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

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**ABSTRACT**

Since the persistence of aromatic compounds, which have carcinogenic and mutagenic properties, has become a particular environmental problem, an additional method for their removal from soil is bioaugmentation, which is described as a method for improving the degradation capacity of contaminated areas by introducing specific competent strains or consortia of microorganisms. Therefore, for soils where microorganisms have lost or no longer possess the ability to biodegrade these venturous compounds, bioaugmentation is usually recommended as a method to improve the bioremediation size ratio. Bioaugmentation is gaining attention as an approach to enhance catabolic potential at contaminated sites and stimulate biodegradation of recalcitrant priority pollutants. Several promising results have been reported from previous studies with microbial and white rot fungi and their ability to degrade petroleum hydrocarbons. 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.

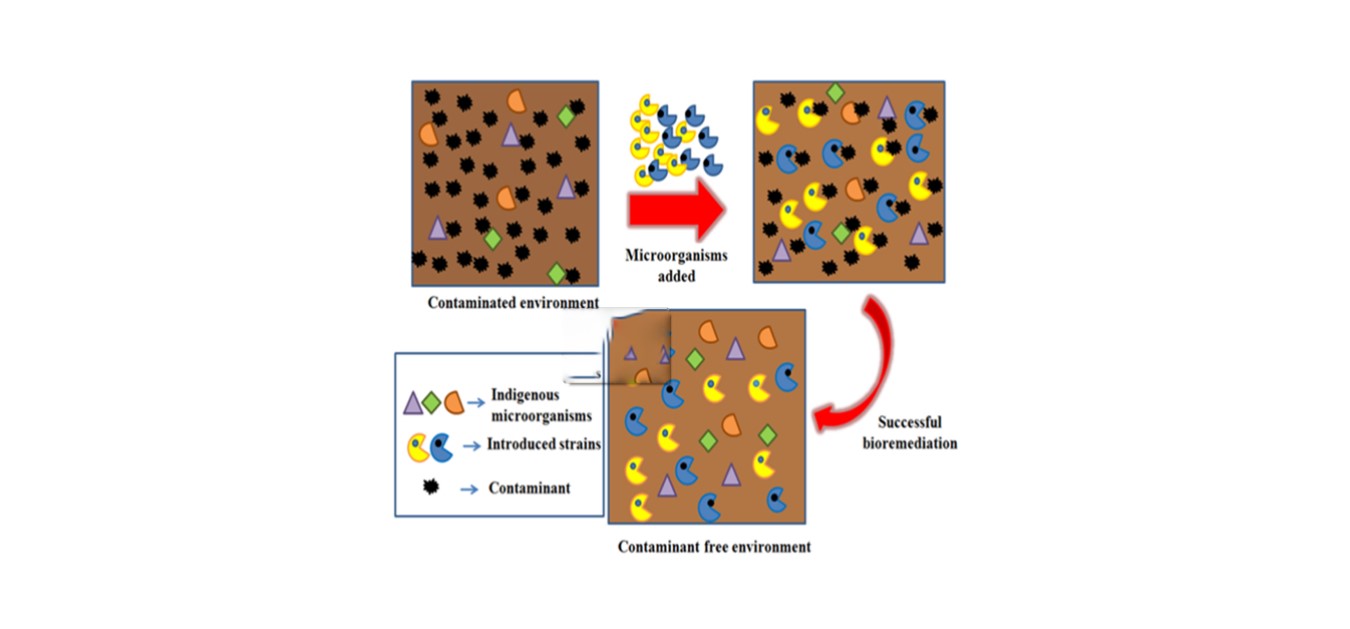
**Keywords: biodegradation, bioaugmentation, white rot fungi, PAHs, fungus, bioremediation, mycoremediation.**

1. **Introduction**

The growth and proliferation of oil consuming microorganisms in contaminated soil is greatly retarded by the provision of nutrients and their hydrocarbon consuming property. The moldering capabilities of fungi are combined with a number of naturally-occurring compounds that serve as potential carbon sources. Hydrocarbon pollutants have similar or analogous molecular structures that cause the fungi to additionally engulf them. Therefore, once a portion is contaminated, the ability to traumatize and convert the contamination into an energy source is selected by the highly potent fungal population capable of metabolizing the contamination. Since the persistence of aromatic compounds, which have carcinogenic and mutagenic properties, has become a particular environmental concern, an additional method for their removal from soil is bioaugmentation, which is described as a method of improving the degradation capacity of contaminated land by introducing specific competent strains or consortia of microorganisms.

