



Review Article

From contamination to cleanup: The role of microorganisms in modern bioremediation practices

Vijayalaxmi Rajiv Shinde^{1*}, Neha Nitin Pai², Pranav Suresh Tambe^{2,3}, Asif Sandu Shaikh⁴, Siraj Babulal Shaikh⁵

¹Dept. of Environmental Science, M.C.E. Society's Abeda Inamdar Senior College of Arts, Science and Commerce, Pune, Maharashtra, India

²Dept. of Biotechnology, M.C.E. Society's Abeda Inamdar Senior College of Arts, Science and Commerce, Pune, Maharashtra, India

³Advanced Scientific Research Laboratory, Dept. of Chemistry, Abeda Inamdar Senior College, of Arts, Science & Commerce, Pune, Maharashtra, India

⁴Dept. of Botany, M.C.E. Society's Abeda Inamdar Senior College of Arts, Science and Commerce, Pune, Maharashtra, India

⁵Dept. of Chemistry, M.C.E. Society's Abeda Inamdar Senior College of Arts, Science and Commerce, Pune, Maharashtra, India

Abstract

Environmental pollution is increasing cause of concern. Introduction of petroleum products and toxic materials into the environment causes disruption of ecosystems and pose serious problem to biodiversity. Hydrocarbons are the chemical compounds found in petroleum-based products, can contaminate soil and water leading to toxic effects on both terrestrial and aquatic habitat. Bioremediation process provides an effective approach to eradicate these components and an alternative effective method for cleaning up toxic waste from polluted areas. This process involves uses various microorganisms of different genera like *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Acinetobacter* either single or consortia to treat these pollutants. Microorganisms play a crucial role by eliminating, these pollutants by degrading them or by utilizing them as nutrient source by different enzymatic pathways. The primary objective of bioremediation is to degrade pollutants and convert them into less harmful forms. Considering on various parameters either ex situ or in situ bioremediation methods are chosen. This review highlights the latest advancements in bioremediation process, by which microorganisms can be used to break down different pollutants, and the future prospects for bioremediation in reducing pollution levels to attain environmental sustainability.

Keywords: Bioremediation, Biodiversity, Consortia, Enzymatic pathways, Environmental sustainability.

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1. Introduction

The process of industrialization has contributed to environmental pollution, which poses risks to aquatic and soil microflora. Human activities have further contributed to these issues by releasing pollutants into the environment, where they persist for prolonged periods of time.¹ These pollutants are harmful to both humans and ecosystems, causing environmental changes and genetic mutations in humans which lead to various disorders.² The accumulation of these pollutants also reduces crop quality and affect different cycles.

To address these challenges, contaminated soils are being treated with various remediation approaches.³ Among these, bioremediation offers a promising alternative for removing environmental contaminants.⁴ Soil is home to a diverse array of microorganisms that play a vital role in promoting plant growth, conserving soil, recycling nutrients, and reducing organic and inorganic pollutants.⁵ Advances in bioremediation have improved its efficiency, cost-effectiveness, and social acceptance.⁶

Research has largely focused on bacterial processes, which play a significant role in remediation by detoxifying pollutants under different physicochemical conditions.^{7,8} Recent studies suggest that combining living organisms

*Corresponding author: Vijayalaxmi Rajiv Shinde
Email: shindevijayalaxmi@azamcampus.org

enhances bioremediation efficiency and microbial diversity in these affected areas.^{8,9} Various researchers have successfully utilized bioremediation to remove organic and inorganic pollutants.¹⁰⁻¹² For instance, *Aspergillus sydowii* has been used to treat pesticide contamination,^{13,14} while *Cymbella* sp. demonstrated 97.1% efficiency in detoxifying polluted water, thereby protecting aquatic flora and fauna.¹⁵

Bioremediation involves the degradation and detoxification of harmful substances under controlled conditions using by single organisms or consortia. This process reduces the environmental impact of pollutants. Enzymes play a critical role in each stage of this metabolic process, aiding degradation.^{24,43} These enzymes belong to various classes like hydrolases, dehydrogenases, proteases, and lipases.^{38,43}

Bioremediation can occur naturally or be facilitated by adding living organisms, transforming harmful organic pollutants into non-toxic or naturally occurring inorganic compounds.⁴⁴ Strategies for bioremediation involve using microbial enzymes to break down hydrocarbons into less harmful substances. Current research is focuses on genetically modified microorganisms capable of degrading pollutants more effectively.¹⁶

Environmental factors also significantly influence bioremediation outcomes. Emerging pollutants, especially organic compounds, predominantly enter ecosystems through human activities.¹⁷ This review explores recent advancements and strategies in bioremediation, highlighting its role in achieving environmental sustainability.

