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ECO-REJUVINATION: ADVANCING BIOREMEDIATION APPROACHES FOR MITIGATING POLLUTION AND ENVIRONMENTAL RESTORATION

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ABSTRACT

In recent decades, there has been a noticeable increase in environmental pollution due to human activities. One promising method for cleaning up contaminated environments is bioremediation. The core principle revolves around converting harmful substances into less dangerous forms. Bioremediation can be conducted on-site or off-site, depending on factors such as cost, site characteristics, and the nature and concentration of pollutants present. The choice of the most suitable bioremediation approach depends on these factors. Additionally, various strategies like biostimulation, bioaugmentation, bioventing, bioattenuation, phytoremediation etc. are employed to enhance the process, with their effectiveness influenced by environmental conditions. Bioremediation is widely regarded as a highly effective, cost-efficient and environmentally friendly method for managing polluted environments. Conducting thorough site assessments, including analyzing the type and extent of contamination, soil properties, hydrogeological conditions and potential ecological impacts, is essential for selecting the most appropriate bioremediation strategy. Continuous monitoring of remediation progress and environmental parameters is vital to assess the efficacy of the chosen techniques and make any necessary adjustments in real-time. Overall, adopting a holistic and adaptive approach, is key to maximizing the benefits of bioremediation while minimizing pollution and enhancing environmental restoration.

Key words : Biostimulation, Bioaugmentation, Biopiles, Microorganism, Phytoremediaton.

Introduction

Over the last few decades, human activities have surged, leading to a concerning escalation in environmental pollution. Factors contributing to this alarming trend include population explosion, unsustainable agricultural practices, haphazard urbanization, rampant deforestation, accelerated industrialization, and indiscriminate exploitation of energy resources. These anthropogenic actions have culminated in the release of various pollutants into the environment, posing significant threats to both ecological balance and public health.

Many pollutants, including chemical fertilisers, heavy metals, nuclear waste, pesticides, herbicides, insecticides, greenhouse gases, and hydrocarbons are causing widespread concerns about the environment and human health. These pollutants have far-reaching effects, making problems like soil erosion, water contamination, air pollution, and biodiversity loss worse. In addition, thousands of hazardous waste sites have already been identified, and more are expected in the years to come, making the proliferation of these sites a widespread problem.

Bioremediation stands out as a promising solution for addressing emerging contaminant issues, harnessing the power of microbes to restore contaminated environments. This technique involves employing microorganisms to either break down or contain waste materials (Shanahan, 2004). Microorganisms, including aerobic and anaerobic bacteria as well as fungi, play pivotal roles in this process. This detoxification process aims to neutralize harmful chemicals through mineralization, transformation, or alteration (Shannon and Unterman, 1993). Their remarkable ability to degrade, eradicates, immobilize or detoxify various chemical and physical pollutants makes them invaluable agents in environmental cleanup efforts.

At its core, bioremediation functions by utilizing microorganisms to break down pollutants and convert them into less harmful forms. This process occurs through the collective action of diverse microbial communities, working together to neutralize environmental contaminants. Central to bioremediation's effectiveness are the biotic and abiotic factors that influence the rate of pollutant degradation. Biotic factors encompass the types and abundance of microorganisms present, while abiotic factors include environmental conditions such as temperature, pH, oxygen levels and nutrient availability. These factors collectively dictate the efficiency and success of the bioremediation process.

Concept of bioremediation

The remarkable technique known as "bioremediation" uses living things to use their metabolic processes to remove or neutralise pollutants from the environment. These microorganisms, which include bacteria, fungi and algae, help to clean up contaminated areas. Microorganisms are widespread in the Earth's biosphere and can be found in a variety of habitats, including soil, water, plants, animals, and even harsh settings like deep sea habitats and freezing ice. They are perfect for cleaning up the environment because of their abundance and capacity to metabolise a variety of chemicals.

In essence, bioremediation is a waste management method that uses living things to eliminate or lessen the number of pollutants found in contaminated areas. It is regarded as a therapeutic strategy in which hazardous materials are broken down into less toxic or non-toxic materials by naturally occurring organisms. Bioremediation technologies have been widely used and are still developing quickly over the last few decades. This method's eco-friendly features have made it dependable and successful.

It offers significant advantages over chemical and physical remediation methods, including its environmental friendliness and cost-effectiveness. The process aims to reduce, detoxify, degrade, mineralize, or transform more toxic pollutants into less harmful forms. The specific pollutants targeted for removal vary widely and may include pesticides, agrochemicals, chlorinated compounds, heavy metals, xenobiotic compounds, organic halogens, greenhouse gases, hydrocarbons, nuclear waste, dyes, plastics and sludge.

Role of microorganism in bioremediation

Microorganisms greatly contribute to biological balance and are essential to the complex web of life's food chains. These microscopic organisms—fungi, algae, yeast and bacteria—play a crucial role in bioremediation by helping to remove contaminated materials. Microbes are remarkably versatile; they can survive in conditions with high temperatures and potentially harmful substances, which emphasises their applicability to remediation procedures. The need for carbon, which is necessary for microbial growth and metabolic processes, is fundamental to their activity.

