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

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**ABSTRACT**

Bioaugmentation, which is defined as a method of improving the degradation capacity of contaminated land by introducing specific competent strains or consortia of microorganisms, is another method for their removal from soil. The persistence of aromatic compounds, which have carcinogenic and mutagenic properties, has become a particular environmental concern. Therefore, bioaugmentation is typically advised as a way to increase the bioremediation size ratio in soils where microorganisms have lost or no longer possess the ability to biodegrade certain exhaust-related substances. As a method to increase catabolic potential in contaminated sites and encourage the biodegradation of resistant priority pollutants, bioaugmentation is gaining popularity. Previous research on the potential of white rot fungi and microbial fungi to break down petroleum hydrocarbons has produced a number of promising findings. This chapter explores the concept of bioaugmentation and provides case studies and instructions for the effective application of this strategy for fungi-based bioremediation of contaminated soils.

Keywords: PAHs, fungus, bioremediation, mycoremediation, biodegradation, bioaugmentation.

1. **Introduction**

The presence of nutrients and their ability to consume hydrocarbons significantly reduce the growth and proliferation of microorganisms that consume oil in polluted soil. The ability of fungi to decompose organic matter is combined with a variety of naturally occurring substances that could act as carbon sources. Due to their comparable or equivalent chemical structures to hydrocarbon contaminants, fungus also consume them. Therefore, once a piece of the soil is contaminated, the very robust fungal population that can metabolize the contamination selects the capacity to traumatize and turn the contaminant into an energy source. Bioaugmentation, which is defined as a method of enhancing the degradation capacity of contaminated land by introducing particular competent strains or consortia of microorganisms, is a further method for their removal from soil because the persistence of aromatic compounds, which have carcinogenic and mutagenic properties, has become a particular environmental concern.

In bioaugmentation, the biological component is created or engineered to have the ability to break down specific chemicals. Once a colony of microorganisms has established itself in a contaminated site, it can strengthen the biological material's capacity to behave in a way that destroys pollutants that were already challenging to breakdown. Therefore, bioaugmentation is typically advised as a way to boost the size ratio in bioremediation for soils when the microbes are no longer able to biodegrade these hazardous chemicals.

To hasten the clearance of unwanted substances from contaminated locations, bioaugmentation is the use of autochthonous or allochthonous wild type or genetically engineered microorganisms [1]. The process of bioaugmentation is shown in Figure 1, which makes it clear that it is mostly used as an optional technique for bioremediation in areas that are oil-polluted [2].



**Figure 1 is a diagram that illustrates bioaugmentation [2].**

1. **The bioaugmentation principle**

This method's justification is to introduce microorganisms that can degrade contaminants to speed up the process of degrading difficult pollutants [3,4]. A contaminated site's microbiota can be improved, which not only aids in the removal of pollutants from the current site but also boosts the genetic performance of the ideal site. Therefore, bioaugmentation results in an increase in the site's genetic diversity and factor pool. In essence, increasing microbial diversity can increase this genetic diversity [5,6].

1. **Fungus-Based Modification**

**A. Bioaugmentation using tiny fungus (lower fungi)**

Essabri [7] used fungus isolated from olive oil effluent to perform bioaugmentation and biostimulation of petroleum- hydrocarbon breakdown in a petroleum-contaminated soil. 35 isolates from 11 genera were remedied during the degrading process, and three isolates and their consortium were made to proliferate on petroleum hydrocarbon as the only carbon source under in vitro circumstances. The three organisms with the best capacity to decrease petroleum hydrocarbons without producing hostile behaviors were Aspergillus niger, Penicillium ochrochloron, and Trichodema viride. These fungus developed extracellular enzymes, gathered noticeably more biomass, and destroyed every petroleum hydrocarbon. These isolates demonstrated rapid total petroleum hydrocarbon degradation over a 60-day period, according to GC-MS analytical results.

Using the bioaugmentation technique, Ebele [8] investigated the efficacy of the fungus Candida tropicalis and Aspergillus clavatus in the bioremediation of soil contaminated with old motor oil. In Mgbuka-Nkpor, Nigeria, soil samples from auto repair shops were used to isolate the fungi. The highest amount of UEO biodegradation was detected and confirmed by Candida tropicalis and Aspergillus clavatus. Finally, the soil that had been contaminated with oil and had been injected with a mixed culture of the isolates (C. tropicalis and A. clavatus) degraded UEO at the maximum rate, or 95.42%. In comparison to the non-inoculated control, a faster rate of biodegradation and a shorter half-life of total petroleum hydrocarbon (TPH) were seen in the soil microcosm containing the isolates. It was discovered that C.tropicalis and A. clavatus kept away from auto repair facilities can aid in the bioremediation of UEO-contaminated soil.

Penicillium chrysogenum and Aspergillus nudilans were shown to be effective bioaugmentation agents at the site of a crude oil spill (surface and sunsurface soil) in Qio Tai, Ogoni area, according to Nrior & Mene [9]. Additionally, he had stated that bioremediation employing fungal isolates can efficiently remove petroleum hydrocarbons and cut down on the amount of time needed for cleanup.