In bioaugmentation, the biological material is engineered to be able to degrade certain compounds. Once a microorganism attaches to the contaminated space, it’s able to improve the ability of the biological material to behave in such a way that it degrades contaminants that were already difficult to degrade before. Therefore, for soils in which the microorganisms have lost or no longer have the ability to biodegrade these venturous compounds, bioaugmentation is usually recommended as a method to improve the bioremediation size ratio.

Bioaugmentation is the application of autochthonous or allochthonous wild type or genetically modified microorganisms to contaminated sites to accelerate the removal of undesirable compounds [1]. Figure 1 depicts the process of bioaugmentation which clarifies that bioaugmentation is mainly introduced in oil polluted environments as an optional strategy for bioremediation [2].



**Figure 1 Diagrammatic reprsentation of bioaugmentation [2].**

1. **Principle of bioaugmentation**

The explanation for this approach is the enhancement of the degree or rate of degradation of the complicated pollutants by the addition of pollutant-degrading microorganisms [3,4]. Improving the microbiota of a contaminated site not only improves the elimination of the pollutants from the current site, but also increases the genetic performance of the required site at the same time. Therefore, bioaugmentation corresponds to an increase in the factor pool and, thus, the genetic diversity of the site. Essentially, this genetic diversity can be increased by increasing microbial diversity [5,6].

**2. Fungal Bioaugmentation**

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

Essabri [7] 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. Aspergillus niger, *Penicillium ochrochloron*, and *Trichodema viride* possessed utmost potentiality to reduce petroleum hydrocarbon without emerging antagonistic activities. These fungi accumulated significantly higher biomass, produced extracellular enzymes, and degraded total petroleum hydrocarbon. GC-MS analysis data confirmed that these isolates displayed rapid total petroleum hydrocarbon biodegradation within a period of 60 days.

Ebele [8] evaluated the effectiveness of fungi *Candida tropicalis* and *Aspergillus clavatus* in bioremediation of used engine oil contaminated soil using bioaugmentation technique. Fungi were isolated from soil samples collected from automobile workshops in Mgbuka-Nkpor,Nigeria. Preliminary identification was done using the cultural and microscopic characteristics and verified using the 18SrRNA gene sequence. *Candida tropicalis* and *Aspergillus clavatus* were identified and confirmed with the highest extent of biodegradation of UEO. Lastly, oil contaminated soil inoculated with the mixed culture of the isolates (C. tropicalis and A. clavatus) displayed the highest depletion in concentration of UEO (95.42%). Higher biodegradation rate and shorter half-life of total petroleum hydrocarbon (TPH) was recorded in soil microcosm containing the isolates as compared to the un-inoculated control. Investigation concluded that *C.tropicalis* and *A. clavatus* secluded from automobile workshops can promote the bioremediation of UEO contaminated soil.

Nrior & Mene [9] recorded 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. He had also reported that bioremediation with the use of fungal isolate can effectively remove the petroleum hydrocarbons and shorten the remediation period.

Ma [10] 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. Covino [11] 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. Fan [12] studied the effect of Biostimulation-Bioaugmentation on saturate and aromatic hydrocarbon degradation applied to a silty-loam soil polluted with complex mixture of total petroleum hydrocarbons (TPH) especially engine oil bioaugmentation was performed with Rhizopus oryzae, isolated from aged soils.

Microcosms were established with a PAH contaminated soil using biostimulation (addition of ground corn cob) and bioaugmentation (inoculated with *Monilinia* sp. W5-2) [13]. The degradation of polycyclic aromatic hydrocarbons and the microbial community were studied at the end of the 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 were verified by genetic toxicity assay, suggesting an important role of fungal remediation. Molecular fingerprint profiling and selective enumeration showed that biostimulation with ground corn cob increased both number and abundance of native degraders of aromatic hydrocarbons and altered the composition of soil microbial community, which is beneficial for natural attenuation of PAHs. At the same time, bioaugmentation with *Monilinia* strain W5-2 had negligible effects on the native microbial community. This could mean that fungal remediation (bioaugmentation) could be considered a promising tool for PAHs removal.

Garon & Sage [14] 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.