Various microbial consortia are employed in bioremediation across different environments. These microorganisms encompass a diverse range, including *Achromobacter, Arthrobacter, Alcaligenes, Bacillus, Corynebacterium, Pseudomonas, Flavobacterium, Mycobacterium, Nitrosomonas, Xanthobacter*, among others. They exhibit degradative capacities essential for breaking down complex compounds found in contaminants.

Aerobic bacteria are one of the microbial groups used in bioremediation because of their capacity to break down a wide range of materials, including pesticides, hydrocarbons, and polyaromatic compounds. *Pseudomonas, Acinetobacter, Sphingomonas, Flavobacterium, Rhodococcus* and *Mycobacterium* are a few examples. These microorganisms break down and eventually remove pollutants by using them as carbon and energy sources. The lignocellulolytic qualities of two types of mushroom fungi, *Pleurotus ostreatus* and *Trametes versicolor* have been investigated for their potential in bioremediation and decomposition of dangerous materials, like caffeine residues (Fan *et al.*, 2000). Additionally, these fungi have been explored for their ability to degrade various toxic compounds found in polluted soils or contaminated groundwater, including pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and chlorinated ethenes (Perez *et al*., 2008; Rigas *et al*., 2007).

In contrast, anaerobic bacteria, while less frequently utilized, are gaining interest for their potential in bioremediation. Particularly, they show promise in dechlorinating compounds like polychlorinated biphenyls and trichloroethylene, converting them into less toxic forms. Despite their less frequent use compared to aerobic counterparts, anaerobic bacteria hold significance in addressing specific pollutants and contributing to comprehensive remediation efforts.

Factors affecting bioremediation process

The effectiveness of bioremediation hinges on numerous factors, spanning both biotic (biological) and abiotic (environmental) realms. Biotic factors play a pivotal role in the degradation of organic compounds by microorganisms, influenced by factors such as microbial population density, accessibility of contaminants to microbial communities and interactions among microorganisms, as well as with other organisms like protozoa and bacteriophages. Enzyme activity, competition, succession, predation, mutation, horizontal gene transfer, biomass production and population size and composition are among the key biological factors that influence contaminant degradation rates.

On the other hand, abiotic factors encompass the interactions between environmental contaminants and the metabolic activity and physicochemical properties of targeted microorganisms. The success of microbial-pollutant interactions is contingent upon various environmental conditions, including temperature, pH, moisture levels, soil structure, water solubility, nutrient availability, oxygen content, redox potential, and resource availability. Additionally, factors such as pollutant concentration, chemical structure, solubility, and toxicity also influence biodegradation kinetics.

Some important points to be considered are as follows

1. **Contaminant concentrations:** The level of contaminants directly affects the activity of microbes. High concentrations can harm bacteria, while low concentrations may not trigger the production of enzymes needed for degradation.
2. **Contaminant bioavailability:** This depends on how much contaminants bind to solids or are trapped by molecules in the environment. Contaminants that are tightly bound, dispersed widely, or in Non-Aqueous Phase Liquid (NAPL) form are less accessible for microbial reactions.
3. **Site characteristics:** The properties of the site greatly influence the success of bioremediation. Factors like pH (between 6 -8), temperature, moisture, nutrient availability, and redox potential play crucial roles.
4. **Redox Potential and oxygen content:** The environment's oxidizing or reducing conditions, influenced by electron acceptors like nitrate or iron oxides, affect microbial activity.
5. **Nutrients:** Microbes require nutrients for growth and reproduction. Although trace nutrients are usually present, additional nutrients can be supplied in usable forms or through organic substrates to stimulate bioremediation.
6. **Moisture content:** Microbial growth requires sufficient moisture in the environment, typically ranging from 12% to 25%.
7. **Temperature:** Temperature directly impacts microbial metabolism and activity. Warmer temperatures generally increase biodegradation rates, while cooler temperatures slow them down.

Techniques involved in Bioremediation

Ex-situ and *in-situ* applications are the two general categories into which bioremediation techniques can be divided. Many factors, such as the type of pollution, the degree and depth of contamination, the location and environment, financial constraints and compliance with environmental laws, all influence the choice of the most suitable technique. Furthermore, bioremediation processes depend on performance indicators like temperature, pH, oxygen and nutrient levels and other abiotic variables. Pollutants are removed from contaminated sites using *ex-situ* bioremediation techniques and then transported to another location for treatment. The choice to use *ex-situ* techniques is usually impacted by a number of factors, including the type and depth of contamination, the degree of pollution, the cost of treatment and the location of the contaminated site. Performance criteria also play a crucial role in determining the suitability of *ex-situ* bioremediation methods.

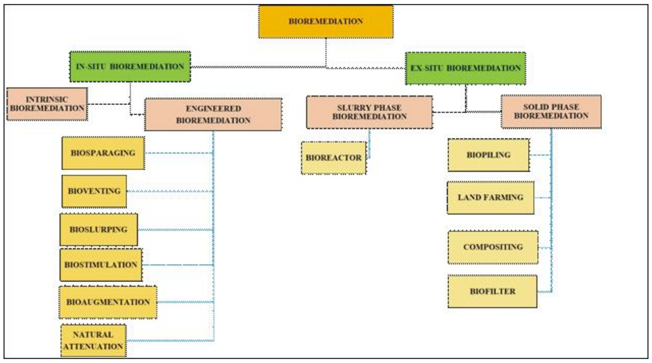

Fig. 1 : Classification of bioremediation types (Image credit: Hridesh Harsha Sarma, MSc. Agri, AAU, Jorhat).