Ma [10] looked into the bioremediation of high-grade petroleum-contaminated soil using mixed cultures, including Acremonium sp. They found that there is a considerable risk to human health and that the extreme contamination of soil with petroleum has a negative impact on the environment. In a clay soil that had previously been contaminated with medium and long chain aliphatic hydrocarbons (AH), Covino [11] isolated and identified the major mycobiota members before assessing their capacity to break down AH. Using Rhizopus oryzae bioaugmentation isolated from old soils, Fan [12] investigated the impact of biostimulation bioaugmentation on the breakdown of saturated and aromatic hydrocarbons in a silty loam soil contaminated with a complex combination of total petroleum hydrocarbons (TPH).

A PAH-contaminated soil was used to create microcosms, which were then biostimulated (by adding pulverized corncobs) and bioaugmented (by inoculating with Monilinia sp. W5-2) [13]. The microbial community and polycyclic aromatic hydrocarbon degradation were investigated. According to this, the total amount of PAHs in the bioaugmented microcosms reduced by 35 % after 30 days of incubation, while the total amounts of PAHs in the biostimulated and control microcosms decreased by 16 % and 3 %, respectively. The levels of benzo[a]pyrene and anthracene decreased in bioaugmented microcosms by 70 8% and 72 2%, respectively, but they decreased substantially less in biostimulated and control microcosms. Genetic toxicity assay results showed that biostimulated microcosms' soils had been detoxified, highlighting the significant contribution of fungi to remediation. This could imply that fungus remediation (also known as bioaugmentation) is a potentially effective PAH elimination method.

In order to hasten the biodegradation of fluorene in soil slurries, Garon & Sage [14] looked at the effects of adding maltosyl-cyclodextrin and the possibility of bioaugmentation by fungi. The biodegradation of fluorene was examined in 47 fungus strains that were obtained from a contaminated site. The findings demonstrated that "adapted" soil-isolated fungus had greater potential than the reference strains from the lab collection.

In a work by Ataikiru [15], light crude oil-contaminated Niger Delta soils were bioengineered using yeast isolates. Species of Alternaria, Aspergillus, Fusarium, Mucor, Penicillium, Rhizopus, Trichoderma, Candida, Rhodotorula, and Saccharomyces were among the endemic fungal isolates found. The bioaugmentation of petroleum-contaminated marsh mud with two fungi species, Candida tropicalis and Penicillium chrysogenum, was examined by Nrior and Onwuka [16]. Over the course of a 28-day period, Penicillium chrysogenum and Candida tropicalis were added to the native microorganisms found in the mucky soil to speed up the rate of deterioration.

The biodegradation of polycyclic aromatic hydrocarbons (PAHs) by Trichoderma reesei and the impact of bioaugmentation on an old PAH-contaminated soil were both investigated by Yao [17]. A substantial difference between the treatments was also revealed by principal component analysis (PCA), demonstrating that bioaugmentation preserved the microbiological function of the PAH-contaminated soil. The findings imply that bioaugmentation by T. reesei may be a promising bioremediation approach for soil that has been contaminated with PAH for a long time.

In order to bioremediate a burned forest soil contaminated with hazardous hydrocarbons, Andreolli [18] undertook a comparison study of the techniques of bioaugmentation and biostimulation. During the course of the project, two different bioremediation strategies—bioaugmentation by inoculating the soil with a suspension of Trichoderma sp. mycelium and biostimulation by supplying the soil with a microbial growth-promoting formulation—were compared with the strategy of natural attenuation (no soil improvement). It was determined that 60 days after soil treatment with the biostimulation protocol, the best performance in reducing the significantly amended hydrocarbons was achieved when about 70% of the initial concentration of significantly amended hydrocarbons was reduced. This was most likely because the improved nutrient balance in the burned soil improved microbial degradation, which led to the reduction of significantly amended hydrocarbons.

Aspergillus niger, Penicillium glabrum, and Cladosporium cladosporioides spore suspensions were also successful in removing petroleum hydrocarbons from a polluted soil [19], and a genetic toxicity test confirmed the soils' detoxification in bioaugmented microcosms, highlighting the crucial role of fungi in remediation.

Mancera-Lopez [20] used a combination of biostimulation and bioaugmentation with filamentous fungi to accomplish bioremediation on an ancient soil that had been contaminated with hydrocarbons, and tests were carried out with Rhizopus sp.

1. **Macrofungi used in bioaugmentation (Mushrooms- Higher Fungi)**

Using periodic biostimulation and bioaugmentation (PBB), methods to maintain enzymatic oxidation during prolonged bioremediation of oleaginous soil microcosms were examined (Yanto [21]). It was intended to investigate how two or three different fungal strains affected the breakdown of crude oil. The findings demonstrated that PBB sparked the biodegradation of crude oils, and all of the fungi used in the study's co-culture systems displayed increased enzymatic activity. This study offers a crucial method for cleaning up PHC-contaminated areas using PBB, particularly when combined with mixed fungal cultures for accelerated biodegradation.

