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Bioaugmentation and Biostimulation: Optimizing In-situ and Ex-situ Bioremediation Strategies for Complex Soil Contaminants

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Abstract

The increasing level of environmental contamination has led to a surge in the need for long- lasting and efficient cleanup techniques. Utilizing living creatures to break down or eliminate pollutants is a promising approach known as bioremediation. Particularly successful approaches for dealing with complicated soil pollutants are in-situ or ex-situ bioremediation techniques like bioaugmentation and biostimulation. Whereas biostimulation concentrates on promoting native microbial activity, bioaugmentation adds exogenous microbial strains to improve pollutant breakdown. When compared to traditional techniques, optimizing these procedures can minimize environmental effect, lower costs, and increase cleanup efficiency. The concepts, applications, difficulties, chances for innovation, and suggestions for maximizing bioaugmentation and biostimulation in in situ and ex situ bioremediation methods for complicated soil pollutants are all examined in this review study.

Keywords

Bioremediation, in-situ bioremediation, ex-situ bioremediation, bioaugmentation, biostimulation, soil contaminants, microbial engineering, remediation efficiency

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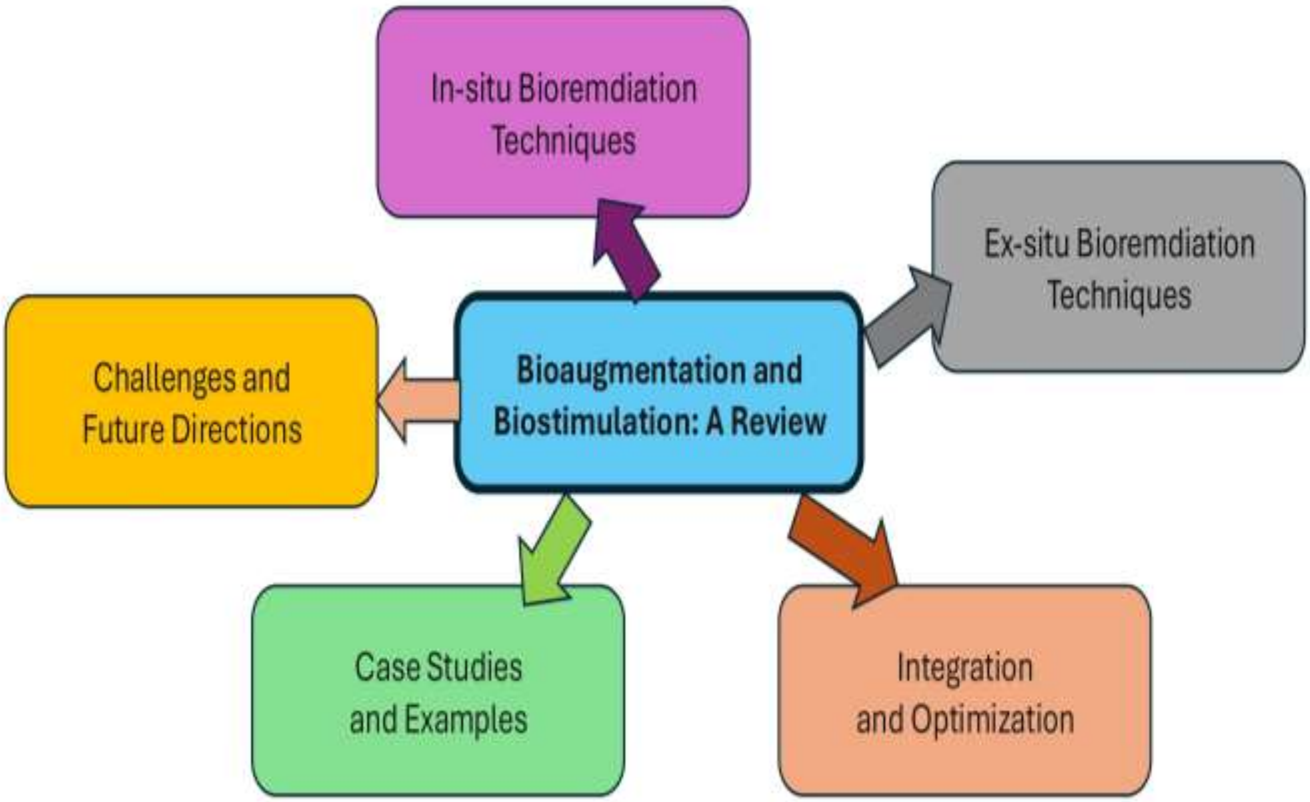
Graphical Abstract



Introduction:

The need for sustainable and efficient remediation solutions has grown critical due to the increasing levels of environmental contamination. Metals and inorganic compounds are the main culprits that are responsible for this contamination. For example, soil cadmium pollution is a worldwide problem. All soils contain cadmium, a naturally occurring metal that comes from both geogenic and human sources. Cadmium has an impact on people, plants, and soil microorganisms.(Qasim, 2024). Similarly, sodium dodecyl sulfate (SDS) is also known as sodium lauryl sulfate; it is an organic compound and anionic surfactant used in most sectors. SOD is a detergent that is used primarily extensively in soap manufacturing industry. It is also used in personal care products in concentrations of 0.1-1%, and in laboratory applications, its concentrations increased up to 2-10%. However, the widespread use of SDS has raised environmental concerns and has become a hazard to soil microbial communities, crucial to soil health and fertility (Haidri et al., 2024).