Solid-phase treatment

As a type of *ex-situ* bioremediation known as "solid-phase treatment," contaminated soil is excavated and subsequently stacked. In addition to soil, these piles may also include organic wastes like leaves, dung from animals, leftovers from agriculture and different kinds of waste from homes, businesses and cities. A network of pipes that distribute air—essential for ventilation and microbial respiration—allows bacteria to grow within these piles.

In contrast to slurry-phase techniques, solid-phase bioremediation systems require a large amount of space to operate and the cleanup procedure is typically time-consuming. Among the common solid-phase treatment methods are land farming, composting, windrows and biopiles (Kulshreshtha *et al.*, 2014). Every one of these approaches has particular benefits and difficulties when it comes to cleaning up contaminated areas.

Slurry-phase bioremediation

In comparison to other techniques, slurry-phase bioremediation provides a relatively quicker treatment approach. This method creates the ideal conditions for microorganisms to break down the contaminants in the soil by combining contaminated soil with water, nutrients, and oxygen inside a bioreactor. This procedure separates the contaminated soil from the stones and debris. The physicochemical characteristics of the soil, the rate of biodegradation and the concentration of pollutants all influence the amount of waterto be added.

Once the bioremediation process is complete, the soil is removed and dried using various techniques such as vacuum filters, pressure filters and centrifuges. Subsequently, the treated soil is disposed of, while the resulting fluids undergo further treatment. This method allows for efficient and rapid remediation of contaminated soil, minimizing environmental impact.

Types of bioremediations

Bioremediation technologies can be broadly classified as *ex situ* or *in situ* (Hatzinger *et al.*, 2002; Talley and Sleeper, 2006). There are far more than nine types of bioremediations, but the following are the most common ways in which it is used.

Ex-situ bioremediation techniques

1. **Biopile:** Aeration and nutrient supplementation are used to increase microbial metabolic activity, and the contaminated soil that has been excavated is stacked above ground. This technique includes leachate collection, treatment beds, irrigation, nutrient application and aeration systems. Because of its affordability and capacity to sustain ideal biodegradation conditions, including pH, nutrient levels, temperature and aeration, it is becoming more and more well-liked. Biopiles can even be used in very cold environments and are especially useful for treating volatile, low molecular weight pollutants. Heating systems can be incorporated into such designs to increase microbial activity and contaminant availability, which will speed up the remediation process by accelerating the rate of biodegradation. Furthermore, the simultaneous delivery of heat and air can be facilitated by the introduction of heated air, which will enhance bioremediation.
2. **Windrows:** To increase microbial degradation activity, windrow bioremediation entails turning piled contaminated soil on a regular basis. This procedure speeds up the rate of bioremediation by increasing aeration and promoting uniform distribution of water, nutrients, pollutants and microbial activity. While windrow treatment has demonstrated greater hydrocarbon removal rates than biopile treatment, it might not be the optimal option for volatile compound-contaminated soils because of the possibility of anaerobic zone formation, which could result in the release of greenhouse gases like methane (CH_4).
3. **Land farming :** Land farming is a low-tech, low-cost bioremediation method that needs little equipment. Depending on the extent and location of contamination, it is often used in both *ex-situ* and *in-situ* remediation scenarios. Excavated and tilled, polluted soils are then thinly applied over a prepared area to allow native microorganisms to engage in aerobic biodegradation. Treating large amounts of contaminated soil with little energy and environmental impact is made possible by land farming.
4. **Bioreactor:** A bioreactor is a device that uses biological reactions to transform raw materials into targeted products. For bioremediation, a variety of operating modes—such as batch, fed-batch, sequencing batch, continuous, and multistage—allow for the best possible growth conditions. In order to achieve effective bioremediation processes, bioreactors provide precise control over parameters like pH, temperature, agitation, aeration, substrate and inoculum concentrations. Because of its flexibility, non-biological losses are kept to a minimum while biological degradation is maximised.

In-situ bioremediation techniques

By treating contaminated materials right at the

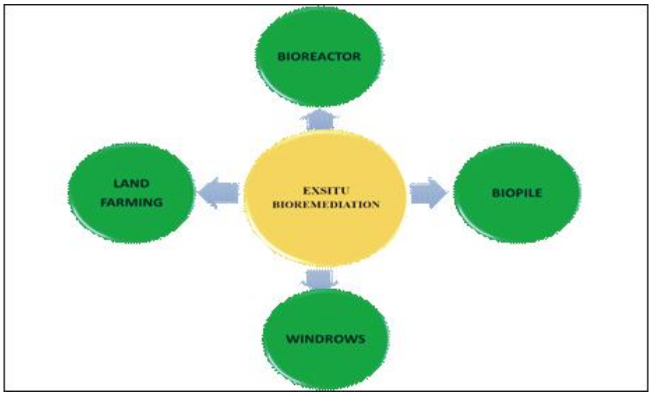

Fig. 2 : *Ex situ* bioremediation techniques (Image credit: Hridesh Harsha Sarma, MSc. Agri, AAU, Jorhat).

pollution site, these techniques reduce soil disturbance and do away with the need for excavation. These methods should ideally be less expensive than ex-situ bioremediation strategies. While some in-situ bioremediation techniques, like intrinsic bioremediation or natural attenuation, may advance without the need for extra improvements, others, like bioventing, biosparging, and phytoremediation can be further improved to increase their efficacy. The efficacy of *in-situ* bioremediation techniques in treating sites contaminated with hydrocarbons, heavy metals, dyes and chlorinated solvents has been demonstrated.

