**BIOREMEDIATION AS A STRATEGY TO COMBAT SOIL POLLUTION**

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**ABSTRACT**

 Presently, soil pollution stemming from ongoing anthropogenic activities, swift industrialization, and the introduction of heavy metals constitutes a significant menace to human well-being and the environment, necessitating the implementation of effective remediation approaches. Notably, hydrocarbons and heavy metals represent recurring soil contaminants, posing detrimental repercussions for both the ecosystem and human health. Recent strides in bioremediation strategies strive to ecologically reinstate polluted environments, embodying advancements towards sustainability. Diverse bioremediation techniques, offering cost-effective, sustainable, and safer alternatives, have surfaced to counteract the escalation of environmental contamination. Employing microbes to expunge pollutants from tainted sites is a potent and dependable tactic, encompassing ex-situ and in-situ methods contingent upon site attributes, pollutant type, and quantity. Thus, the selection of an appropriate bioremediation approach becomes pivotal in achieving substantial reduction of pollutant levels. This article aims to furnish insights into varied bioremediation techniques, encompassing their applicability, limitations and future prospects.

**KEYWORDS:** Bioremediation, contaminants, hydrocarbons, insitu, soil pollution

1. **INTRODUCTION**

 As the world's population continues to rise and industrialization and intensive farming become more prevalent, the issue of soil contamination has grown significantly. Soil pollutants have the potential to elicit a range of harmful effects, including those that are carcinogenic, immunotoxic, and mutagenic. These contaminants traverse the environment and food chain, resulting in grave human and ecological health implications. Various methods have been attempted to remediate polluted soil, yet none have yielded complete resolution. It is recognized that microorganisms possess the capability to degrade diverse organic compounds and assimilate inorganic substances. In contemporary times, these microorganisms play a pivotal role in pollution mitigation through a process termed "bioremediation". Bioremediation stands as an emerging technology wherein microorganisms are harnessed to eliminate pollutants from various environmental mediums, encompassing air, water, and soil. Microbes, plants, and their enzymes play pivotal roles in breaking down and degrading environmental contaminants, thereby detoxifying impacted areas. Living organisms inherently possess the capacity to adsorb, accumulate, and degrade pollutants in their surroundings, which has prompted the exploration of biological systems for decontamination efforts. This approach entails the removal of contaminants and toxins from environments such as soil, water, and more. Its applicability extends to addressing environmental challenges like oil spills. In this method, microorganisms can originate from the contaminated site itself or be sourced from external locations and introduced to the affected area. The natural process of bioremediation can be enhanced through

1. **Biostimulation**

 It involves alteration of affected site to stimulate the existing microorganisms which can carry out bioremediation. The growth and activity of inhabitant microorganisms can be enhanced by the addition of nutrients like oxygen, nitrogen, phosphorus, electron donors so that they can degrade the hazardous and toxic contaminants [1][2]. By supplying these nutrients, the efficiency of the microbiota to produce enzymes necessary for breakdown can be increased. This method is most efficient for degradation of hydrocarbons especially petroleum products and its derivatives. Petroleum contaminated site is carbon (C) rich source required by microorganisms for their metabolic activities. So a meagre amount of other rate limiting nutrients can be added onto the soil that significantly boosts up the petroleum degradation rate.

1. **Bioaugmentation**

It is the manual inoculation of specialized microorganisms such as archaea or bacterial cultures capable to detoxifying paricular contaminant in the contaminated areas (soil or groundwater). The technique is designed for the enrichment of microbiota in order to reduce the contamination level by converting toxic compounds to non toxic ones. Acrylic compounds, phenolic compounds, nitrate and other industrial waste can be degraded by the addition of microbiota to the specific targeted site. Bioaugmentation has a wide range of application in the degradation of cyanide, nicotine, synthetic dye and treatment of chlorinated solvent. Some of the bacteria used under this process include *Cryptococcus humicolus* for cyanide degradation, *Shewanella* sp. for dye degradation, *Acinetobacter* sp. for nicotine degradation *etc.* Other microorganisms include *Achromobacter, Anthrobacter, Alcaligenes, Bacillus, Pseudomonas, Mycobacterium, Nitrosomonas, Xanthobacter etc*[3].

