**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 by the highly potent fungal population which 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 Diagrammatic reprsentation 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 [7] performed bioaugmentation and biostimulation of total petroleum hydrocarbon degradation in a petroleum-contaminated soil with fungi Isolated from olive oil effluent. Throughout 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 reported 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.

Ebele [8] 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.

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 [9]. *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. throughout the bioremediation period, it absolutely was ascertained that the augmenting organisms used were able to degrade the petroleum hydrocarbon in the soil. The initial Total Hydrocarbon Content (THC) of the unaugmented or untreated crude oil spill soil samples employed as control (day 1) were: polluted surface soil (142422.14mg/kg), polluted underground soil (74779.29mg/kg); whereas 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 confirmed 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.

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. 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 organism (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.

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. 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 [12] 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) particularly engine oil, which comprises 58% saturate hydrocarbons (sat) and 29% polycyclic aromatic hydrocarbons (PAH) and 13% polar compound. The bioaugmentation was experimented out with *Rhizopus oryzae*, isolated from highly aged soils contaminated with 60,400 mg of TPH per kilogram of dried soil. The indigenous fungi simply grew in a complex solid mixture of hydrocarbons of high molecular weight, after previous acclimatization in liquid culture. The related fungus was able to eliminate additional PAH compared with biostimulation alone.

Microcosms were set up with a PAHs-contaminated soil using biostimulation (supplementation of ground corn cob) and bioaugmentation (inoculated with *Monilinia* sp. W5-2) [13]. 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.

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 experimented for the biodegradation of fluorene. Results revealed the higher potential of “adaptated” fungi isolated from contaminated soil vs. reference strains procured from 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 [15]. [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.

García [16] 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 effect 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 indicated that previously 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.

Bosiljcic[17] studied bioaugmentation using *Pleurotus ostreatus* to remediate Polycyclic Aromatic Hydrocarbons (PAH) contaminated river sediment. The aim of the study was to detect if polyaromatic hydrocarbon degradation in previously contaminated river sediment could be done when treated with the white-rot fungus *Pleurotus ostreatus. P. ostreatus*, cultured on barley, was added to sediment with various amendments and controls, and incubated in triplicate at 25 °C for 42 days. Treatments comprised sawdust, shredded newspaper, a nitrogen source, and cyclodextrin. The most effective treatment included the supplementation 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 supplemented 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 [18].

Bioaugmentation of tar-contaminated soils under field conditions using *Pleurotus ostreatus* refuse from commercial mushroom production performed by Hestbjerg[19] 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. No vital PAH degradation was observed in the B&W soil.

Lestan & Lamar [20] 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.

**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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