At first, physical and chemical methods were used to tackle the problem of environmental pollution but in recent years, utilization of microorganisms for the purpose of environmental health reclamation is in trend. One possible method for minimizing ecological harm and reestablishing the natural balance of ecosystems is bioremediation, which involves the use of biological agents such as plants and microbes to remove or lessen the harmful effects of environmental pollutants (Dehnavi et al, 2022). Biological agents such as fungi, bacteria, and archaea are used as bioremediators (Sanjana et al., 2024). Frequently used microorganisms include *Achromobacter*, *Arthrobacter*, *Alcaligenes*, *Bacillus*, *Corynebacterium*, *Pseudomonas*, *Flavobacterium*, *Mycobacterium*, *Nitrosomonas*, *Xanthobacter*, etc (Singh R, et al 2014). Oil-contaminated soils can be effectively treated at a reasonable cost with bioremediation. For instance, the two primary bioremediation techniques frequently employed for soil cleanup are bioaugmentation and biostimulation (Yaman, 2020).

The specific contaminants and environmental conditions present at the cleanup site determine the mechanisms underlying biostimulation approaches. There are two methods of bioremediation: in-situ bioremediation and ex-situ bioremediation. The most promising methods for effectively cleaning up the environment, including soils, water, and sediments contaminated with inorganic and/or organic contaminants, include biostimulation and bioaugmentation, which are environmentally friendly in situ bioremediation techniques (Kuppusamy et al, 2020). In-situ bioremediation is the biological treatment of contaminated soil and groundwater without the need to pump and treat groundwater above soil or excavate the soil. In situ bioremediation is known to be accomplished through three well-established technological processes: (1) bioattenuation, which uses

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natural degradation processes to disperse contaminants through biotransformation; (2) biostimulation, which involves adding nutrients, water, electron donors, or acceptors to promote microbial growth; and (3) bioaugmentation, which involves the inoculation of native, allochthonous, or genetically modified microorganisms with particular abilities to degrade or biotransform the pollutant of interest (Cheng et al., 2016). In situ bioremediation techniques have been successfully used to treat chlorinated solvents, dyes, heavy metals, and hydrocarbons polluted sites (Kim S et al, 2014), (Frascari D et al, 2015), (Roy M et al, 2015).

"Ex-situ bioremediation" of a contaminated site refers to moving the contaminated material to another area for cleaning and treatment. After treatment, the remediated material may be safely disposed of, returned to its original position, or used for another purpose, depending on the regulations and the type of contaminants. The choice between in situ and ex situ methods is based on a number of factors, such as the type and extent of pollution, site characteristics, regulatory requirements, and economic feasibility.

Bioaugmentation:

The process of bioaugmentation entails the introduction of exogenous microorganisms to augment the natural microbial breakdown of environmental pollutants. There are two approaches to bioaugmentation: (1) isolating microorganisms that can eliminate pollutants from contaminated soils, culturing them in a lab, and then reintroducing the bacteria to the original site (reinoculation of native bacteria), or (2) inoculating microorganisms derived from various contaminated sites that have been shown to be able to break down the contaminants of concern. The addition of microbial biomass (bacteria, fungi, and their secreted enzymes) to contaminated areas, i.e., the process of bioaugmentation, can be adapted to the green environment and can notably improve an area's pollutant removal efficiency (RE), as well as reduce their removal time and costs. (Muter, 2023)

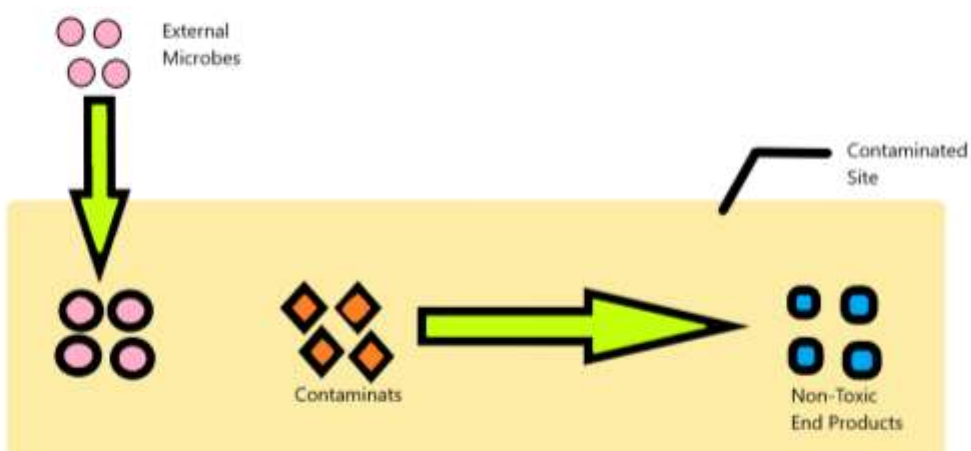

Fig 1: Process of bioaugmentation

In-situ bioaugmentation techniques:

Direct inoculation, bioventing, bioaugmentation using solid carriers, plasmid remediated bioaugmentation and rhizoremediation are examples of in-situ bioaugmentation techniques. Microorganisms are directly injected into the contaminated soil at the location of contamination during a direct inoculation. One method to accomplish this is by injecting the necessary microorganisms into the soil using microbial cultures or solutions. In an effort to increase the ability of native or foreign microorganisms to break down pollutants, bioventing is a technique that involves injecting nutrients and air into polluted soil. Although bioaugmentation has been used extensively to support bioremediation, it has also frequently been associated with significant challenges and poor results, most likely as a result of incomplete knowledge that led to improper application strategies

To improve the natural bioremediation abilities of contaminated soils, plasmid-carrying bacteria are directly introduced into the soil by a technique known as "in-situ plasmid bioaugmentation" (Chakraborty et al., 2018). More research is required to improve the selection of donor bacterial strains and accompanying plasmids, acquire extensive knowledge of native soil bacterial populations, understand how environmental factors affect plasmid acquisition, and understand how the target catabolic genes are expressed and function, ensuring that plasmid-mediated bioaugmentation can be consistently and reliably replicated (Garbisu et al, 2017).

Microorganisms are immobilized on solid carriers, like pellets, beads, or biofilms, and then injected into the contaminated soil as part of the bioaugmentation process using solid carriers. By protecting the introduced bacteria from outside threats, this method allows them to stay active for extended periods of time. In rhizoremediation, specific plants are used to promote the growth of specific microbial communities in the rhizosphere—the soil zone that is affected by plant roots. These microorganisms facilitate the degradation of contaminants in the soil.

Ex-situ bioaugmentation techniques:

Ex-situ bioaugmentation techniques include landfarming, composting biopiling, and processing by bioreactors, chemical, and physical processes. In bioreactors, in order to create and preserve the ideal conditions for microbial activity, polluted soil must first be excavated and then put within bioreactors. The bioreactors are then filled with microorganisms and the nutrients and additives required to speed up the breakdown of contaminants. Biopiles involves the use if an excavation method, in which polluted soil is piled in windrows or piles, much like in bioreactors. To improve microbial activity and aerate the soil, microbial inoculants are applied and the piles are saerated on a regular basis. In addition to being utilized to remove volatile low molecular weight contaminants, the biopile is also a useful remediation strateg for extremely cold, contaminated areas. (Gomez F et al, 2014; Dias RL, 2015; Whelan MJ 2015). The biopile's adaptability allows for a reduction in remediation time by increasing microbial activity and contaminant availability while also increasing biodegradation rate (Saroj et al., 2022).

Landfarming is a method that includes tilling and covering contaminated soil with a layer to promote microbial activity and aeration. Fertilizers and microbial inoculants can be added to stimulate decomposition. Excavating the soil and adding organic materials—wood, hay, manure, and vegetal waste—to the polluted soil in a controlled setting allows microorganisms to break down the toxins while producing heat to hasten the process. This is known as composting. Ex situ thermal remediation methods are the best choice for eliminating polycyclic aromatic hydrocarbons (PAH), petroleum hydrocarbons (TPH), phenolic compounds, cyanides, benzene, toluene, ethylbenzene, xylenes (BTEX), and chlorinated compounds, such as polychlorinated pesticides, polychlorinated hydrocarbons, polychlorinated dibenzodioxins (PCDD), and polychlorinated dibenzofurans (PCDF). Case studies provide important insights into the benefits and practical implementation of bioaugmentation in soil remediation.

Case study 1: Petroleum hydrocarbon remediation through bioaugmentation

"Petroleum hydrocarbon remediation" is one case study concerning bioaugmentation. The current study employed a two-step bioaugmentation technique (TSBS) to improve the degradation of total petroleum hydrocarbons (TPH) from petroleum refinery sludge (PRS) by means of an indigenous bacterial consortia. Four native isolated strains of bacteria—Dietzia sp. IRB191, Dietzia sp. IRB192, Staphylococcus sp. BSM19, and Stenotrophomonas sp. IRB19 from PRS—were used to create a bacterial consortium. Using a one variable at a time approach, the ideal values for pH, temperature, and sludge concentration were 7, 34°C, and 2% (w/v), respectively, for maximum TPH degradation. In order to adopt a novel TSBS, the formed consortium was twice injected with the culturing medium under ideal culture conditions: once on the first day (0th day) and once again on the tenth day.