1. **Biorestoration**

It involves restoration of contaminated site by the use of indigenous microorganisms and also by providing them favorable conditions like optimizing temperature, pH, oxygen and moisture content for the degradation of contaminants.

1. **TECHNIQUES OF BIOREMEDIATION**

 The selection of a bioremediation approach hinges on factors such as the pollutant's properties, quantity, and classification, the costs associated with the remediation method, and adherence to environmental regulations [4]. Bioremediation methods can be categorized into in-situ and ex-situ practices, determined by considerations like waste handling for treatment and the geographic site of remediation. These techniques are briefed in Figure 1 and further elucidated in subsequent sections:

1. **In Situ bioremediation**

 In situ bioremediation is the in-site treatment of contaminated soil or groundwater using biological agents with the intension of minimal damage to soil structure. This direct clean-up approach does not require the removal of contaminated materials and purely relies on natural or engineered biological processes for complete degradation, transformation and complete removal of pollutants from the environment which may be soil or water. Site relevancy for bioremediation technique depends on biodegradability of contaminant and geological and chemical parameters of the affected site. The best possible site for in situ bioremediation must be easy to controllable and interpret [5].

There are two major types of in situ bioremediation.

**Intrinsic bioremediation**

 Also referred as "natural" or "passive" bioremediation [5]. This process uses microbes already present in the environment to convert the pollutant degrades from toxic to non-toxic forms. The success of the process totally depends on immanent abilities of microbial population already present at the site and does not require engineering steps to enhance the process. The innate ability of native microbes to metabolize the contaminant must be demonstrated in the laboratory tests. It is extremely effective process with respect to soil and ground water contaminants. As no human intervention is involved so considered less expensive and most commonly used process.

**Engineered bioremediation**

 The another approach involves the enhancement of microbial metabolic activities by using engineered site-modification processes like installed system to flow nutrients. In this method, isolation of contaminated site can be done to create in situ bioreactors  for rapid growth of more microorganisms under optimum physico-chemical growth conditions. The method is employed when there is urgent need for the rapid removal of contaminant or when there is alarming threat to human health or ecosystem due to the contaminants.

**Bioventing**

 Bioventing is a promising in-situ remediation technology that stimulates indigenous microbial degradation of contaminants by the addition of air or oxygen to native soil microbes. In this technique, bacterial activity is accelerated by instigating air or oxygen into the unsaturated zone with the use of injection wells. Another way is the addition of nutrients which further accelerates the microbial activities and convert the pollutants to a non-toxic form [6]. With this technique, it is possible to treat all aerobically biodegradable contaminants and is also successful in degradation of petroleum wastes and volatile organic compounds (VOCs). More commonly it is implemented at sites contaminated with mid weight petroleum wastes (jet and diesel fuel) than low weight (gasoline). A typical bioventing setup includes a blower or compressor to which air-supply wells and soil-gas monitoring wells remains connected. Bioventing is comparatively, a low-cost technique as there is no need of lot of equipments instead it requires less supporting infrastructure and monitoring. But one condition should be taken care of that the oxygen availability throughout the site should be more in order to increase the rate of pollutant biodegradation.

**Biosparging**

The in-situ biological approach is implemented in sites polluted with benzene, toluene, ethylbenzene, xylene and naphthalene. To achieve the goal, specific aerobic bacteria are loaded to the deeper anaerobic layers of soil and groundwater with injection filters providing oxygen to the bacteria which ultimately enhances their ability to degrade minerals oils and aromatic compounds. The effectiveness of biosparging technique depends on soil permeability and pollutant degradability[7].

**Bioslurping**

The technique mergesbioventing and vacuum-enhanced free-product recovery approaches. The technique is commonly implemented to target hydrocarbon contaminated areas and to remediate sites contaminated with volatile and semi-volatile organic compounds. The process of bioslurping involves the installation of dual-phase extraction wells having sections for injecting oxygen and extracting groundwater to stimulate the aerobic biodegradation of contaminants into harmless byproducts like CO2 and water. In this technique, vacuum-enhanced pumping causes lifting light non-aqueous [phase](http://www.cpeo.org/techtree/glossary/P.htm#phase) liquids off the water table and released from the capillary fringe. This reduces changes in the elevation of water table. Bioslurping is a cost effective method as it pumps only a little quantity of soil vapor and groundwater at a time. Hence for the treatment vapor and free product, small plant can be installed [8].

