**Bioaugmentation via Fungus: An approach to enhance soil bioremediation**

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**ABSTRACT**

As persistence of aromatic compounds that exhibit carcinogenic and mutagenic properties has become specific environmental concern, an extra methodology of their removal from soil is bioaugmentation that is outlined as a method for improvement of the degradative capability of contaminated areas by introduction of specific competent strains or consortia of microorganisms. Therefore, soils in which the microorganisms have lost or didn’t possess the power to biodegrade those venturous compounds, bioaugmentation is usually recommended as a method for improvement of the magnitude relation of bioremediation. Bioaugmentation is receiving increasing attention as an approach to reinforce the catabolic potential at contaminated sites and stimulate the biodegradation of recalcitrant priority pollutants. According to the previous explorations involving micro fungi and white rot fungi including their capability to degrade petroleum hydrocarbons, several auspicious results have been reported. This chapter discusses the principle of bioaugmentation, and presents case studies and guidelines for its successful implementation as a bioremediation approach of contaminated soil via fungus

**Keywords: biodegradation, bioaugmentation, white rot fungi, PAHs, fungus, bioremediation, mycoremediation.**

**1.Introduction**

The growth and proliferation of oil consuming microorganisms in contaminated soil is greatly laid low with the provision of nutrients and their hydrocarbonoclastic property. Moldering capacities of fungi get together with the array of naturally-occurring compounds that function potential carbon sources. Hydrocarbon pollutants have similar or analogous molecular structures that change the fungi to gulped up them additionally. Therefore, once a part is contaminated, the capability to traumatize the contamination and convert it into an energy supply is chosen for within the fungal population that ends up in a population that's higher able to metabolize the contamination. As persistence of aromatic compounds that exhibit carcinogenic and mutagenic properties has become specific environmental concern, an extra methodology of their removal from soil is bioaugmentation that is outlined as a method for improvement of the degradative capability of contaminated areas by introduction of specific competent strains or consortia of microorganisms.

Bioaugmentation develops the biological material so as to swimmingly break down sure compounds. Once a microorganism is adscititious to the contaminated space, they're able to improve the biological material’s capability to behave during a manner on break down contamination that was already choppy before. Therefore, soils in which the microorganisms have lost or didn’t possess the power to biodegrade those venturous compounds, bioaugmentation is usually recommended as a method for improvement of the magnitude relation of bioremediation.

Bioaugmentation is the method of application of autochthonous or allochthonous wild type or genetically modified microorganisms to polluted hazardous waste sites in order to accelerate the elimination of undesired compounds [1] Figure 1 depicts the process of bioaugmentation. Bioaugmentation is mainly introduced in oil contaminated environments as an optional strategy for bioremediation [2].



**Figure 1 The pictorial diagram of bioaugmentation [2].**

1. **Principle of bioaugmentation**

The explanation of this approach is to reinforce the degree or rate of degradation of the complicated pollutants by the addition of pollutant-degrading microorganisms [3,4]. Enhancing the microbiota of a contaminated website won't solely enhance the elimination of the pollutants from the actual website however conjointly at an equivalent time will increase the genetic capability of the required website. Therefore, bioaugmentation corresponds to a rise within the factor pool and, thus, the genetic diversity of the location. in essence, this genetic diversity might be increased by augmenting the microbic diversity [5,6].

 **2. Fungal Bioaugmentation**

 **A. Bioaugmentation by micro fungi (lower fungi)**

Essabri *et al*. (2019) performed bioaugmentation and biostimulation of total petroleum hydrocarbon degradation in a petroleum-contaminated soil with fungi Isolated from olive oil effluent. During the process of degradation, 35 isolates belonging to 11 genera were sanitized and 3 isolates as well as their consortium were initiated to be able to raise in association with petroleum hydrocarbon as sole supply of carbon under in vitro circumstances. The isolated strains were grounded on internal transcribed spacer (ITS) rDNA sequence analysis. *Aspergillus niger*, *Penicillium ochrochloron*, and *Trichodema viride. possessed* utmost potentiality to reduce petroleum hydrocarbon without emerging antagonistic activities. *P. ochrocholon for its growth* on petroleum hydrocarbon gained weight of 44%, *A. niger* 49%, and *T. viride* 39% within the first 30–40 days. These fungi accumulated significantly higher biomass, produced extracellular enzymes, and degraded total petroleum hydrocarbon as compared to those of controls and *A. niger* firmly degraded total petroleum hydrocarbon with a degradation of about 71.19%. GC-MS analysis data confirmed that these isolates displayed rapid total petroleum hydrocarbon biodegradation within a period of 60 days and the half-life showed that *A. niger* was the shortest with *t*1/2 = 21.280 day−1 parallel to the highest percent degradation of 71.19% and first-order kinetic suited into the present study. By multivariate analysis, factor analysis (FA) identified five main factors. The first factor (F1) of the fungi species justify 20.0% which manifests that fungi species controls the degradation of petroleum variability and hierarchical cluster analysis (HCA) as a dendrogram with five observations and three variables displays two predominant clusters order cluster 1 > 2.