Types of *in-situ* bioremediation

1. **Intrinsic bioremediation :** Natural attenuation, another name for intrinsic bioremediation, is an in-situ method of cleaning up contaminated areas without the need for outside assistance. This technique depends on the natural microbial populations that are native to the contaminated area being stimulated. Intrinsic bioremediation is a technique that uses both anaerobic and aerobic microbial processes to promote the biodegradation of pollutants, including difficult-to-degrade substances. Compared to other in-situ techniques, this one is more affordable because it doesn't require outside assistance.
2. **Engineered In-situ bioremediation:** Another approach involves the introduction of specific microorganisms to the contaminated site. Engineered microorganisms are utilized to enhance the degradation process by optimizing physicochemical conditions conducive to microbial growth.
3. **Bioventing:** Bioventing techniques entail the controlled delivery of airflow to the unsaturated (vadose) zone, promoting the activity of indigenous microbes for bioremediation. Nutrients and moisture may be added to enhance microbial transformation of pollutants, ultimately rendering them harmless. Bioventing has gained popularity as a cost-effective *in-situ* bioremediation method.
4. **Bioslurping:** Bioslurping combines vacuum-enhanced pumping, soil vapor extraction, and bioventing to remediate soil and groundwater contamination (Gidarakos and Aivalioti, 2007). By indirectly providing oxygen and stimulating contaminant biodegradation, this technique is effective for treating soils contaminated with volatile and semi-volatile organic compounds.
5. **Biosparging:** Similar to bioventing, biosparging involves injecting air into the subsurface soil to enhance microbial activity and facilitate pollutant removal. Biosparging is particularly useful for treating aquifers contaminated with diesel and kerosene.
6. **Phytoremediation :** Phytoremediation harnesses plant interactions to detoxify contaminated soils through physical, chemical, biological, microbiological, and biochemical processes. Various mechanisms, including extraction, degradation, filtration, accumulation, stabilization and volatilization, are employed to mitigate pollutant toxicity. Phytoremediation is effective for removing pollutants such as heavy metals and organic compounds.

Arsenic hyperaccumulators : Arsenic hyper accumulators accumulate arsenic to levels exceeding 2,000 mg/kg in plant tissues (Bondada and Ma, 2003).

Moreover, an effective species for arsenic phytoextraction should preferentially accumulate arsenic in its shoots rather than its roots. This facilitates easier harvesting or removal of the arsenic-laden above-ground biomass. Typically, these plants exhibit very high concentrations of contaminants when grown in polluted environments. To assess the levels of arsenic bioconcentration and distribution in plants, bioconcentration factor (BF) and transfer factor (TF) can be utilized. The BF represents the ratio of arsenic concentration in plants to that in the soil, while the TF indicates the ratio of arsenic concentration in roots to that in shoots.

Greenhouse studies have shown that *Pteris vittata* accumulated arsenic concentrations in above-ground plant tissues more than 200 times higher than the majority of other plant species tested using arsenic-contaminated soil

(Salido *et al*., 2003).

One notable distinction between *Pteris vittata* and arsenic non-accumulating species is the significant transport of arsenic from roots to shoots in *P. vittata*, resulting in the accumulation of up to 95% of arsenic in above-ground tissues (Doucleff and Terry, 2002).

Phytostabilization : To reduce the movement of metals in the subsoil, metal-tolerant plants are used in the process of phytostabilization of inorganic substances. Because of physical disturbance or the harmful effects of contamination, toxic soils are usually devoid of vegetation. Metal-contaminated exposed soils are frequently more prone to mobility due to processes like leaching and wind- and water-borne transportation. Plants that are tolerant of metals can be useful in reducing the mobility of metals brought on by these processes. This strategy has been successfully used by a Liverpool team to stabilise metalliferous mine wastes (Cunningham and Berti, 1993). Salt *et al.* (1995) demonstrated with success that *B. juncea* seedlings could reduce the amount of lead that leached into groundwater from contaminated soils.