**Phytoremediation**

 In this in situ approach, plants are used toremediate contaminated soil. Phytoremediation is well suited for areas with low-level contaminated sites. Deep rooted trees, grasses, legumes, and aquatic plants plant significant role in eliminating the contaminants in phytoremediation field. The approach is implemented to remove TPH, BTEX, PAH, 2,4,6-trinitrotoluene (TNT), and hexahyro-1,3,5-trinitro-1,3,5 triazine (RDX) [9]. Several mechanisms of phytoremediation like extraction, degradation, stabilization, volatilization and filtration are known to remediate the soil. Plants like willow and alfalfa hydrocarbons and chlorinated compounds can be eliminated by the mechanism of degradation and mineralization [10] [11]. For successful outcome of this approach plant must be free from pests and diseases [12]. The biggest advantages of phtoremediation is that some valuable accumulated metals can be recovered following a process known as phytomining.

1. **Ex-situ bioremediation**

 The approach involves the transference of contaminants or pollutants from their polluted site to the different treatment site. The contaminated soil is excavated or removed and transported to a controlled environment where bioremediation can take place. The technique is successfully considered when favored by the concentration and type of pollutant, depth of pollution, treatment cost and location of polluted site [6]. Proper oxygen, moisture and nutrients conditions must be maintained on offsite. Some common ex-situ techniques include:

**Biopile**

The technique involves the excavation of soil and piled above ground followed by aeration and nutrient amendment to stimulate the metabolic activities of microorganisms. Straw, saw dust, wood chips can also be loaded into the biopile in order to accelerate the microbial activity for effective biodegradation. The soil microbial activity is stimulated by the addition of nutrients, moisture, oxygen and minerals. This type of approach is implemented to treat volatile, low molecular weight contaminants [13] [14] pH, temperature, aeration and nutrients are some of the factors responsible for the rate of degradation. Due to cost effectiveness, the method is more preferred.

**Land farming**

 It is a well practiced technique that usesvolatilization and biodegradation for reducing hydrocarbon concentrations. In this technique the contaminated soil after excavation is allowed to spread over a prepared bed and periodically turned over until pollutants are degraded. Proper soil conditions must be maintained to optimize the rate of pollutant degradation. Moisture content must be controlled by spraying or through irrigation, aeration by tilting and mixing and pH must be maintained near neutral by using lime.

**Composting**

 Composting is a self heating biological process during which organic materials are degraded by microorganisms which lead to the production of organic and inorganic by-products and energy is releases in the form of heat. The technique is applicable to treat soil contaminated with petroleum hydrocarbons, solvents, pesticides and heavy metals. This techniques uses indigenous microorganisms, bacteria, fungi to sequester or breakdown pollutants in soil and water thus, helps soil to regain pre-contaminated state. Degradation efficiency cannot be increased only by oxygenation and irrigation but also by monitoring temperature and moisture content. The technique involves two methods windrow and aerated static pile. In the former, composed is arranged in long piles mixed with mobile equipments while in the latter, compost is arranged in heaps and provided with aerated blowers. Compost acts as soil conditioner and provide a variety of nutrients to plants.

1. **Challenges to Bioremediation of Soil Pollution**

 Bioremediation, a sustainable approach aimed at combating soil pollution, encounters several obstacles that demand careful consideration for successful implementation. The restricted availability of pollutants presents a challenge, especially with strongly sorbed compounds like pesticides and polycyclic aromatic hydrocarbons (PAHs), necessitating strategies to enhance pollutant bioavailability [15][16]. Moreover, composting organic matter can alter pH, potentially impacting pollutant stability, thereby requiring measures to counteract pH-related adverse effects. Additionally, the adaptation of introduced exogenous microorganisms to contaminated soils can hinder pollutant degradation, making it essential to understand and address factors influencing microbial adaptability for effective bioaugmentation [16][17][18].