Ebele *et al*. (2018) evaluated the effectiveness of fungi *Candida Tropicalis* and *Aspergillus Clavatus*) in bioremediation of used engine oil contaminatedsoil using bioaugmentation technique. Fungi were isolated from soil samples collected from automobile workshops in Mgbuka-Nkpor, Nigeria. The isolates were screened for used engine oil (UEO) biodegradation

potentials in mineral salt broth. Preliminary identification was done using the cultural and microscopic characteristics and verified using the 18SrRNA gene sequence. The capability of the isolates in bioremediation of UEO contaminated soil was also investigated out employing bioaugmentation technique. A sum of 8 fungal isolates were attained from this experiment. *Candida tropicalis* and *Aspergillus clavatus* were identified and confirmed with the highest extent of biodegradation of UEO. Lastly, oil contaminated soil inoculated withthe mixed culture of the isolates (*C. tropicalis* and *A. clavatus*) displayed the highest depletion in concentration ofUEO (95.42%). Higher biodegradation rate and shorter half-lifeof total petroleum hydrocarbon (TPH) was recorded insoil microcosm containing the isolates as compared tothe uninoculated control. Investigation concluded that *C.tropicalis* and *A. clavatus* secluded from automobileworkshops can promote the bioremediation of UEOcontaminated soil.

In one of the studies conducted by Ataikiru*, et al*. (2018) yeast isolates were used to bio augment bonny light crude oil polluted soil in Niger Delta. Yeast isolates procured were *Candida adriatica* ZIM 2468 and *Candida taoyuanica* MYA-4700. The endemic fungal isolates identified were species of *Alternaria, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Trichoderma, Candida, Rhodotorula* and *Saccharomyces*. Firstly, 1 kg of fresh soil sample was polluted with crude oil (10%) and later, physico-chemical analysis was carried up on the fresh soil before and after pollution. *Candida adriatica* ZIM 2468, *Candida taoyuanica* MYA-4700 and a pool comprising each isolate were inoculated into the different microcosms to increase the number of microorganisms. After every fourteen days, samples were analyzed for total petroleum hydrocarbon (TPH) and microbial counts were performed. At 56th day total petroleum losses of 84.6% (A - consortium of *Candida adriatica* ZIM 2468 *and Candida taoyuanica* MYA4700), 77.3% (B - *Candida adriatica* ZIM 2468), 73.4% (C - *Candida taoyuanica* MYA-4700) and 28.7% (Control – unamended) were documented in the bioaugmentation set-up. On the whole, amending with both Candida species proved to be more effective in hydrocarbon utilization.

Bioremediation of crude oil contaminated Marshland muddy soil by bioaugmentation approach using two fungal species *Candida tropicalis* and *Penicillium chrysogenum* were evaluated (Nrior,& Onwuka, 2017). *Penicillium chrysogenum* and *Candida tropicalis* were employed to augment the indigenous microorganisms present in the muddy soil to enhance the degradation rate for a period of 28days, sampling and analysis were carried out at weekly interval (1, 7, 14, 21, and 28 days). The indigenous fungi isolate from the soil were identified to be of the following genera, *Mucor* sp., *Penicillium* sp., *Aspergillus* sp., *Candida* sp., and *Fusarium* sp. During the bioremediation process, it was observed that the augmenting organisms used were able to degrade the petroleum hydrocarbon in the soil. Four batches were setup; Control (contaminated mud without augmenting microbes), Contaminated mud with *Penicillium Chrysogenum*, Contaminated mud with *Candida tropicalis*, Contaminated mud with *Penicillium Chrysogenum* with *Candida tropicalis.* The initial concentration of Total Hydrocarbon Content (THC) of crude oil marsh mud samples setup at day 1 was 938mg/kg; while on the day 28, the residual value were as follows: Contaminated mud with *Penicillium Chrysogenum* with *Candida tropicalis* (148mg/kg) < Contaminated mud with *Candida tropicalis* (247mg/kg) < Contaminated mud with *Penicillium Chrysogenum* (360mg/kg) < Control (646mg/kg). The percentage bioremediation rates of the fungal species were as follows: Contaminated mud with *Penicillium Chrysogenum* with *Candida tropicalis* (84.22%) > Contaminated mud with *Candida tropicalis* (70.79%) < Contaminated mud with *Penicillium Chrysogenum* (61.62%) < Control (31.13%). Comparatively, the mixed consortium *of Candida tropicalis* with *Penicillium Chrysogenum* express higher bioremediation potential; while in relation to individual organism bioaugmenting potential *Candida tropicalis* is higher than *Penicillium chrysogenum*. This study therefore reveals that *Pencillium chrysogenium* and *Candida tropicalis* can be used in treatment of contaminated marshland muddy soil thereby minimizing the adverse environmental risks and human health hazards associated with the toxic effects of this petroleum hydrocarbon in marshland ecosystem.