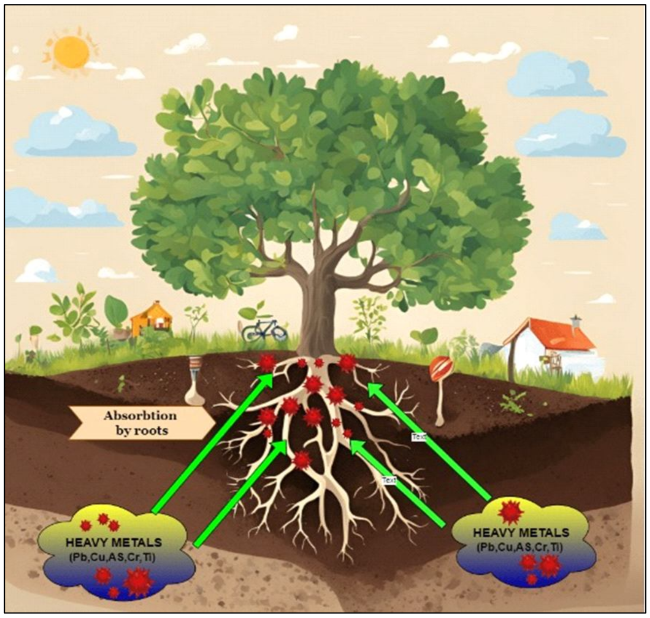

Fig. 3 : Phytoremediation (Image credit: Hridesh Harsha Sarma, MSc. Agri, AAU, Jorhat).

At NEERI, Nagpur, extensive research using IBA has been done on the phyto-stabilization of coal mine dumps, manganese mine dumps, fly ash dumps and metalliferous mine wastes (Juwarkar *et al*., 2000). Legumes have a great ability to stabilise soils that have been weakened by metals and to replenish vegetation. This is explained by a number of factors: first, they collect nitrogen in a form that is easily mineralized through symbiosis with rhizobia, which benefits non-leguminous plants; second, they do well in low-nutrient environments; and third, they can live in arid environments that are vulnerable to flooding and strong winds (Jha *et al*., 1995).

- **Phytodegradation :** The process by which organic materials taken up by a plant are broken down into smaller molecules and incorporated into the plant's tissues is known as phytodegradation. Enzymes found in plants have the ability to degrade and change various herbicides, ammunition wastes and chlorinated solvents (such as trichloroethylene). Usually, these enzymes are reductases, oxygenases, and dehalogenases (Black, 1995).
- **Phytovolatilization :** By using plants and plant-associated soil microbes, phytovolatilization removes pollutants from the soil, transforms them into volatile forms, and releases them into the atmosphere (Lin, 2008). When trees and other plants take in water and other organic and inorganic pollutants from their environment, a process known as phytovolatilization takes place. Through methylation processes, metalloids such as selenium, arsenic, and tin can be transformed into volatile compounds or mercury, which can subsequently undergo biological transformation to become elemental mercury. The primary applications of phytovolatilization have been in the elimination of selenium and mercury.
- **Rhizofiltration :** Root accumulation mechanisms encompass: (1) surface sorption, where physical or chemical processes like chelation and ion exchange facilitate sorption onto the root surface; and (2) biological processes, such as intracellular uptake, vacuolar deposition, and subsequent translocation to the shoots (Chaney, 1983) and (3) root remediated precipitation which mainly involves the release of root exudates (Dushenkov *et al*., 1995).

The capacity of *Brassica juncea*, a high biomass crop plant, to accumulate lead (Pb) in its roots was compared to 24 other plant species including *Helianthus annuus* (sunflower) and various grasses such as colonial *Bentgrass* and *Poa pratensis* (Kentucky bluegrass). While all tested species exhibited significant root accumulation of Pb, *Brassica juncea* (with 14% of dry weight Pb in the roots) displayed the most favorable combination of metal accumulation ability and high biomass production. Additionally, *Brassica juncea* was found to accumulate substantial amounts of copper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni) and zinc (Zn).

Considering that the extent of metals accumulated via surface sorption correlates with root mass, the rapid and cost-effective generation of a large root mass by *Brassica juncea* makes it a promising candidate for rhizofiltration (Dushenkov *et al*., 1995)

1. **Permeable Reactive Barrier (PRB) :** PRB serves as a physical method for remediating contaminated groundwater, utilizing biological mechanisms such as precipitation, degradation, and sorption for pollutant removal. This *in-situ* technique is commonly used to remediate heavy metals and chlorinated compounds in groundwater pollution.
2. **Air-sparging :** By introducing oxygen into the subsurface, air-sparging improves the aerobic biodegradation of contaminated groundwater (Johnson *et al*., 2007). By pumping air beneath the water table, this is accomplished. Air sparging is a novel technique for cleaning up contaminated aquifers. It was primarily created to treat groundwater contamination brought on by fuels, non-halogenated volatile organic compounds (VOCs), semi-volatile organic compounds (SVOCs), pesticides, organics and herbicides. It exhibits potential for replenishing aquifers contaminated by petroleum hydrocarbons and other volatile and/or biodegradable pollutants (Gidarakos and Aivalioti, 2008; Heron *et al*., 2002).