After 15 days of incubation, the maximal TPH degradation of 91.5 ± 2.28% was recorded with TSBS, 1.18 times greater than that of SSBS (77.3 ± 2.6%). The bacterial consortium with TSBS successfully decomposed the TPH present in the PRS, as demonstrated by the GC-FID research. With a rate constant of 0.155 d^{-1}, the TPH degradation utilizing TSBS proceeded in accordance with first-order kinetics. Therefore, it can be said that using a TSBS for biodegradation is a safe, efficient, and environmentally beneficial method of disposing of petroleum refinery sludge. Heavy metal-contaminated soils may be treated through microbial augmentation using fungi or bacteria resistant to metals. (Muter, 2023)

Case study 2: Remediation of farmland heavy metals

The removal of heavy metals identified in farms is the subject of another case study. Bacillus subtilis 38 (B38) is a UV-irradiated mutant strain of Bacillus subtilis that has a high resistance to cadmium. According to this study, B38 was an effective biosorbent for the adsorption of lead, mercury, chromium, and cadmium, among other heavy metals. B38 and NovoGro (SNB) applied simultaneously showed a synergistic effect on heavy metal immobilization in soil. When compared to the control, the amounts of heavy metals in the edible portions of the three examined plants—lettuce, radish, and soybean—dropped by 55.4–97.9% when treated with SNB. Mehlich 3 (M3), the first stage of the Community Bureau of Reference technique (BCR1), and diethylenetriamine pentaacetic acid (DTPA) were three single extraction methods that demonstrated good prediction capacities for metal bioavailability to foliage, rhizome, and leguminous plant, respectively. The results of the polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) demonstrated that NovoGro may promote the growth of native and alien B38 bacteria. When the technology was finally tested in the field, the amounts of heavy metals in the edible portion of radish were reduced from 30.8% to 96.0% following bioremediation using SNB treatment. This study reveals an effective strategy for cleaning up farmland that has been affected by several heavy metals. (Wang et al., 2021)

Factors affecting bioaugmentation:

Case studies show that the degree to which bioaugmentation is successful in remediating soil depends on a number of parameters. Microbial competency is one such component. Microbial competency is the capacity for metabolism and adaptability of introduced bacteria. In addition, there are environmental variables (pH, temperature, moisture content, and nutrient availability), microbial interactions (interactions between introduced and indigenous microbial populations), and substrate availability (a sufficient supply of electron donors, acceptors, and co-substrates). The development and survival of introduced microbes can be impacted by predatory organisms, antagonistic relationships, and competition for resources. A number of factors, including temperature, the composition of the pollutant, and the microbial inoculum, affect bioaugmentation and must be taken into account for the treatment process to function as seamlessly as possible (Chettri et al, 2024)

Table 1. Specific bioaugmentation researches (Omokhagbor Adams et al., 2020)

Type of contaminant	Initial TPH concentration	Microorganisms added	Source of Microorganisms	Duration of research	TPH reduction (%)	Comment	References
TPH (Crude oil)	4200mg/kg	Acinetobacter baumannii T30C	Tapis crude oil contaminated soil of oil refinery plant	35 days	43%	TPH induction was not entirely induced by the introduction of A. baumannii T30C as the reduction observed wan not significantly different from the control	Chang et al. (2011)
TPH (Crude oil)	>100mg/kg	Serratia Sp BF40	Crude oil contaminated saline soils	Unknown	>60%	BF40 Strain of Serratia showed high utilized potential for biodegradation of crude oil contaminated saline soils due to its high surface activity and salt tolerance	Wu et al (2012)
TPH, contaminated by parking trucks, human activities and diesel	10,000mg/kg	Bacillus cereus, Gordoni rubripertincta, Kociria rosea, Bacillus subtilis (Strains 7A and 9A), Aspergillus terreus, Aspergillus carneus	Isolated from pre contaminated sites and assayed for ability to degrade TPH	90days	52.0%	Soil TPH degradation was not significantly different from reduction observed in control, although there was a significant reduction (P<0.05) relative to initial TPH concentration	Diaz-Ramirez et al (2013)
TPH (diesel)	16,300mg/kg	Candida tropicalis SK 21	Petroleum contaminated soil	120days	83%	Inoculation of yeast resulted in 83% TPH removal as against 61%using indigenous microorganisms	Fan et al. 2013
TPH (Diesel from Tank top accident)	>5,000mg/kg	Pseudomonas fluorescens	Isolated from previously contaminated soil	270days	>60%	Phospholipid Fatty Acids (PLFA)released was measured as an indication of living microbial biomass. Augmentation with P. fluorescens markedly improved the PLFA and degradation as compared to samples relying on autochthonous microbes	Kuran et al (2014)
TPH (Biodesel and Diesel	Unknown	*Pseudomonas aeruginosa*, *Arthrobacter xylosoxidans* and *Ochrobacterium intermedium*	Exogenous and identified through sequencing of 16s rRNA gene	32 days	32.2%	Successive bioaugmentation displayed positive effects on biodegradation with substantial reduction in TPH levels	Colla et al 2014