 Comprehensive knowledge of microbial metabolic pathways during bioremediation of organic pollutants and heavy metals is crucial for successful genetic modification of exogenous microorganisms, underscoring the importance of employing advanced techniques like high-throughput sequencing and synthetic biology. Enzymatic action plays a pivotal role in pollutant degradation, but a detailed investigation of enzymatic aspects, including kinetics, molecular structure, activity, and inhibition processes, remains limited. Furthermore, the complex interactions between different pollutants in the same soil/compost mixture can influence their degradation, calling for further in-depth analysis [16][19].

 Lastly, ensuring the quality of end products released post-bioremediation is vital, demanding meticulous monitoring and quality control measures to adhere to stringent environmental standards. By tackling these challenges and integrating advanced technologies and approaches, bioremediation holds the promise of providing an efficient and sustainable solution for soil pollution cleanup [20].

1. **Future Perspectives in Bioremediation of Soil Pollution**

 As society's demand for effective and sustainable solutions to soil pollution continues to rise, the potential of bioremediation appears remarkably promising. With biotechnology and genetic engineering leading the way, exciting possibilities emerge for enhancing the bioremediation capacity of microorganisms, thus facilitating more efficient and targeted cleanup processes.

1. **Genetically Engineered Microorganisms (GEM)**

 The utilization of genetically engineered microorganisms (GEM) is poised to revolutionize bioremediation practices. By crafting designer biocatalysts that target specific pollutants, including recalcitrant compounds, researchers can expand the substrate range of existing metabolic pathways and bolster the stability of catabolic activities. Furthermore, the parallel gene transfer and multiplication of GEM offer an encouraging approach to augment bioremediation efficiency, enabling tailor-made solutions for diverse soil pollution scenarios[21][22].

1. **Nano Materials for Enhanced Bioremediation**

 The integration of nano materials in bioremediation processes shows significant promise for improving efficiency while reducing the time and cost of cleanup. Nano materials enhance the surface area and lower the activation energy of microorganisms, thus increasing their resilience to pollutant toxicity. Leveraging nano materials can elevate the performance of microorganisms in degrading contaminants and expedite the overall bioremediation process.

1. **Hologenomics and Bioremediation**

 The exploration of hologenomics, the study of genomic sequences of plants and their associated microorganisms, holds immense potential for future bioremediation research. Understanding the diversity and evolutionary relationships among bioremediators will aid in developing more efficient and robust bioremediation systems. By manipulating microbial niches through hologenomics, resistance against toxic metal contamination can be enhanced, elevating the overall effectiveness of bioremediation strategies.

1. **Advancements in Plant-Based Bioremediation**

 Plant-based bioremediation, particularly using hyperaccumulator plants, has shown promise in removing heavy metals from contaminated soil. To further advance this field, researchers can explore two main avenues. First, the discovery and validation of new hyperaccumulator plant species can broaden the range of applications. Second, harnessing genetic engineering to enhance the hyperaccumulation capacity of plants will open new possibilities for efficient soil cleanup.

1. **Collaboration and Development**

 To fully unlock the potential of bioremediation, collaboration between researchers, scientists, policymakers, government agencies, industrial sectors, and individuals is indispensable. Continued research, assessment, and investigation are essential for developing best management practices and novel approaches for efficient bioremediation of soil pollution. Enhancing stakeholder coordination and engagement will amplify bioremediation's success and reliability as a sustainable environmental solution [18]. The horizon for bioremediation is promising, with genetic engineering, nanotechnology, and plant-based approaches set to enhance soil cleanup efficiency and effectiveness. Through advanced technologies and collaborative endeavors, bioremediation can play a pivotal role in securing a healthier, sustainable planet for generations to come.

1. **Environmental and Regulatory considerations**

 In bioremediation endeavors, environmental considerations hold utmost significance due to the potential risks associated with the release of transformed pollutants or byproducts into the ecosystem. While the primary goal is to diminish pollutant concentrations in soil, cautious measures must be taken to avert contaminants from leaching into groundwater or volatilizing into the atmosphere. Thorough site characterization, encompassing an evaluation of soil properties and hydrogeological conditions, stands as a pivotal step in comprehending the possible risks tied to the chosen bioremediation method. Furthermore, a meticulous assessment of the compatibility between selected microorganisms and the native soil microbiota is imperative to forestall unintended ecological disruptions.