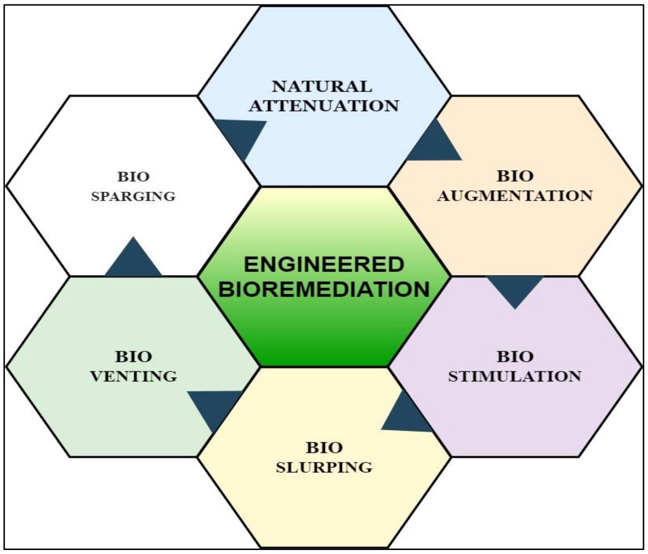

Fig. 4 : Engineered bioremediation techniques (Image credit: Hridesh Harsha Sarma, MSc. Agri, AAU, Jorhat).

Guidelines for executing bioremediation at a polluted site

1. **Site characterization :** This is a crucial first step as it provides detailed information about the nature and extent of contamination at the site. It involves collecting soil, water, or sediment samples from various locations and depths within the contaminated area. These samples are then analyzed to determine:

- Types of contaminants present (e.g., petroleum hydrocarbons, heavy metals, pesticides, etc.) and their concentrations.
- Physical and chemical properties of the site (pH, moisture content, nutrient availability, etc.).
- Microbial population density and diversity, including the presence of contaminant-degrading microorganisms.

Advanced molecular techniques like DNA/RNA extraction and analysis can identify specific microbial species and biodegradation pathways present. This information guides the selection of appropriate bioremediation strategies.

2. **Biostimulation :** If the site characterization reveals the presence of contaminant-degrading microbes, their activity can be stimulated by optimizing environmental conditions. Biostimulation involves introducing the following:

- Oxygen: Bioventing (injecting air) or biosparging (injecting air/oxygen under pressure) can increase oxygen levels for aerobic biodegradation.
- Nutrients: Fertilizers containing nitrogen, phosphorus, and other essential nutrients can be added to support microbial growth and metabolism.
- Moisture: Water may be added to maintain optimal moisture levels for microbial activity.
- Other amendments: Surfactants, bulking agents, or other additives can increase contaminant bioavailability or improve soil conditions.

3. **Bioaugmentation :** In cases where the indigenous microbial population lacks the necessary biodegradation capabilities, specialized microorganisms can be introduced. This process involves:

- Isolating and culturing microbes (bacteria, fungi, or consortia) known to degrade the specific contaminants.
- Introducing these microbes into the contaminated site through inoculation or injection.
- Providing suitable conditions (nutrients, oxygen, etc.) to support the growth and activity of the

introduced microbes.

Bioaugmentation may be necessary for sites contaminated with recalcitrant or complex contaminants that require specific metabolic pathways not present in the native microbial community.

4. **Optimization and monitoring :** Once bioremediation is underway, regular monitoring and optimization are essential. This involves:

- Periodic sampling and analysis of contaminant concentrations to track degradation progress.
- Monitoring microbial population dynamics, nutrient levels, and other environmental parameters.
- Adjusting conditions as needed by introducing additional nutrients, oxygen, or microbial cultures.
- Implementing physical interventions like tilling or mixing to improve contaminant bioavailability or aeration.

Continuous monitoring and optimization ensure that the bioremediation process proceeds efficiently and effectively.

5. **Post-treatment assessment :** After the bioremediation process is completed, a final assessment is conducted to verify that the site meets the desired clean-up goals and regulatory standards. This involves:

- Collecting and analyzing soil, water, or sediment samples from various locations within the treated area.
- Comparing the contaminant concentrations to the established remediation targets or regulatory limits.
- Evaluating the overall success of the bioremediation process and identifying any remaining areas of concern.

If the post-treatment assessment indicates that the clean-up goals have been met, the site can be declared remediated and potentially suitable for future use or development.

It's important to note that bioremediation techniques may be used in combination with other physical or chemical treatment methods for more complex or challenging contamination scenarios. Additionally, site-specific factors, such as contaminant types, soil characteristics, and regulatory requirements, may influence the specific bioremediation approach and strategies employed.

Advantages of bioremediation strategies

1. **Natural Process:** Bioremediation harnesses natural microbial processes to degrade contaminants, making it a sustainable and environmentally friendly approach.
2. **Time Efficiency:** Bioremediation typically requires less time compared to conventional methods, offering a relatively quick solution for treating contaminated materials such as soil.
3. **Excavation-Free:** *In-situ* bioremediation eliminates the need for excavation of contaminated soil, reducing site disruption and minimizing environmental impact.
4. **Volumetric Treatment:** This method provides comprehensive treatment by targeting both dissolved and solid contaminants throughout the entire volume of the affected area.
5. **Expedited Treatment:** Accelerated *in-situ* bioremediation processes often require less time to treat subsurface pollution compared to conventional pump-and-treat methods.
6. **Reduction of Contaminants :** Microbes involved in bioremediation can effectively degrade contaminants, reducing their concentrations and minimizing environmental risks.
7. **On-Site Treatment**: Bioremediation can often be conducted on-site without the need for transporting contaminated materials elsewhere, reducing costs and logistical challenges.
8. **Minimal Disruption:** The process of bioremediation generally does not disturb normal microbial activities, allowing for continued ecosystem functioning and minimizing disruption to the environment.
9. **Cost Effectiveness:** Bioremediation is often a cost-effective solution compared to other conventional cleanup methods, especially for treating large-scale contamination such as oil spills.
10. **Transformation of Harmful Chemicals:** Bioremediation transforms harmful chemicals into water and harmless gases, effectively destroying them and reducing environmental hazards.
11. **Non-chemical Approach:** Unlike some other remediation methods, bioremediation does not rely on the use of dangerous chemicals, contributing to safer and more sustainable cleanup efforts.