Biostimulation:

Biostimulation techniques have been applied to many soil contamination scenarios, including the control of heavy metal and petroleum hydrocarbon pollution. In polluted soils resulting from oil spills or underground storage tank leaks, the microbial breakdown of petroleum hydrocarbons has been enhanced through the application of biostimulation, which entails the addition of nutrients like nitrogen or phosphorus. Biostimulation techniques have been used in heavy metal-contaminated soils (e.g., lead, cadmium, arsenic) to promote microbial metal immobilization or transformation. Adding organic matter and adjusting pH are two of these strategies.

At bioremediation sites, assessments of the overall metabolic and growth response of the community have been obtained by incorporating radiolabeled acetate and thymidine into lipids and DNA, respectively. For the remediation of old organic

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pollutants, biostimulation has frequently shown to be more effective than bioaugmentation, requiring no immediate intervention. (Udume et al., 2023).

To increase the activity of native or naturally occurring microbial communities, additives such bulking agents, moisture, nutrients, oxygen (or other electron acceptors), and/or biodegradable carbonaceous substrates are supplied. Nitrogen salts must frequently be added as an addition to the soil by either injecting them into the soil above the contaminated soil zone or sprinkling a nutrient solution on top of the soil.

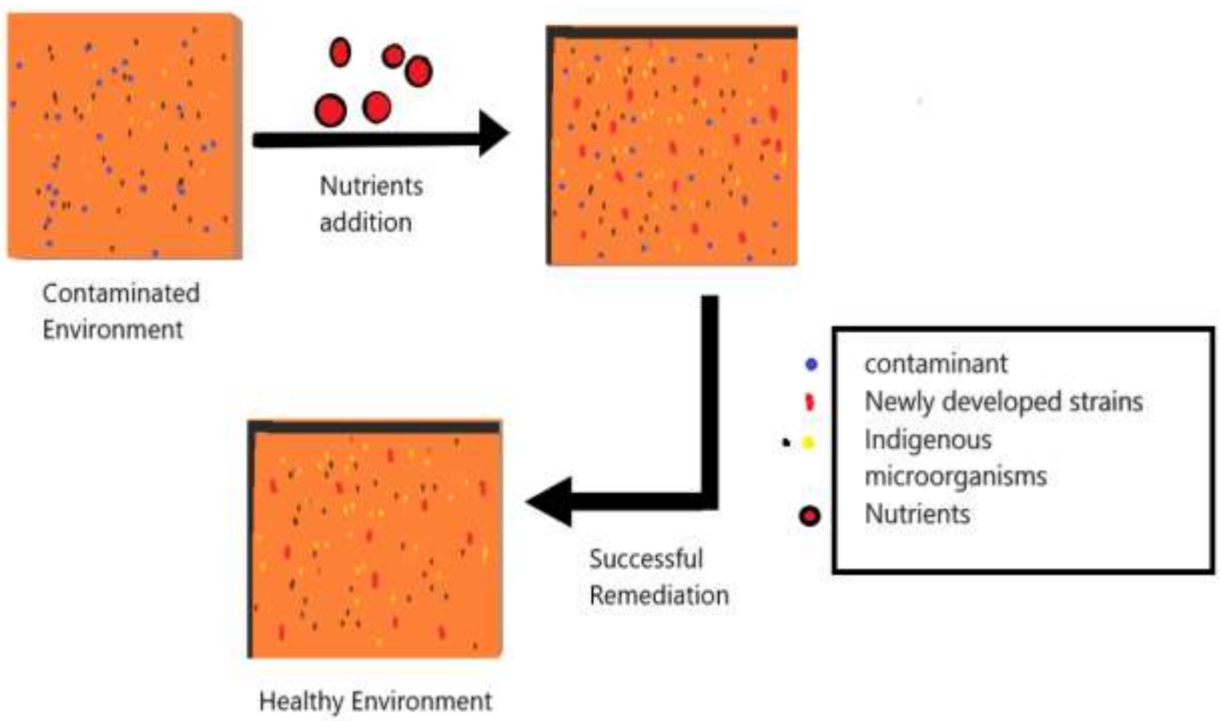

Fig 2. **BiostiFFig 2. Biostimulation Process**

Importance of abiotic factors:

According to a study (Chikere et al., 2012), agitation and homogenization from stirring are important abiotic factors in the reducing the hydrocarbon content. Typical sources of nutrients include organic amendments, inorganic fertilizers, and electron donor/acceptor supplementation, which consists of giving microbial communities organic or inorganic materials that act as electron donors. These compounds facilitate the metabolism of microorganisms and promote the removal of contaminants. Similar to this, electron acceptor supplementation—which

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involves adding oxygen, nitrate, or sulfate as an alternative electron acceptor to improve contaminant degradation under anaerobic conditions and facilitate microbial respiration—and pH adjustments—which involve changing the pH of soil to create ideal conditions for microbial activity and degradation. Contaminants may exhibit pH dependence in terms of solubility or susceptibility to microbial decomposition. An appropriate pH adjustment can promote the transformation of pollutants and microbial activity. Lime and other acidifying substances may be necessary for alkaline soils, although alkaline minerals can be introduced into acidic soils to raise pH levels. For a number of pollutants, slow release electron donors with low solubility have also been employed to regulate biostimulation.

Effectiveness of biostimulation strategies:

It has been well studied and evaluated how well biostimulation techniques work to increase microbial activity and speed up the degradation of pollutants in soil. The evaluation approach typically includes field size testing, pollutant degradation dynamics, and microbiological activity monitoring. In order to assess changes in microbial biomass, activity, and community composition following biostimulation treatments, monitoring the activity of microorganisms entails using techniques including microbial biomass measurement, enzyme tests, and molecular studies (e.g., DNA sequencing). Contaminant degradation dynamics estimates the rates of degradation and transformation products of contaminants over time to evaluate the effectiveness of biostimulation treatment.

Analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) are commonly used for the assessment of pollutants. One approach to assessing the practicality and effectiveness of biostimulation techniques in real-world settings is to carry out field-scale trials. Field trials enable assessments of variables such as long-term sustainability, hydrogeological attributes, and variation by region.

Case study 1: Copper immobilization through biostimulation of the carbonate precipitation

"Copper immobilization through biostimulation of the carbonate precipitation process in soil" is one case study pertaining to the use of biostimualation. The first application of the biostimulation strategy to accelerate MICP in copper (Cu) immobilization in soil is discussed in this case study. After biostimulation, the number, composition and diversity of the bacterial community were assessed using MiSeq Illumina sequencing analysis. This study revealed that the number and types of ureolytic and calcifying bacteria had increased dramatically compared to untreated soil, which led to the MICP process. The results demonstrated that biostimulation in soil, which mainly immobilized Cu in the carbonated portion of the soil, was responsible for the production of calcite precipitation. Meanwhile, the soil's soluble-exchangeable copper percentage dropped from 45.54 mg kg^{-1} to 1.55 mg kg^{-1}. Energy-dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM) were used to evaluate the structure and elemental content of Cu immobilization following biostimulation. In order to carry out Cu remediation from soil, X-Ray Diffraction (XRD) identified the main crystalline phases or biominerals generated during biostimulation. Fourier Transform-Infra Red (FTIR) spectroscopy demonstrated functional chemical groups involved in copper immobilization. (Xueyan Chen et al,2019).

Case Study 2: Biostimulation technique of high temperature

"Biostimulation technique of high temperature on microbial community structure and cis-1,2-dichloroethene dechlorination" is another case study concerning the use of biostimulation. Thermally accelerated anaerobic dechlorination is a revolutionary remediation technology to dechlorinate vinyl chloride, cis-1,2-dichloroethene, and tetrachloroethene, as well as its intermediate metabolites. Extensive studies were carried out at 15 and 30 °C on soil and groundwater samples from three distinct contaminated sites to clarify the effects of high temperature on cis-1,2-dichloroethene dechlorination and related microbial communities under biostimulation settings. At 30°C, cis-1,2-dichloroethene dechlorinated more quickly than it did at 15°C, regardless of the source of the sample.

The higher temperature resulted in an improvement in the first-order degradation rate constant and a reduction in the lag time before the onset of dechlorination.

It was discovered that Dehalococcoides grew more easily at 30 °C than at 15 °C, which might have contributed to the speedier dechlorination of cis-1,2-dichloroethene and vinyl chloride. Conversely, subsequent subculturings led to similar compositions in the microbial community structures in four consortia enriched from two samples at two distinct temperature conditions, indicating that the overall effect of elevated temperature on microbial communities was not significant. The findings of this work show that cis-1,2-dichloroethene-contaminated soil and groundwater can be successfully treated by thermally increasing anaerobic dechlorination under biostimulation Yamazaki (Yuji Yamazaki et al,2020). Biostomulation is used to treat contamination from chlorinated solvents. Electron donor supplements (e.g., hydrogen and lactate) have been used to induce microbial reductive dechlorinated solvents (e.g., trichloroethylene and tetrachloroethylene) in polluted soils and groundwater.