 Evaluation of bioaugmentation efficiency of two fungal species *Penicillium chrysogenum* and

 *Aspergillus nudilans* species on crude oil spill site (surface and underground soil) in Qio Tai,

 Ogoni land was appraised by Nrior & Mene (2017). *Penicillium chrysogenum* and *Aspergillus*

 *nudilans* were utilized to augment the indigenous microorganisms residing the soil toenhance the degradation rate for a period of 28days at weekly interval (1, 7, 14, 21, and 28 days). The indigenous fungi isolate from the soil were identified to be of the following genera, *Penicillium* sp., *Aspergillus* sp., *Histoplasma* sp., *Cladosporium* sp., *Mucor* sp. and *Alternaria* sp. During the bioremediation process, it was observed that the augmenting organisms used were able to degrade the petroleum hydrocarbon in the soil. The initial Total Hydrocarbon Content (THC) of the un-augmented crude oil spill soil samples used as control (day 1) were: polluted surface soil (142422.14mg/kg), polluted underground soil (74779.29mg/kg); while on 28th day, the residual value were as follows: soil polluted with *Penicillium chrysogenum* (79279.28mg/kg) < soil surface polluted with *Aspergillus nudilans* (79422.14mg/kg) < surface soil control (92279.28mg/kg), polluted underground soil were as follows: surface soil polluted with *Aspergillus nudilans* (44636.43mg/kg) < soil polluted with *Penicillium chrysogenum* (47636.42mg/kg) < underground soil control (53993.59mg/kg). The percentage bioremediation rates of the fungal species for surface soil were as follows: polluted surface soil with *Penicillium chrysogenum* (36%) > polluted surface soil with *Aspergillus nudilans* (35%) > and polluted surface soil as control (29%); while underground soil: underground soil polluted with *Aspergillus nudilans* (38%) > underground soil polluted with *Penicillium chrysogenum* (35%) > control polluted underground soil (27%). Comparatively, *Penicillium chrysogenum* (36%) express higher bioremediation potential than *Aspergillus nudilans* (35%) in the crude oil polluted surface soil while in the underground soil; *Aspergillus nidulans* (38%) had higher bioremediation potential than *Penicillium chrysogenum* (35%). This type of bioremediation shows that biologically cultured organisms aid the degradation of soil polluted with hydrocarbon and this method could be adopted for the remediation of a crude oil spill site. Summarily, bioremediation with the use of fungal isolate can effectively remove the petroleum hydrocarbons and shorten the remediation period, with the use of *Penicillium chrysogenum* for surface soil crude oil spill clean-up and *Aspergillus nidulans* for crude oil polluted underground soil bioremediation process.

Ma *et al*. (2015) studied bioaugmentation of soil contaminated with high-level crude oil through inoculation with mixed cultures including *Acremonium* sp. They studied that heavy contamination of soil with crude oil has caused significant negative environmental impacts and presents substantial hazards to human health. In order to explore the most efficient bioaugmentation strategy for these contaminations, experiments were conducted over 180 days in soil heavily contaminated with crude oil (50,000 mg kg−1), with four treatments comprising *Bacillus subtilis* inoculation with no any other inoculation (I), or reinoculation after 100 days with either *B. subtilis* (II), *Acremonium* sp.(III), or a combination of each organisms (IV). The removal values of total petroleum hydrocarbons evaluated were 60.1 ± 2.0, 60.05 ± 3.0, 71.3 ± 5.2 and 74.2 ± 2.7 % for treatment (I–IV), respectively. Treatments (III–IV) significantly strengthened the soil bioremediation as compared with treatments (I–II) (*p* < 0.05). Furthermore, significantly (*p* < 0.05) greater rates of degradation for petroleum hydrocarbon fractions were discovered in treatments (III–IV) compared to that of treatments (I–II), and this was particularly the case with the degradative rates for polycyclic aromatic hydrocarbons and crude oil heavy chunks. Dehydrogenase activity in treatment (III–IV) comprising *Acremonium* sp. displayed a constant increase until the end of experiments. Therefore, reinoculation with pure fungus or fungal-bacterial consortium must be employed as an effective strategy for bioaugmentation of soil heavily contaminated with crude oil.

 A study presented on the effect of combined bio stimulation-bioaugmentation system applied to a silty-loam soil polluted with 60,400mg kg\_1of a complex mixture of total petroleum hydrocarbons (TPH) especially engine oil, which comprises 58% saturate hydrocarbons (sat) and 29% polycyclic aromatic hydrocarbons (PAH) and 13% polar compound. The bioaugmentation was performed with *Rhizopus oryzae*, isolated from aged soils contaminated with 60,400 mg of TPH per kilogram of dried soil. The indigenous fungi easily grew in a complex solid mixture of hydrocarbons of high molecular weight, after previous acclimatization in liquid culture. The related fungus was able to remove more PAH in comparison with bio stimulation alone (Leyla & Sonakebriaeezadi, 2015).

Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by *Trichoderma reesei* FS10-C and effect of bioaugmentation on an aged PAH-contaminated soil was studied by Yao *et al*. (2015). They investigated co-metabolism of benzo[*a*]pyrene (B[a]P) and the capacity of the fungus *Trichoderma reesei* FS10-C to bioremediate an aged polycyclic aromatic hydrocarbon (PAH)-contaminated soil. The fungal isolate eliminated about 54% of B[a]P (20 mg L−1) after 12 days of incubation, enriched with glucose (10 g L−1) as a co-metabolic substrate. Bioaugmented microcosms showed a 25% decrease in total PAH concentrations in soil after 28 days, and the degradation percentages of 3-, 4-, and 5(+6)-ring PAHs were 36%, 35%, and 25%, respectively. Additionally, bioaugmented microcosms showed higher dehydrogenase (DHA) and fluorescein diacetate hydrolysis (FDAH) activities and increased average well-color development (AWCD), Shannon-Weaver index (*H*), and Simpson index (*D*) significantly. Principal component analysis (PCA) also remarked clear distinction between treatments, indicating that bioaugmentation retained the microbiological function of the PAH-contaminated soil. The results suggest that bioaugmentation by *T. reesei* FS10-C can be an auspicious bioremediation strategy for aged PAH-contaminated soils.