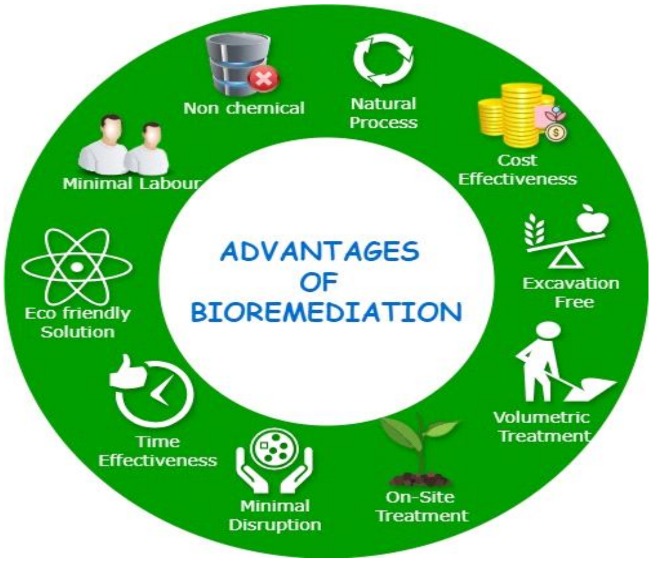

Fig. 5 : Advantages of bioremediation (Image credit: Hridesh Harsha Sarma, MSc. Agri, AAU, Jorhat).

12. **Minimal Labour Intensity:** Bioremediation processes are relatively simple and require less labour compared to some other remediation techniques, reducing overall costs and resource requirements.
13. **Eco-Friendly Solution:** Bioremediation offers an eco-friendly and sustainable approach to remediate contaminated environments, aligning with broader goals of environmental conservation and sustainability.
14. **Enhanced Biodegradation:** Biodegradation processes can be more effective in a controlled bioreactor system compared to *in-situ* methods or solid-phase treatments. The controlled environment allows for better management and predictability, resulting in higher rates of contaminant removal.

Limitations

1. **Biodegradability Constraints:** Bioremediation is only effective for compounds that are biodegradable, limiting its applicability to certain types of contaminants. Additionally, some biodegradation products may pose persistence or toxicity issues in the environment, posing challenges for complete remediation.
2. **Specificity Requirements:** Biological processes in bioremediation are highly specific, requiring the presence of suitable microbial populations, optimal environmental conditions and adequate levels of nutrients and contaminants for successful remediation.
3. **Scale-up Challenges :** Scaling up bioremediation processes from laboratory or pilot-scale studies to large-scale field operations can be challenging, hindering its widespread application in real-world scenarios.
4. **Technological Advancements Needed :** Further research is needed to develop advanced bioremediation technologies capable of addressing complex contaminant mixtures found in various environmental matrices, including solids, liquids and gases.
5. **Time-Intensive Process:** Bioremediation typically requires more time to achieve remediation compared to other treatment options such as excavation and removal of contaminated soil. This time factor can prolong cleanup efforts and delay site restoration.
6. **Regulatory Uncertainty:** The lack of standardized criteria for determining the completion of bioremediation treatments contributes to regulatory uncertainty. Without clear endpoints for evaluation, assessing the effectiveness of bioremediation and ensuring regulatory compliance can be challenging.

Novel trendsin Bioremediation

1. Genetic engineering approaches

- Deng *et al.* (2005) developed a genetically modified *E. coli* SE5000 strain that expressed both a nickel transport system and metallothionein. This modification enabled the bacteria to accumulate nickel ions from water. In contrast to the original *E. coli* cells, which could bind 1.62 mg/g of Ni^{2+}, the engineered *E. coli* showed a significant improvement, binding 7.14 mg/g of Ni^{2+}. Furthermore, it demonstrated effective nickel accumulation across a wide pH range (4–10), with the most optimal pH being 8.6
- Special focus is directed towards genetically modifying bacteria with bacterial hemoglobin (VHb) to address aromatic organic compound treatment in low oxygen conditions (Urgun-Demirtas *et al*., 2006). The utilization of VHb technology has the potential to enhance the remediation of polluted sites where oxygen scarcity hampers the growth of aerobic bioremediating bacteria and the activity of oxygenases essential for the breakdown of various organic pollutants (Urgun-Demirtas *et al*., 2006).