 The efficacy and safety of deploying bioremediation technologies are significantly influenced by regulatory frameworks. Adherence to local, national, and international environmental laws ensures that bioremediation efforts remain within acceptable pollution thresholds, without introducing new hazards. Securing the necessary permits and approvals before embarking on bioremediation projects serves as a crucial demonstration of the proposed methods adequacy and their absence of substantial adverse effects on the environment and human health. Engaging with regulatory authorities and stakeholders throughout the planning and implementation stages fosters transparency and instills public trust in the bioremediation process [17].

 Sustained monitoring and verification of bioremediation progress are imperative to evaluate the effectiveness of the chosen approach and ensure a gradual reduction of pollutant levels over time. Regular monitoring allows for timely adjustments in response to unexpected challenges and aids in identifying potential environmental risks during the remediation process. Implementing comprehensive monitoring protocols that encompass the degradation of pollutants and the health of the surrounding ecosystem is essential for long-term success and to provide reliable data for regulatory compliance.

1. **CONCLUSION**

 In conclusion, the battle against soil pollution stands at a crossroads, with bioremediation as a beacon of hope against industrialization and population-induced contamination. In this chapter we explored bioremediation, which employs microorganisms and plants to restore soil health. From biostimulation to genetic engineering, bioremediation techniques offer versatile approaches. Success hinges on microbe-environment interactions. Genetic engineering, nano materials, and plant-microorganism synergy promise efficient soil cleanup. Challenges include microbial adaptability and pollutant interactions, demanding careful management. Collaboration between stakeholders is crucial for responsible bioremediation. Environmental considerations and regulations emphasize effectiveness and safety. Bioremediation's journey must be responsible and accountable. Sustained monitoring and engagement ensure a greener future. This journey is one of innovation, resilience, and shared responsibility, weaving technology, nature, and human determination. Each microbial action and plant's growth bring us closer to renewing our planet's vitality.

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**Figure 1: Flowchart: Bioremediation Techniques for Soil Pollution Cleanup**

**Table 1: Applications and limitations of different bioremediation techniques.**

| **Bioremediation Techniques**  | **Applicability** | **Limitations** |
| --- | --- | --- |
| In-situ techniques |
| Bioventing | soils contaminated by petroleum hydrocarbon wastes, non-chlorinated solvents, VOCs and other organic chemicals. | High moisture soil, extremely low moisture soil, soil permeability, depth of water table to ground surface, low temperature, vapor monitoringNo effective degradation of chlorinated compounds even in presence of conmetabolite  |
| Biosparging | Targets mineral oils, benzene, toluene, ethylbenzene, xylene, and naphthalene (BTEXN) | Soil permeability, Contaminant biodegradability  |
| Bioslurping | Soil contaminated with petroleum hydrocarbons and volatile organic compounds | Less efficient for low permeable soil |
| Phytoremediation | Phytomining | Decreased effectiveness in diseased conditionContaminant transference through food chain.Invasive species may affect biodiversity |
| Ex-situ techniques |
| Land farming | diesel fuel, jet fuel, oily [sludge](http://www.cpeo.org/techtree/glossary/S.htm#sludge), wood-preserving wastes and pesticides | large space is required. [off-gas](http://www.cpeo.org/techtree/glossary/O.htm#off-gas) control may be required. [leaching](http://www.cpeo.org/techtree/glossary/L.htm#leachate) of metals is one of the major concern |
| Biopiling |  [petroleum hydrocarbon](https://www.drdarrinlew.us/xenobiotics/role-of-microbes-in-petroleum-hydrocarbon-degradation.html)s | Can not remove inorganic pollutants and radionuclides Not effective in case of high concentrations (>50,000 ppm) of total petroleum hydrocarbonsHigh metal concentration may inhibit microbiological development |
| Composting | petroleum hydrocarbons, solvents, chlorophenols, pesticides, herbicides, PAHs, perchlorate and nitroaromatic explosives | Requirement of large space, excavation of large contaminated soil and additional treatment equipments for VOCsAddition of bulk materialsNon-applicable if heavy metal is present in excessive amount. |