Andreolli *et al*. (2015) performed a comparative study on Bioaugmentation and biostimulation as strategies for the bioremediation of a burned woodland soil contaminated by toxic hydrocarbons. During the tenure of work, the [natural attenuation](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/natural-attenuation) strategy (no soil amendments done) was compared with two different [bioremediation](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/bioremediation) approaches, namely [bioaugmentation](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/bioaugmentation) via soil [inoculation](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/inoculation) with a suspension of *Trichoderma* sp. mycelium and bio stimulation via soil supplementation with a [microbial](https://www.sciencedirect.com/topics/engineering/microbial) growth promoting formulation, in order to verify the effectiveness of these methods in terms of degradation efficiency towards toxic [hydrocarbons](https://www.sciencedirect.com/topics/social-sciences/hydrocarbons), with special attention towards the high molecular weight (HMW) fraction, in a forest area smashed by recent [wildfire](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/wildfire) in Northern Italy. The area under investigation, divided into three parcels, was monitored to figure out the dynamics of decay in soil concentration of C12-40 hydrocarbons (including isoalkanes, [cycloalkanes](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/cycloalkane), alkyl-benzenes and alkyl-naphthalenes besides PAHs) and [low molecular weight](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/low-molecular-weight) (LMW) [PAHs](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/polycyclic-aromatic-hydrocarbon), following the adoption of the foregoing different [remediation](https://www.sciencedirect.com/topics/engineering/remediation) strategies. Soil hydrocarbonoclastic potential was even investigated by characterizing the endemic microbial cenoses. Field experiments confirmed that the best performance in the abatement of HMW hydrocarbons was reached 60 days after soil treatment via the biostimulation protocol, when about 70% of the initial concentration of HMW hydrocarbons was reduced. Within a similar time, near about 55% degradation was obtained through the bioaugmentation protocol, whilst natural attenuation allowed only a 45% removal of the starting C12-40 hydrocarbon fraction. Therefore, biostimulation seems to significantly reduce the time required for the remediation, most likely because of the [enhancement](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/augmentation) of microbial degradation through the improvement of nutrient balance in the burned soil.

Covino *et al*. (2015) performed isolation and identification of the main members of the mycobiota of a clay soil historically contaminated by mid- and long-chain aliphatic hydrocarbons (AH) and to subsequently assess their hydrocarbon-degrading ability. All the isolates were Ascomycetes and, among them, the foremost fascinating was *Pseudoallescheria* sp. 18A, which exhibited both the ability to use AH as the sole carbon supply and to copiously colonize a wheat straw:poplar wood chip (70:30, w/w) lignocellulosic mixture (LM) selected as the modification for subsequent soil remediation microcosms. After mycoaugmentation performed with *Pseudoallescheria* sp. of the aforesaid soil, mixed with the sterile LM (5:1 mass ratio), a 79.7% AH depletion and a major detoxification, inferred by a drop in mortality of *Folsomia candida* from 90 to 24%, were observed. However, similar degradation and detoxification outcomes were discovered out in the non-inoculated incubation control soil that had been amended with the sterile LM. This was due to the biostimulation exerted by the modification on the resident microbiota, fungi specially, the activity and density of which were low, instead, in the non-amended incubation control soil.

Fan *et al*. (2014) studied the effect of Biostimulation-Bioaugmentation on saturate and aromatic hydrocarbon degradation applied to a silty-loam soil polluted with 60,400mg kg\_1of a complex mixture of total petroleum hydrocarbons (TPH) especially engine oil, which comprises 58% saturate hydrocarbons (sat) and 29% polycyclic aromatic hydrocarbons (PAH) and 13% polar compound. The bioaugmentation was performed with *Rhizopus oryzae*, isolated from aged soils contaminated with 60,400 mg of TPH per kilogram of dried soil. The indigenous fungi easily grew in a complex solid mixture of hydrocarbons of high molecular weight, after previous acclimatization in liquid culture. The related fungus was able to remove more PAH in comparison with biostimulation alone.

Elimination of petroleum hydrocarbons from a polluted soil [65,000 mg total petroleum hydrocarbons (TPH)/kg soil] that had been exposed to tropical environmental conditions for more than 20 years in southeast Mexico, was investigated out via filamentous fungi. Batch reactors (60 mL) containing a substrate consisting of polluted soil and sugar cane bagasse pith as bulk agent (80:20, w/w) were used throughout the experiment. Both the sterile and non-sterile batch reactors were inoculated with spore suspensions from *Aspergillus niger, Penicillium glabrum*, and *Cladosporium cladosporioides*. The TPH were determined at the beginning and at the end of the experiments, and the CO2 production and accumulation were monitored by gas chromatography. All the fungal species encountered were associated with the removal of TPH, either on sterile or non-sterile treatments. A bioaugmentation process was noticed due to the synergistic effect of *C. cladosporioides* and well-adapted indigenous microbial populations from the contaminated soil, as the highest removal of TPH (78.5%) and CO2 accumulation (14.3%) were recorded in this non-sterile treatment. By contrast, the lowest TPH removal was documented in the same species, but in the sterile treatment (62.3%) disclosing that the absence of adapted indigenous microbiota significantly reduced fungal metabolism (CO2 accumulation: 9.1%), as well as the removal of TPH (Pérez *et al*., 2010).

