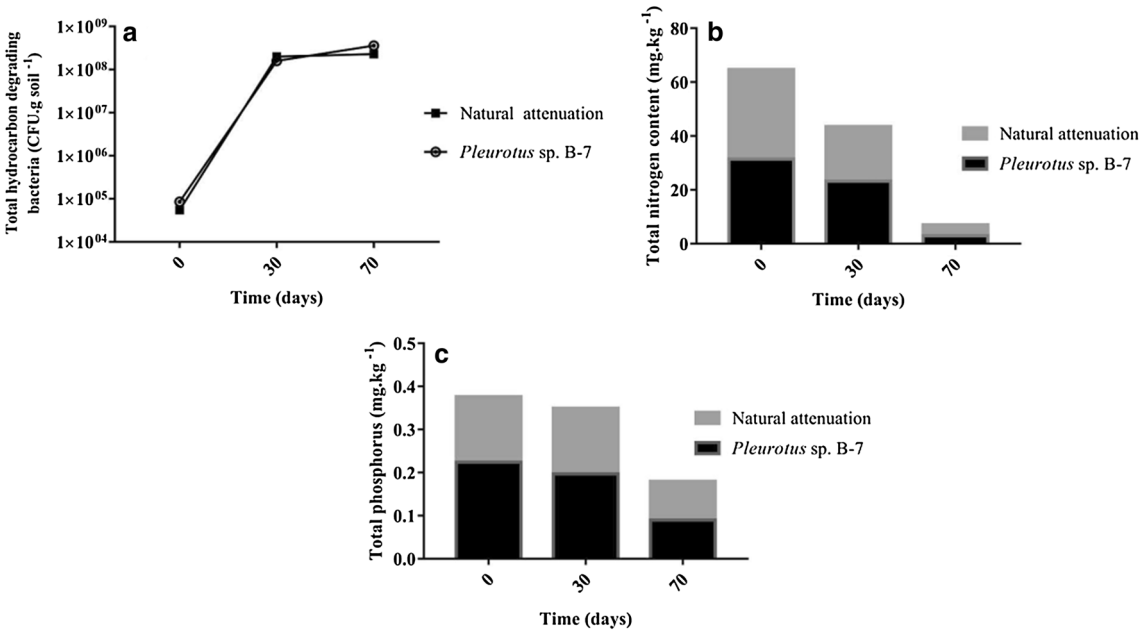

Fig. 3 Microbial count, nitrogen and phosphorus levels in natural attenuation control and B-7 treated parcels. Total hydrocarbon-degrading bacteria (**a**), total nitrogen (**b**), total phosphorus (**c**)

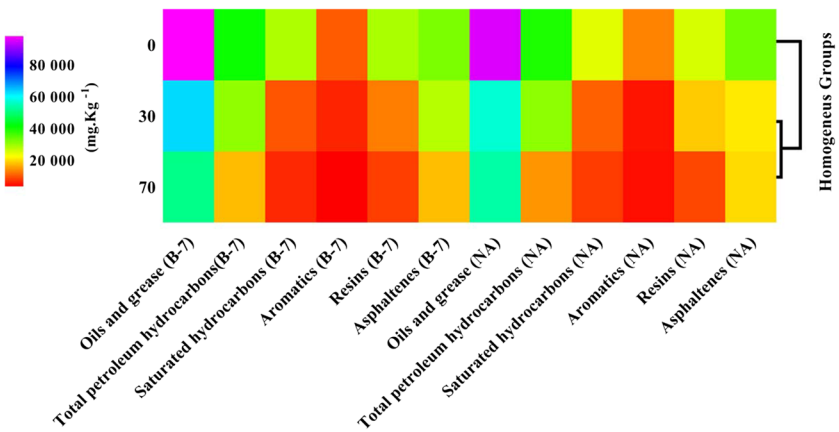

Fig. 4 Two-way joining graph of the oil-fractions concentration (mg kg^{-1}) in the field assay for the natural attenuation control and B-7 strain during 0, 30 and 70 days of treatment (left side on the heat map). Significant temporal differences between treatments are clustered in the right side dendrogram homogeneous groups (see text for *P* values details and Online Resource 1)