In one of the studies conducted by Ataikiru [15] yeast isolates were used to bio augmentbonny light crude oil polluted soil in Niger Delta. The endemic fungal isolates identified were species of Alternaria, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Trichoderma, Candida, Rhodotorula and Saccharomyces. Bioremediation of crude oil contaminated Marshland muddy soil by bioaugmentation approach using two fungal species Candida tropicalis and Penicillium chrysogenum were evaluated by Nrior and Onwuka [16]. 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 28 days.

Similarly, biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by Trichoderma reesei and effect of bioaugmentation on an aged PAH-contaminated soil was studied by Yao [17]. 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 can be an auspicious bioremediation strategy for aged PAH-contaminated soils.

Andreolli [18] 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 strategy (no soil amendments done) was compared with two different bioremediation approaches, namely bioaugmentation via soil inoculation with a suspension of Trichoderma sp. mycelium and bio stimulation via soil supplementation with a microbial growth promoting formulation. It was 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, most likely because of the enhancement of microbial degradation through the improvement of nutrient balance in the burned soil.

Elimination of petroleum hydrocarbons from a polluted soil was also achieved by spore suspensions of *Aspergillus niger,* *Penicillium glabrum*, and *Cladosporium cladosporioides* [19] and detoxification of soils in bioaugmented microcosms was verified by genetic toxicity assay, suggesting important role of fungal remediation.

Mancera-Lo´pez [20] performed bioremediation of an aged hydrocarbon-contaminated soil by a combined system of biostimulation-bioaugmentation with filamentous fungi and assay was performed via Rhizopus sp., Penicillium funiculosum and Aspergillus sydowii strains isolated from two aged soils.

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

Strategies to maintain enzymatic oxidation during the extended bioremediation of oily soil microcosms were examined using periodic biostimulation and bioaugmentation (PBB) was reported by Yanto [21]. In order to compare the effects of two or three fungal strains 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. 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 [11] compared the efficacy of 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. 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.

García [22] 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. The 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 forheavy-oil-contaminated soil was adopted, in order to improve our understanding ofthe biodegradability of pollutants, microbial community dynamics and ecotoxicological effects of various bioremediation strategies [23]. The bioremediation assays were achieved about 50% of total petroleum hydrocarbon (TPH), via the T. versicolor inoculation. Bosiljcic [24] 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. Similarly substrate level bioaugmentation of Waste Engine Oil (WEO) polluted soil performed by Ikhajiagbe & Anoliefo [25] investigated the impact of substrate microbial augmentation on the bioremediation of Waste Engine Oil (WEO) polluted soil by use of Pleurotus tuberregium.

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 by Olutayo [26]. Hestbjerg [27] 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. Lestan & Lamar [28] 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 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 [28]. Phanerochaete chrysosporium and Trametes versicolor introduced into PCP-contaminated soil on pellets with higher biological potential and higher nitrogen content, did not remove PCP more efficiently when the fungi were imported on pellets with a lower biological potential. However, under the latter conditions most of the PCP was transformed converted into pentachloroanisole (PCA).

**3. Research Needs and conclusion**

Although considerable progress has been made in selecting acceptable inocula and developing formulations to promote their activity for a variety of bioremediation applications, as well as in understanding of however environmental factors and growth conditions that influence microbial transport and adhesion, further analysis is needed to improve our incomplete understanding of the factors that impede the distribution, survival and sustained performance of exogenous microorganisms. This requires an improved understanding of bacterial adhesion and filtration through the porous medium, both toward target pollutants, and how these processes are regulated which may lead to better strategies to enhance microbial intromission and distribution.

There is a great opportunity for natural genetic breeding to provide strains that not only have broad catabolic specificity and can degrade mixtures of priority pollutants, but are also tolerant of environmental stresses such as unfavorable pH or chemical reaction conditions encountered in the field. In addition, to such abiotic stresses, the performance of other strains could be affected by biological stresses such as competition for nutrients with autochthonous strains and amensalistic or predatory microbial interactions. Therefore, exploring approaches to inhibit species that hinder the performance of the other strains (e.g., through strain-specific bacteriophages) could be fruitful avenue of analysis. In addition, there is a need for improved mathematical modeling and rhetorical analysis tools such as transcriptional analysis of catabolic genes and various biomarkers to assess the performance of the other strains and ensure their participation in the remediation method.

Overall, as our empirical information for implementing bioaugmentation grows rapidly, so does our mechanistic understanding of the physicochemical, ecological and genetic factors that influence the semipermanent efficacy of otherwise strains. This could ultimately lead North American nation to higher hep decisions about once and the way to use bioaugmentation while a reliable way to treat a good type of rectification desires.

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