Microcosms were set up with a PAHs-contaminated soil using biostimulation (supplementation of ground corn cob) and bioaugmentation (inoculated with *Monilinia* sp. W5-2) (Wu *et al*., 2008). Degradation of polycyclic aromatic hydrocarbons and microbial community were investigated at the end of incubation period. After 30 days, bioaugmented microcosms displayed a 35 ± 0% decrease in total PAHs, whereas biostimulated and control microcosms revealed 16 ± 9% and 3 ± 0% decrease in total PAHs, respectively. Bioaugmented microcosms also revealed 70 ± 8% and 72 ± 2% decrease in benzo[a]pyrene and anthracene, respectively, while the values observed for biostimulated and control microcosms were much lower. Detoxification of soils in bioaugmented microcosms was varified by genetic toxicity assay, suggesting important role of fungal remediation. Molecular fingerprint profiles and selective enumeration exhibited biostimulation with ground corn cob increased both number and abundance of native aromatic hydrocarbons degraders and altered the composition of microbial community in soil, which is profitable for natural attenuation of PAHs. Simultaneously, bioaugmentation with *Monilinia* strain W5-2 levied negligible effect on native microbial community. This it could be recommended that fungal remediation (bioaugmentation) could be considered as promising tool in eliminating PAHs.

Mancera-Lo´pez *et al*. (2008) performed bioremediation of an aged hydrocarbon-contaminated soil by a combined system of biostimulation-bioaugmentation with filamentous fungi. They presented a study of the effect of a combined biostimulation–bioaugmentation treatment applied to a silty-loam soil polluted with 60,600 mg kg−1 of a complex mixture of total petroleum hydrocarbons (TPH), which contains 40% aliphatic hydrocarbons (AH) and 21% polycyclic aromatic hydrocarbons (PAH). bioaugmentation was performed via *Rhizopus sp., Penicillium funiculosum* and *Aspergillus sydowii* strains isolated from two aged soils polluted with 60,600 and 500,000 mg of TPH per kilogram of dried soil. The indigenous fungi grew easily in a complex solid mixture of hydrocarbons of high molecular weight, after previous acclimatization in liquid culture. These three fungi eliminated about 36%, 30% and 17% more PAH as compared to that of biostimulation alone. In the bioaugmented systems with *Rhizopus* sp. and *A. sydowii*, a positive correlation of respirometric activity (CO2 production) with hydrocarbon removal was resulted out (R2=0.75; p(F)=0.001 and R2=0.78; p(F)=0.001, respectively); in contrast, *P. funiculosum* did not exhibit any correlation

Garon & Sage (2004) assessed the potential of fungal bioaugmentation and the effect of maltosyl-cyclodextrin amendment, as an approach to accelerate fluorene biodegradation in soil slurries. 47 fungal strains isolated from a contaminated site were tested in the biodegradation of fluorene. Results revealed the higher potential of “adaptated” fungi isolated from contaminated soil vs. reference strains belonging to the collection of the laboratory. These assays permited to select the most potent strain, *Absidia cylindrospora*, which was employed in a bioaugmentation process. With *Absidia cylindrospora*, more than 90% of the fluorene was eliminated out in 288 h while 576 h were required in the absence of fungal bioaugmentation. Maltosyl-cyclodextrin, a branched-cyclodextrin was selected in order to optimize fluorene bioavailability and biodegradation in soil slurries. Results indicated that *Absidia cylindrospora* and maltosyl-cyclodextrin could be used successfully in bioremediation systems.

1. **Bioaugmentation by macro fungi (Mushrooms- Higher Fungi)**

Strategies to maintain enzymatic oxidation during the extended bioremediation of oily soil [microcosms](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/microcosm) were examined using periodic biostimulation and [bioaugmentation](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/bioaugmentation) (PBB) was reported by Yanto *et al*. (2017). [PBB](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/polybrominated-biphenyl) was used and supplemented with 10 ml malt extract broth (the biostimulation treatment) and 2 g pre-grown [fungus](https://www.sciencedirect.com/topics/immunology-and-microbiology/fungus) in wood meal (the bioaugmentation treatment) to soil artificially tarnished with PHCs 15, 30, 60, and 90 d later the first experiment. Two kinds of fungal [co-cultures](https://www.sciencedirect.com/topics/immunology-and-microbiology/coculture): *Pestalotiopsis* sp. NG007/*[Polyporus](https://www.sciencedirect.com/topics/immunology-and-microbiology/polyporus%22%20%5Co%20%22Learn%20more%20about%20Polyporus%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages)* sp. S133 (1/1) and *Pestalotiopsis* sp. NG007/*Polyporus* sp. S133/*[Trametes hirsuta](https://www.sciencedirect.com/topics/immunology-and-microbiology/trametes-hirsuta%22%20%5Co%20%22Learn%20more%20about%20Trametes%20Hirsuta%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages)* D7 (1/1/1), were tested out in order to compare the effects of two or three [fungal strains](https://www.sciencedirect.com/topics/immunology-and-microbiology/fungal-strain) on the crude oil degradation. Results demonstrated that PBB triggered the biodegradation of crude oils and all fungal co-culture systems employed in this study showed stronger [enzymatic activities](https://www.sciencedirect.com/topics/immunology-and-microbiology/enzyme-activity) (C12O, MnP, and laccase) after PBB. Moreover, PBB with three fungal strains (NG007/S133/D7) demonstrated the most effective degradation, and it was possible to maintain enzymatic activities after extended bioremediation. This study offers an important strategy to remediate PHC-contaminated environments by PBB particularly with mixed fungal cultures for extended biodegradation.