Saturated hydrocarbons Duncan test *P* value: 0.03); Resins Duncan test *P* value: 0.01; Asphaltenic components Duncan test *P* value: 0.01 (α: 0.05)] Online Resource 1. Interestingly, at 70 days of treatment the Resins, Saturated and Aromatics-hydrocarbons reached accepted levels according to National Standardization Body of the Republic of Cuba (NC 819: 2017; NC 1263: 2018) (10,000 mg kg^{-1}). However, this period was not enough to reduce Oils and grease, or Asphaltenes up to standards accepted levels. Probably the high initial content of Oils and grease in treated parcels, interfered drastically with the microbial metabolic processes due to its diverse and complex chemical composition (coming from aged fatty matter, sulfur compounds, and organic pigments) [32]. Thus, longer treatments may be necessary for accomplishing the oil-pollutants levels in impacted soils, required by normativity. Except for TPH, resins and asphaltenes, at 70 days there were no differences between B-7 and attenuation control. The percentage of resins removal achieved by the inoculum and the control, amount to 56% and 27% respectively, while the asphaltenes amounted to degrade a maximum of 14% in B-7 treatment. These findings suggest a greater resins and asphaltenes biodegradation in the parcel with the fungal inoculum, although the contribution of indigenous microbiota is not ruled out, for example, there was a significant increase in the biodegraders count with the B-7 treatment (8.50E+04 CFU g $soil^{-1}$ at 0 day and 3.55E+08 CFU g $soil^{-1}$ at 70 day) (Fig. 3a). This

increase in four magnitude orders may be related to nutrient biostimulation applied to the treated parcel.

In the parcel's topsoil, the total phosphorus concentrations were extremely low (attenuation control 0.152 mg kg^{-1}, B-7 treatment 0.224 mg kg^{-1}) at the beginning of the experiment (Fig. 3c), maybe due to the historical characteristics of the field, intrinsically dry, geologically young, little fertile with sparse vegetation mostly composed by Gramineae, spiny crawling plants like *Ricinus communis.* After 70 days, we noticed a decrease of phosphorous to almost undetectable levels (~ 0.09 mg kg^{-1}). The total nitrogen content in both plots also suffered a significant decrease over 70 days (Fig. 3b), maybe as a response to the increased metabolic demands of autochthonous and aloctone populations. Phosphorus and nitrogen are essential nutrients required by the microorganisms in the synthesis of cellular structures and for maintenance of adequate metabolic equilibria. The low levels detected in this study may be caused by a weak ability to release parent material rock-derived, like phosphorus, or by sequestration effects mediated by hydrophobic soil matrix contaminants [33]. This suggest that biostimulation plays an important role in bioremediation so maybe it should be considered for treatments at field level by adding nutrients every 70 days (in the case of this study).

Several reports suggest that the *Pleurotus* genus can effectively degrade petroleum hydrocarbons in the presence of diverse indigenous microflora [27, 34]. In this study, we tested the behavior of four strains of *Pleurotus* sp. on the degradation of polluted soils with petroleum hydrocarbons and drill cuttings. Our experimental approximation contemplated several scales to select the strain with the best performance in the bioremediation of contaminated soil. Finally, the selected strain (B-7) was tested in the field against a natural attenuation control in order to evaluate its possible use on an industrial scale to the bioremediation of soils impacted with wastes from the petroleum industry. Our strictly controlled laboratory and microcosm studies yielded promising results for biodegradation of the most of the hydrocarbon fractions in crude oil and waste. Furthermore, the field study achieved significant decrease of certain contaminants to acceptable levels at 70 days, according to the Cuban standards for crude oil residues and water-based drilling cuttings treatment (< 10,000 mg kg^{-1}: NC 819: 2017 and NC 1263: 2018). As other studies have pointed out, the combination of several bioremediation strategies could improve the hydrocarbon removal [35, 36]. Remarkably, asphaltenic components in the polluted soils, were consistently modified by *Pleurotus* B-7 in the different experiments performed, supporting the possibility to implement an asphalt bioremediation scheme with this strain. However, these kinds of realistic field approaches require extensive environmental monitoring to weigh the real effectiveness of the soil bioremediation process in order to meet international standards. This study is the first large-scale approach to clean up soils contaminated with petroleum hydrocarbons through combined natural attenuation and bioaugmentation in the Cuban oil industry and confirms that long-term monitoring is a necessary condition in the current efforts for bioremediation of petroleum hydrocarbons polluted soils.