It could be challenging to remove old hydrophobic pollutants from fine-textured soils during cleanup. In order to remove aliphatic hydrocarbons (TAH) and assess their toxicity from a historically polluted clay soil, Covino [11] compared the efficiency of augmentation treatments with that of biostimulation and assessed their impact on the local microbial population. Botryosphaeria rhodina, Pleurotus ostreatus, and combinations of both were employed as inoculants, and a sterile lignocellulosic mixture was added to the soil (1:5, w/w) as a biostimulation method.

Garca [22] investigated the impact of microbial community and polycyclic aromatic hydrocarbon biodegradation on the biostimulation and bioaugmentation of polluted soils using spent fungal substrate (Agaricus bisporus). Two bioaugmentation strategies were examined; the first entailed using the waste directly, which implied using A. bisporus in addition to the waste's natural microbiota. To evaluate its capacity to serve as a fungal carrier, SAS was sterilized and once more injected with the fungus. The outcomes clearly show SAS's appropriateness for environmental remediation and pave the way for a tempting waste recovery option.

A multifaceted approach was used to assess biostimulation and bioaugmentation strategies for heavy oil polluted soils in order to better understand the biodegradability of pollutants, microbial community dynamics, and ecotoxicological impacts of various bioremediation strategies [23]. T. versicolor inoculation in the bioremediation assays produced roughly 50% of the total petroleum hydrocarbons (TPH). Pleurotus ostreatus was explored by Bosiljcic [24] as a bioaugmentation method for the treatment of river silt contaminated with polycyclic aromatic hydrocarbons (PAH). The study's objective was to ascertain whether the white-rot fungus Pleurotus ostreatus could decompose polyaromatic hydrocarbons in historically contaminated river sediments. Similar research was conducted by Ikhajiagbe & Anoliefo [25] on the impact of microbial substrate supplementation on the bioremediation of used oil-contaminated soils using Pleurotus tuberregium.

Olutayo [26] reported the impacts of sawdust and cotton waste as soil amendments as well as Pleurotus pulmonarius (pp) bioaugmentation on the development of cowpea (Vigna ungiculata (L.) Walp) on soils contaminated with crude oil (COIL), automotive gasoline (AGO), and waste oil (SEO). According to Hestbjerg [27], certain polycyclic and heterocyclic aromatic hydrocarbons (also known as polycyclic aromatic hydrocarbons [PAHs]) in soils investigated in the field under northern temperate zone conditions were affected by the white rot fungus Pleurotus ostreatus.

The fungus inocula for bioaugmentation of polluted soils was created by Lestan & Lamar [28]. Innovative fungal inocula for bioaugmentation of soils contaminated with dangerous organic chemicals were reported in their report. The biological potential (i.e., fungal biomass produced per unit substrate) of a solid, pelleted fungal inoculum designed for bioaugmentation of polluted soils with white-rot fungus was examined by Letan [28] using the fluorescein diacetate hydrolysis activity test (FDA). When put into PCP-contaminated soils on pellets with higher biological potential and higher nitrogen content, Phanerochaete chrysosporium and Trametes versicolor did not eliminate PCP more effectively than when the fungus were placed on pellets with lower biological potential. But in the latter circumstances, the majority of the PCP was changed into pentachloroanisole (PCA).

1. **The conclusion and research needs**

Although significant progress has been made in terms of choosing suitable inocula and creating formulations to encourage their activity for a variety of bioremediation applications, as well as in terms of comprehending the environmental aspects and growth circumstances that affect microbial transport and adhesion, more research is necessary to complete our incomplete understanding of the aspects that obstruct the distribution, survival, and sustained performance of exogenous microorganisms. In order to enhance strategies for microbial intromission and distribution, it is necessary to have a better understanding of bacterial adherence and filtering through the porous medium, both in terms of the target pollutants and how these processes are regulated.

Natural genetic breeding has a significant possibility to produce strains that not only have broad catabolic specificity and can breakdown mixtures of priority pollutants, but are also resilient to environmental challenges such unfavorable pH or chemical reaction conditions found in the field. In addition to such abiotic factors, biological stresses such as competition for nutrients with native strains and mentalistic or predatory microbe interactions may also have an impact on the performance of other strains. Therefore, investigating methods to inhibit species that interfere with other strains' performance (for example, using strain-specific bacteriophages) could be a useful line of inquiry. To evaluate the performance of the other strains and ensure their involvement in the remediation procedure, there is also a need for enhanced mathematical modeling and rhetorical analytical techniques, like as transcriptional assessments of catabolic genes and different biomarkers.

Overall, our mechanistic understanding of the physicochemical, ecological, and genetic aspects that affect the semi-permanent efficacy of other strains, as well as our practical knowledge of the application of bioaugmentation, are expanding quickly. As a result, the North American region may decide more wisely when and how to apply bioaugmentation while also seeking a trustworthy way to handle a decent kind of rectification

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