Elimination of aged hydrophobic pollutants from fine-textured soils could be a difficult issue in remediation. Covino *et al*. (2016) compared the efficacy of [augmentation](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/augmentation) treatments to that of biostimulation in terms of total aliphatic hydrocarbon (TAH) and toxicity removal from a historically polluted clay soil and to assess their impact on the resident [microbial community](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/microbial-community). *Pleurotus ostreatus*, *Botryosphaeria rhodina* and a combination of each were used as the inoculants whereas the addition of a sterilized lignocellulose mixture to soil (1:5, *w*/*w*) was used as a biostimulation approach. As hostile towards the untreated control soil, where TAH concentration was remained unchanged and residual toxicity were observed after 60 days, the participation of specialized bacteria was observed in the biostimulated [microcosms](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/microcosm) resulting in significant TAH elimination (79.8%). The bacterial community structure in *B. rhodina*-augmented microcosms did not differ from the biostimulated microcosms due to the inefficiency of the [fungus](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/fungus) to be restored within the resident [microbiota](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/microbiota). Higher TAH eliminations were observed in microcosms inoculated with *P. ostreatus* only (*Po*) and in binary consortium with *B. rhodina* (BC) (86.8 and 88.2%, respectively). In these microcosms, degradation of contaminant proliferated their [bioavailability](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/bio-availability) thresholds resolved by sequential supercritical CO2 extraction. Illumina metabarcoding of 16S rRNA gene revealed that the augmentation with *Po* and BC led to lower relative abundances of Gram (+) taxa, particularly Actinobacteria, than those in bio stimulated microcosms. Best [detoxification](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/detoxification), with respect to the non-amended [incubation](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/incubation) control, was observed in *Po* microcosms where a drop in collembola mortality (from 90 to 22%) occurred. After incubation, in both *Po* and BC, the relative abundances of *P. ostreatus* sequences were higher than 60% thus revealing the [compatibility](https://www.sciencedirect.com/topics/earth-and-planetary-sciences/suitability) of this fungus for application in bioaugmentation-based remediation applications.

García *et al.* (2015) studied implications of polluted soil biostimulation and bioaugmentation with spent mushroom substrate (*Agaricus bisporus*) on the microbial community and polycyclic aromatic hydrocarbons biodegradation. Various applications of spent *Agaricus bisporus* substrate (SAS), considered as a widespread agro-industrial waste, were explored with respect to the remediation of a historically polluted soil with Polycyclic Aromatic Hydrocarbons (PAH). Two bioaugmentation approaches were studied out; the first ramified the use of the waste itself which implied the application of *A. bisporus* additionally with the inherent microbiota of the waste. In the second treatment, SAS was sterilized and inoculated again with the fungus to test its ability to act as a fungal carrier. All these treatments were compared with natural attenuation in terms of their impact on soil heterotrophic and PAH-degrading bacteria, fungal growth, biodiversity of soil microbiota and ability to affect PAH bioavailability and ensuing degradation and detoxification. Results clearly showed that historically PAH contaminated soil was not amenable to natural attenuation. Conversely, the addition of sterilized spent *A. bisporus* substrate to the soil accelerated resident soil bacteria with ensuing high removals of 3-ring PAH. Both augmentation treatments were more effective in eliminating highly condensed PAH, among which some of them were considered as carcinogenic agent. Regardless of the mode of application, the present results strongly support the adequacy of SAS for environmental remediation purposes and open the way to an attractive recycling option of this waste.

A diversified approach to evaluate biostimulation and bioaugmentation strategies for heavy-oil-contaminated soil was adopted, in order to improve our understanding of the biodegradability of pollutants, microbial community dynamics and ecotoxicological effects of various bioremediation strategies (Lladó *et al*., 2012). In order to improve hydrocarbon degradation, the following bioremediation treatments were detected: i) supplementation of inorganic nutrients; ii) addition of the rhamnolipid-based biosurfactant MAT10; iii) inoculation of polycyclic aromatic hydrocarbon-degrading microbial consortium (TD); and iv) inoculation of a familiar hydrocarbon-degrading white-rot fungus strain of *Trametes versicolor*. Followed after 200 days, all the bioremediation assays were achieved between 30% and 50% total petroleum hydrocarbon (TPH) biodegradation, via the *T. versicolor* inoculation degrading it the most. Biostimulation and *T. versicolor* inoculation endorsed the *Brevundimonas* genus concurrently with other α-proteobacteria, β-proteobacteria and Cytophaga-Flexibacter-Bacteroides (CFB) as well as Actinobacteria groups. highest hydrocarbon degradation in soil achieved via *T. versicolor* inoculation promoted autochthonous Gram-positive bacterial groups, such as Firmicutes and Actinobacteria. An acute toxicity test using *Eisenia fetida* proved the improvement in the quality of the soil after all biostimulation and bioaugmentation strategies.