Table 2. Biostimulation using organic nutrients (Omokhagbor Adams et al., 2020)

Nutrient Added	Type of contaminant	Initial TPH concentration	Percentage Removed	Duration	Comment	References
Compost made from wood chips and sewage sludge	Petroleum Hydrocarbon-crude oil	38,000mg/kg	100% removal of 2-3 ringed PAHs within the first 3 months	570 days	Showed 100% removal over a 19 month period with removal linked to the native microbial population and improved growth in the system	Atagana (2008)
Brewery spent grains, banana skin and spent mushroom compost	Petroleum hydrocarbons-spent lubricating oil	Not indicated	79% and 92% for 5% oil contamination linked to the presence of organic waste and low contamination while reduction was between 17% and 24% in 15% w/w oil contamination initially and 36% to 55% after 84 days linked to high initial concentration	84 days	Showed significant removal of TPH using the organic nutrient sources	Abioye *et al.*(2012)
Poultry droppings	Petroleum hydrocarbons-contaminated marine sediments	106.4ppm-116ppm TPH and 96.6 – 104ppm PAH	95.35% for TPH and 98.92% for PAH	56 days	Significant degradation of PAH and TPH in a bioreactor using 20g poultry litter and 1 litter seawater	Chikere *et al* (2012)
Cowdung	Hydrocarbon polluted mangrove swamp	14,103.02mg/kg	62.96%	70 days	Significant reduction observed when compared with control using cowdung as Biostimulation agent	Orji *et al* (2012)
Tea leaf, soy cake and potato skin	Petroleum hydrocarbon-diesel fuel	100000mg/kg and 200000mg/kg variation in concentration	Between 25% and 82%	126 days	Showed significant degradation of TPH for the treatment with soycake	Dadrasnia and Agamuthu (2013)
Oil palm empty fruit bunch and sugar cane bargasses	Petroleum hydrocarbon crude oil	Not indicated	100% for sugarcane bargasse and up to 97% for empty palm fruit bunch	20 days	Showed significant biodegradation using these supplements for stimulating microbial growth	Hamzah *et al* (2014).

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Integration and optimization of bioaugmentation and biostimulation strategies:

Bioremediation strategies are an eco-friendly, economical, and successful alternative to physiochemical procedures. However, their remediation process takes time, which restricts their use in large-scale applications (Dehnavi et al, 2022). Combining the benefits of bioaugmentation and biostimulation offers a synergistic approach to bioremediation that may produce more long-lasting and efficient pollution remediation outcomes. When many microbial strains or consortia are added through bioaugmentation and paired with microbial proliferation caused by biostimulation, the microbial community's redundancy and resilience are increased. Redundancy ensures the availability of multiple microbial species capable of decomposing the contaminants, hence reducing the likelihood of treatment failure due to environmental changes or microbial competition.

To optimize microbial consortia and treatment conditions for in-situ or ex-situ bioremediation, bioaugmentation and biostimulation must be applied together. Elements like site characterization, microbial consortia selection, optimization of nutrient addition, and monitoring and control are also critical to consider.

Case study 1: Integration of biostimulation and bioaugmentation techniques for degradation of PHE in soil:

In order to simulate a flooded soil environment, a long-term (63-day) batch experiment was established and PHE was chosen as the PAH model pollutant in the investigation. In order to repair PHE-contaminated soil, it is necessary to determine whether the anaerobic microbial community and granular biochar nurtured by PHE can enhance the anaerobic biodegradation of PHE in soil, whether biochar can strengthen the anaerobic microbial community, and whether biostimulation and bioaugmentation can work in combination to promote the anaerobic biological removal of PHE. These findings would offer theoretical and technical support for the microbial regulation and remediation of actual PAH-contaminated soil (Xue et al., 2024). The study's findings demonstrated that PHE could be effectively removed from soil, and using biochar improved outcomes.

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Case Study 2: Remediation of hydrocarbon contaminated lake sediments using the synergistic effects of bioaugmentation and biostimulation

To improve biodegradation efficiency, a combination of bioaugmentation and biostimulation was used in a study on the remediation of hydrocarbon-contaminated lake sediments. Three sets involved in the 32-day batch incubation: unamended (K), biostimulated and bioaugmented (NB), and biostimulated (N). The abundance of proteobacteria, especially betaproteobacteria, was found to be higher in all sets of results. In the biostimulated set (N), Pseudomonas was the most prominent genus. Aeromonas, Pseudomonas, Citrobacter, and Klebsiella were among the microorganisms that were added. Hydrocarbon concentrations in the biostimulated and bioaugmented sets were considerably lower during the incubation period in comparison to the unamended set. The study emphasizes how bioaugmentation and biostimulation synergize to improve hydrocarbon biodegradation in lake sediments, highlighting the significance of acknowledging microbial interactions to develop successful bioremediation techniques.