- In an effort to improve the effectiveness of plants in phytoremediation of mercury pollution, Nagata *et al.* (2009) genetically modified tobacco plants to express both a mercury transporter (*MerT*) and a mercury chelator (Kiyono and Pan-Hou, 2006). They achieved this by integrating the bacterial *merT* gene into the polyphosphate kinase gene (*ppk*) in transgenic tobacco plants. The aim was to assess the plant's capacity to remove mercury from the environment. The study found that while the integration of the *merT* gene did not significantly alter the tobacco plants' resistance to mercury or their production of polyphosphate, the transgenic expression of *MerT* in these plants led to a faster and more efficient uptake of mercury

2. Nanotechnology applications

- Mace´ *et al.* (2006) investigated the effectiveness of nano-particle hydroxyapatite in remedying soil heavy metal contamination using the Toxicity Characteristic Leaching Procedure in a cultivation experiment. Their findings showed that nano-particle hydroxyapatite notably decreased the availability of soil copper (Cu) and zinc (Zn) compared to the control condition.
- Elliott *et al.* (2008) also showcased the promise of zerovalent iron nanoparticles in treating and remediating persistent organic pollutants (POPs).
- Varanasi *et al.* (2007) employed nanoparticles in their investigation to address soil contaminated with polychlorinated biphenyls (PCBs). Their findings indicated that nanoparticles facilitated the dechlorination process, leading to high efficiency in PCB destruction, with a minimum total PCB destruction efficiency recorded at 95%.

3. Fungal and bacterial degradation

- Yang *et al.* (2010) documented that they genetically modified *Stenotrophomonas* sp. strain *YC-1*, an indigenous soil bacterium producing methyl parathion hydrolase (MPH), to expand its substrate range to include organophosphates (OPs). Findings suggest that this engineered strain's wider substrate specificity, coupled with its rapid degradation capability, positions it as a viable option for the *in-situ* remediation of sites contaminated with Ops.
- Sood *et al.* (2010) found that the robustness of the *C. digboiensis* strain enabled it to effectively degrade acidic oily sludge on-site, likely developed through extended exposure to the contaminants. Therefore, they demonstrated the capability of *Candida digboiensis* TERI ASN6 to bioremediate hydrocarbons at low pH conditions.

4. Electro-remediation

- Electro-remediation involves applying direct current between electrodes in the soil in a controlled manner. This system consists of three sections: two electrode compartments and a central soil compartment positioned between the electrodes. In the process of treating soil contaminated with mercury (Hg), ions are transported from the soil to the electrodes via an ion exchange membrane (Pazos et al., 2010).
- In their investigation of electro-dialytic soil remediation, Hansen *et al.* (1997) found that adding oxidising agents and chloride to the soil promoted the mobilisation of mercury (Hg) and increased the rate at which Hg was removed from the soil. According to their research, adding chelating agents to the soil increased the solubility of mercury, which increased the electro-remediation process's efficacy.

5. Thermal treatment

- Thermal treatment involves applying high temperatures (320–700°C) to soil in order to remove mercury through the process of volatilization.
- High temperature and low pressure are used to volatilize mercury (Hg). Condensation then follows the volatilization process, turning Hg vapour into liquid Hg^0. The removal of mercury from the soil matrix during the thermal treatment process has an efficiency of 41.99% (Xu *et al.*, 2015). This treatment has the potential to can remove high concentrations of Mercury (up to 34,000 mg/kg) from the soil.

Success story of Bioremediation

In the ongoing battle against lake pollution, government initiatives in collaboration with NGOs have implemented bioremediation techniques, yielding promising results across various water bodies in the city. Lakes such as Chinna Cheruvu, Yerrakunta and Novotel Lake have witnessed substantial improvements in key water quality parameters like Biological Oxygen Demand (BOD) and Dissolved Oxygen (DO) levels. For example, Dhruvansh NGO's phytoremediation efforts at Chinna Cheruvu in 2016 led to a significant reduction in BOD levels from 68 mg/l to 8-10 mg/l. Similarly, Novotel Lake saw an impressive 80% improvement in water quality, despite facing BOD levels over 100 ppm in 2021. These remediation efforts not only enhanced water quality but also rejuvenated marine life, with thriving turtle populations in Chinna Cheruvu. Experts emphasize the scalability of bioremediation, suggesting its potential application in larger lakes like Hussain Sagar for addressing organic pollutants. As bioremediation continues to prove its effectiveness in revitalizing polluted water bodies, it emerges as a sustainable solution for urban environmental restoration and water management.

Conclusion

A pivotal initial step towards successful bioremediation lies in thorough site characterization, which facilitates the identification of the most suitable and promising remediation approach, whether *ex-situ* or *in-situ*. While ex-situ techniques may entail higher costs due to excavation and transportation of contaminated materials, they offer the advantage of treating a broader spectrum of pollutants. Conversely, *in-situ* techniques eliminate the need for excavation, but may incur expenses related to on-site equipment installation and effective control of subsurface conditions. The geological attributes of polluted sites, including soil composition, pollutant characteristics and depth, alongside considerations of human habitation and site-specific performance of each bioremediation technique, should inform the selection of the most appropriate and effective remediation strategy. By integrating these factors, bioremediation endeavors can be optimized to achieve successful treatment and restoration of polluted sites, thereby contributing to environmental sustainability and protection of human health.

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