Substrate Bioaugmentation of Waste Engine Oil Polluted Soil performed by Ikhajiagbe & Anoliefo (2012) investigated the impact of substrate microbial augmentation on the bioremediation of Waste Engine Oil (WEO) polluted soil. Five different concentrations of WEO in soil on weight basis were obtained by completely infusing WEO in measured soil: 1.0, 2.5, 5.0 and 10.0% w/w. The untreated soil was used as the control (0% w/w) in the experiment. The set up was left for 5 months without agitating the soil. After 5 months, the soils were first supplemented with sawdust and then inoculated with mycelia of *Pleurotus tuberregium.* Significant (p = 0.05) decreases in soil physicochemical parameters were documented 9 months after bioaugmentation (9 MAB), excepting total organic carbon and total nitrogen, which displayed significant increases throughout the experiment period. Total (100%) remediation of some PAH compounds - benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, and indeno(1,2,3-c,d)pyrene - was recorded. Over sixty per cent (66.22%) of total individual PAH compounds were absolutely (100%) remediated.

Phytoassessment of a 5-Month Old Waste Engine Oil Polluted Soil conducted by Ikhajiagbe & Anoliefo (2012) after augmentation with *Pleurotus tuberregium* revealed a bioassessment of the effects of substrate microbial augmentation to approach the bioremediation of Waste Engine Oil (WEO) polluted soil. Four varied concentrations of WEO in soil on weight basis were obtained by completely mixing WEO in measured soil: 1.0, 2.5, 5.0, and 10.0% w/w. The untreated soil was used as the control (0% w/w) throughout experiment. The set up was left for 5 months without agitating the soil. After 5 months, the soils were first supplemented with sawdust and then inoculated with mycelia of *Pleurotus tuberregium*. Nine months after bioaugmentation (9 MAB) there occured total (100%) remediation of some PAH compounds (benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g, h, i)perylene, benzo(k)fluoranthene, chrysene, dibenzo(a, h)anthracene, fluoranthene, fluorene, and indeno(1, 2, 3-c,d) pyrene) was recorded. Significant (p=0.05) decreases in heavy metal concentration from 5-9 MAB resulted in significant reductions in Hazard Quotients (HQ), which implied less possibility for ecological risk for heavy metal constituents. Phytoassessment of the polluted soil was performed at 5 MAB, and results displayed that virtually all the cowpea seedlings died within 2 weeks. Only those seedlings in untreated soils survived. Nine months after readjustment of soil treatments, all cowpea plants sustained up to fruiting, with grain production in the highly polluted soil being 15.25g/plant as compared to that of 26.01g/plant in the control experiment.

Bosiljcic (2008) studied bioaugmentation using *Pleurotus ostreatus* to remediate Polycyclic Aromatic Hydrocarbons (PAH) contaminated river sediment. The purpose of the study was to determine if polyaromatic hydrocarbon degradation in historically contaminated river sediment could be done when treated with the white-rot fungus *Pleurotus ostreatus. P. ostreatus*, grown on barley, was added to sediment with various amendments and controls, and incubated in triplicate at 25 °C for 42 days. Treatments included sawdust, shredded newspaper, a nitrogen source, and cyclodextrin. The most effective treatment included the addition of white-rot fungi, sawdust, nitrogen, and cyclodextrin. This treatment showed greater than 50% degradation of 9 of the 11 PAHs with 95% degradation for benzo fluoranthene. Fungal biomass (total mycelia and metabolically active mycelia) increased in all treatments with added fungi. The greatest increase in fungal biomass appeared in the same treatment with the greatest extent of PAH degradation (from 82 ± 10 mg sediment-1 at time 0 to 374 ± 18 mg sediment-1 at 42 days). These data showed that *P. ostreatus* is capable of colonizing highly contaminated Mahoning River sediment and degrading the PAHs present as well as showed potential for remediating historically contaminated river sediment.

The effects of sawdust and waste cotton as soil supplement and bioaugumentation with *Pleurotus pulmonarius* (pp) on soil contaminated with crude oil (COIL), automotive gasoline oil (AGO), and spent engine oil (SEO) on the growth of cowpea (Vigna ungiculata (L.) Walp) was detected. Significant increase (P = 0.05) on the growth of cowpea when polluted soil was amended and bioaugmented with *P. pulmonarius* (pp) after one month of incubation as compared with the result of planting on polluted soil with no amendments and bioaugumentation was observed. Addition of waste cotton as a supplement and *P. pulmonarius* as bioaugumentation agents to crude oil contaminated soil significantly diminished time of seed germination from 8 to 3 days, increased seed germination from 60 to 96%, plant height ranged from 10.3 to 22 cm, number of leaves from 3 to 5 and biomass from 0.5 to1.5 g dry wt. Similarly, reductions in time of germination, increments in percentage germination, plant height, leaf number and total biomass in cowpea plants grown in automotive gasoline oil and spent engine oil polluted soils, supplemented with waste cotton or saw dust and bioaugumented with *P. pulmonarius* were observed in this study (Olutayo, 2007).