Case Study 3: Synergistic Effects of Bioaugmentation and Biostimulation on Total Petroleum Hydrocarbon Degradation

The case study demonstrates how bioaugmentation and biostimulation techniques can be successfully combined. It shows that whereas bioaugmentation by itself decreased culture growth in suspension, biostimulation—the addition of nutrients and surfactants—significantly increased microbial activity and improved total petroleum hydrocarbon (TPH) biodegradation. The efficacy of utilizing specialized microbial consortia in combination with nutrient and surfactant inputs for pollutant removal and site cleanup was demonstrated by the combined strategy of bioaugmentation with biostimulation, which resulted in greater extent of degradation and fewer intermediates (Jasmine & Mukherji, 2014).

Challenges and future directions:

- Even with the application of the bioremediation techniques, research has frequently demonstrated relatively low success rates, with removal efficiencies averaging between 40% and 50% over the course of a normal 6-month period. In an effort to improve the efficacy of these techniques, extensive research has been conducted to

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trigger the microbial activity. As a consequence, specific approaches have been able to achieve rates of removal ranging from 55% to 80% in a shorter duration of time (Jumbo et al., 2024). There might be some challenges regarding the efficiency of treatment.

- The introduction of exogenous microbial strains through bioaugmentation may restrict the establishment and efficiency of the imported strains by competing with native bacteria for resources and habitats.
- Changes in the pH, temperature, moisture content, and organic matter content of the soil can all have an impact on microbial activity and the rate at which pollutants break down. These differences may manifest in terms of place and time.
- It might be challenging to create the appropriate environment for bioaugmentation and biostimulation in different environments.
- The efficiency of bioaugmentation and biostimulation methods can be diminished by specific contaminants, depending on their degree of recalcitrance or their capacity to hinder microbial activity. Because of factors like microbial community dynamics, contaminant persistence, and changes in environmental conditions, maintaining the efficiency of remediation over the long term can be challenging.

Continuous management and monitoring initiatives are required to ensure long-term sustainability. Regulations and approval processes may impose restrictions on the use of bioaugmentation and biostimulation techniques for soil remediation. Complying with legal standards and demonstrating efficacy might bring additional challenges. We can currently sequence metagenomes with fewer than 5 cells in a sample using methods such as multiple displacement amplification.

Opportunities for Innovation and Improvement in Optimization Among the tactics are integrated remediation procedures, biostimulation modifications, microbial engineering, and monitoring technologies. Improvements in microbial engineering techniques, such as genetic modification and synthetic biology, enable the creation of microbial strains with enhanced pollutant-degrading capacities.

By tailoring engineered microbes to specific contaminants and environmental conditions, remediation efficiency can be boosted. Innovative biostimulation additives, such nanomaterials, bio-based chemicals, and microbial stimulants, can

provide alternatives to current approaches for increasing microbial activity and promoting the breakdown of pollutants in soil.
Greater efficacy and environmental advantages might be revealed by comparing these novel additions with more traditional nutrients and electron donors. The effectiveness of pollutant removal can be increased by combining bioaugmentation and biostimulation approaches with other remediation methods such as chemical oxidation/reduction, phytoremediation, and electrokinetic remediation. It allows for the simultaneous treatment of several contaminants or site-specific difficulties. Technological developments in monitoring, such as molecular tools, isotope tracing, and remote sensing techniques, enable real-time monitoring of microbial activity, pollutant concentrations, and remediation processes within soil.

Conclusion:

For sustainable environmental remediation, optimization of in-situ and ex-situ bioremediation approaches by bioaugmentation, biostimulation, and customized solutions offers great potential. As demonstrated in several soil contamination scenarios involving heavy metals, hydrocarbons, and chlorinated solvents, biostimulation increases native microbial activity while bioaugmentation introduces exogenous microbial strains to speed up pollutant breakdown. By minimizing their negative effects on the environment, lowering expenses, and increasing cleanup effectiveness, these optimized approaches provide benefits above conventional techniques. Treatment conditions and microbial consortia performance is optimized by the synergistic effects of combining bioaugmentation and biostimulation. These factors include microbial interactions, substrate availability, ambient conditions, and microbial competency. Important issues to address include microbial competition, environmental unpredictability, pollutant complexity, long-term sustainability, and regulatory constraints. In order to promote practical applications through advanced monitoring technologies, integrated remediation tactics, and enhanced biostimulation formulations, future research should concentrate on resolving microbial dynamics, environmental uncertainties, and sustainability problems. To enhance bioremediation techniques and overcome regulatory obstacles, stakeholders, researchers, practitioners, and regulatory agencies must work together. Long-term monitoring programmes are also crucial for determining the success of remediation and controlling possible pollutant rebound. It is essential to implement public outreach and education programmes to address concerns regarding environmental dangers, liability, and regulatory compliance, and to promote greater awareness and acceptance of both in-situ and ex-situ bioremediation.

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