3.4 Genomics bases for petroleum hydrocarbon degradation in *Pleurotus*

We annotated the genome representative of *Pleurotus ostreatus* PC15 (GenBank assembly accession GCA_000697685.1) to explore the genetic bases that allow degrading petroleum hydrocarbons within *Pleurotus* genus. KEGG's annotation process revealed that several molecular functions hydrocarbon-related were significantly enriched given the abundance of sequences assigned to specifics KEGG Orthology (KO) (Table 2) (see Online Resource 2 for a complete KO list assignments). For example, phenol 2-monooxygenase, and salicylate monooxigenase increased abundance, could be an adaptive dosage response, that allows contend with the degradation of aromatic hydrocarbons derived from crude oil, like toluene, styrene and naphthalene relatives, increasing survival capabilities under selective pressure [37–39]. The presence of an almost complete pathway to degrade alkane-like compounds in the genome of *P. ostreatus* (Fig. 5), suggests a plausibly strategy to degrade

Table 2 Enriched molecular functions abundance for hydrocarbons biodegradation deduced from representative genome of *Pleurotus ostreatus* PC15 according KEGG annotations

Enzyme	Abundance	KO[a]	*P* value	Benjamini–Hochberg adjusted *P* value	Reference pathway
Phenol 2-monooxygenase (EC:1.14.13.7)	4	K03380	0.004584	0.0057	Toluene degradation
Amidase (EC:3.5.1.4)	5	K01426	0.000177	0.00029	Styrene degradation
Salicylate monooxigenase (EC:1.14.13.1)	11	K00480	< 0.000001	2.49E−06	Naphthalene degradation

[a]KO identifier represent an entry of functional orthologues linked with experimental evidence of functionally characterized sequence data

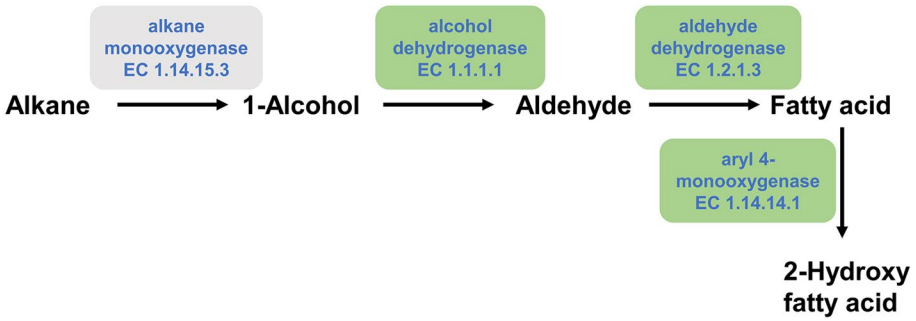

Fig. 5 Alkane-like compounds degradation pathway reconstructed in *Pleurotus ostreatus* genome according to KEGG reference pathway (branch of fatty acid degradation). Green boxes indicate orthologous present in the genome

aliphatic hydrocarbons, and could explain the high efficiency in the degradation of saturated fractions reported in this study. It's worth mentioning a coding sequence for aryl 4-monooxygenase (EC 1.14.14.1), member of the cytochrome P450 system, enzyme related with the oxidation of polycyclic aromatics hydrocarbons [21]. These observations suggest that *Pleurotus* possesses a metabolic potential that allows it to fill niches related to the degradation of petroleum hydrocarbons. However, more studies are needed to map the functionality of most *Pleurotus* genes, which lack functional annotation.

4 Conclusions

One drawback in bioremediation processes lies in achieving comparable results in laboratory as in the field. Although selected and adapted microbial strains are useful in hydrocarbon biodegradation, not all fractions are degraded in similar extension, the combination of different strategies becomes necessary in order to significantly reduce contaminant levels under realistic field conditions. Our results point toward a combined use of bioaugmentation and natural attenuation with the purpose of obtaining improved outcomes in bioremediation processes. The genus *Pleurotus* contains a genetic potential still poorly explored, however functional annotation analysis such as the one developed in this study could help to disclose new metabolic functions useful in bioremediation. Currently, we are carrying out studies on this site to understand how autochthonous microbial communities interact in the hydrocarbon-degradation processes through metagenomic approaches. It is worth to highlight the isolation of a very efficient strain of *Pleurotus* capable of asphaltenes degradation (B-7 strain), since very few organisms can achieve this capacity.

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Data availability Statistical and genomic annotation datasets generated during the current study are available on the Online Resource described on this paper. Other data are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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