Bioaugmentation of tar-contaminated soils under field conditions using *Pleurotus ostreatus* refuse from commercial mushroom production performed by Hestbjerg *et al*. (2003) reported the influence of the white rot fungus *Pleurotus ostreatus* on the degradation of selected poly- and heterocyclic aromatic hydrocarbons (referred to as polycyclic aromatic hydrocarbons [PAHs]) in soil investigated under field conditions representing the Northern temperate zone. *Pleurotus ostreatus* was supplemented to two contaminated soils in the form of homogenized refuse from the profitable production of fungus. The soils were collected from a former shipyard (the B&W soil) and beneath a former coal tar storage at an old asphalt factory in Denmark (the Ringe soil). Treatments (control, soil infused with autoclaved sawdust medium, and soil treated with *P. ostreatus* refuse) were established in triplicate in concrete cylinders (height, 50 cm; diameter, 60 cm). The activity of *P. ostreatus* was assayed as laccase activity and phenanthrene (PHE)- and pyrene (PYR)-degrading bacteria were calculated. Twenty-one different PAHs were quantified. After nine weeks the concentrations of the 3-, 4-, 5-, and 6-ring PAHs in the Ringe soil were reduced by 78, 41, and 4%, respectively. These reductions corresponded with high initial laccase activity, a decrease in pH caused by the fungus, and a rise within the range of PHE- and PYR-degrading bacteria. No vital PAH degradation was observed in the B&W soil. Reasons for the difference in performance of *P. ostreatus* in the two soils are discussed in terms of soil histories and bioavailability. The use of *P. ostreatus* refuse holds promising potential for bioremediation purposes.

Lestan & Lamar (1996) developed the fungal inocula for bioaugmentation of contaminated soils. Their report described novel fungal inocula for bioaugmentation of soils contaminated with hazardous organic compounds. The inocula were in the form of pelleted solid substrates coated with a sodium alginate suspension of fungal spores or mycelial fragments and incubated until over grown with the mycelium of selected lignin-degrading fungi. The organisms detected were *Phanerochaete chrysosporium* (BKM F-1767, ATCC 42725) including *P. sordida* (HHB-8922-Sp), common crust fungus *Irpex lacteus* (Mad-517, ATCC 11245), *Bjerkandera adusta* (FP-135160-Sp, ATCC 62023), and *Trametes versicolor* (MD-277). The pelleted fungal inocula resisted competition and proliferation from endemic soil microbes, were lower in moisture content than current fungal inocula, and had sufficient mechanical strength to allow handling and introduction into the soil without a changing the mechanical consistency of the pellets. On inoculation at a rate of 3% in artificially contaminated nonsterile soil*, I. lacteus, B. adusta,* and *T. versicolor* eliminated 86, 82, and 90%, respectively, of the pentachlorophenol in 4 weeks.

The suitability of the fluorescein diacetate hydrolyzing activity (FDA) assay for determining the biological potential (i.e. fungal biomass produced per unit of substrate) of solid pelleted fungal inoculum designed for use in the bioaugmentation of contaminated soils with white-rot fungi, was evaluated by Leštan *et al*. (1996). FDA activity of the white-rot fungus *Phanerochaete chrysosporium* grown on pelleted substrates and on agar was found to be proportional to quantities of fungal ergesterol and fungal dry matter, respectively. Inoculum biological potential was highly influenced by substrate formulation including structure, and temperature. Biological potential and the type of carrier interfered the ability of *P. chrysosporium* to tolerate pentachlorophenol (PCP). *Phanerochaete chrysosporium* and *Trametes versicolor* introduced into PCP-contaminated soil on pellets with higher biological potential and higher nitrogen content (C:N ratio of 50∶1), did not remove PCP more efficiently when the fungi were imported on pellets with a lower biological potential (C:N ratio of 309∶1). However, under the latter conditions most of the PCP was transformed converted into pentachloroanisole (PCA). In soil inoculated with *T. versicolor* on pellets with high biological potential, higher manganese peroxidase activity was encountered as compared to the soil inoculated with pellets with a lower biological potential.

**3. Research Needs and conclusion**

Although sizable progress has been created is choosing acceptable inocula and advancing recipes to induce their activity for a good style of bioremediation applications, and on the understanding of however environmental factors and growth conditions influence microorganism transport and adhesion, more analysis is required to advance our incomplete understanding of factors that hinder the distribution, survival and sustained performance of exogenous microorganisms. One analysis challenge is to reinforce the transport and distribution of the inoculum throughout the contaminated zone. this needs improved understanding of bacterial adhesion and filtration through the porous medium, moreover as taxis towards or far from target pollutants, and also the regulation of such processes, which can cause higher strategies to reinforce microbic intromission and distribution.

There is vital chance for natural genetic breeding to supply strains that not only exhibit broad catabolic specificity and might degrade mixtures of priority pollutants, but are also tolerant to environmental stress like unfavorable pH or chemical reaction conditions that will be encountered in place. Additionally, to such abiotic stress, the performance of the else strains could be hindered by biological stress like competition for nutrients with autochthonic strains and amensalistic or predatory microbic interactions. Thus, exploring approaches to by selection inhibit species that hinder the performance of the else strains (e.g., victimisation strain specific bacteriophages) may well be a fruitful avenue of analysis. there's additionally a requirement for improved mathematical modeling and rhetorical analysis tools like transcription analysis of catabolic genes and different biomarkers to assess the performance of the else strains and ensure their participation within the cleanup method.

Overall, as our empirical information for the implementation of bioaugmentation is growing fast, as is our mechanistic understanding of the physico-chemical, ecological and genetic factors that influence the semipermanent effectivity of the else strains. this could ultimately lead North American nation to higher hep choices on once and the way to use bioaugmentation during a reliable fashion to handle a good style of rectification desires.

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