2. Microorganisms used in Bioremediation

Various groups of microorganisms are involved in the process of bioremediation, these includes bacteria from different habitats to clean up contaminated environments.¹⁸ Microorganisms have potential to survive at different temperatures, and biological systems which in turn makes them suitable for remediation purpose and environmental cleanup.¹⁹ They have unique enzymes to utilize carbon as their primary nutrient source.²⁰

Aerobic bacteria have been shown to break down organic compounds, by utilizing them as carbon and energy sources.^{20,21}

In contrast, anaerobic bacteria which requires less oxygen levels for their optimum growth, convert pollutants into less toxic forms.²² Bacteria belonging to different species have been applied in anaerobic bioremediation processes. Similarly, dyes can be anaerobically decomposed through reduction reactions involving electrons from the oxidation of organic substrates.^{23,24}

3. Factors Affecting Microbial Bioremediation

Microbes which are used in the cleanup process use various enzymatic metabolic pathways to accelerate enzymatic reactions to break down organic pollutants.^{25,26} Microorganisms to effectively degrade pollutants, their interaction with these compounds for a period of time is quite important. Different factors which are influencing bioremediation process, include chemical, and biological conditions, soil type, sources of carbon and nitrogen, and the type of microorganisms involved.²⁷ Microbial consortia are found to be more effective than single microorganism due to their collaborative ability to utilize all available substrates thereby enhancing effectiveness of the process.²⁸

Research indicates that carbon acts as crucial nutrient source for in situ bioremediation, as it boosts the metabolic activity of microbial communities and enhances the breakdown of pollutants. In anaerobic environments, organic carbon ferments to produce hydrogen gas.²⁹ Various studies signify that soil type significantly impacts bioremediation effectiveness.³⁰

4. Critical Factors Playing Crucial Role for Microbial Bioremediation

4.1. Biological factors

Soil microorganisms and consortia together compete for different carbon sources, which enhances the degradation of organic compounds present in ecosystem as pollutants. Degradation rates are affected by concentration of contaminants. Enzyme activities possessed by microorganisms can either initiate or suppress contaminant degradation. Enzymes involved in contaminant degradation must have affinity to bind and degrade the contaminant.^{31,32}

4.2. Oxygen

Availability of oxygen enhances degradation rates as compared to those conditions working in anaerobic conditions. Aerobic decomposition occurs because most organisms need oxygen to survive. Higher percentage of oxygen can increase hydrocarbon metabolism thereby degrading pollutants occurring in soil or environmental surroundings.³³

4.3. Moisture content

It determines the amount of water content required by organism to grow. Microorganisms need an adequate amount of water to grow and be metabolically active. Excess wet soil conditions can alter the activity of biodegradation agents to some or larger extent.³⁴

4.4. Nutrients

Nutrient requirement play a very crucial role in microbial growth and multiplication, as well as in biodegradation rate and efficiency. Optimization of bacterial carbon-to-nitrogen-to-phosphorus (C: N) ratio can enhance biodegradation

effectiveness, particularly when essential nutrients like nitrogen and phosphorus are available. Adding nutrients in cold environments can significantly enhance microbial metabolic activity and increase the rates of biodegradation.^{35,36}

4.5. Temperature

Temperature tolerance is another physical factor that influences the survival of microorganisms. As temperature influences certain enzyme production which are involved degradation process, with optimal temperatures supports fast and more efficient breakdown of pollutants.^{37,38}

4.6. pH

The pH of contaminants plays a role in microbial metabolism and the degradation process. It can either enhance or inhibit microbial activity depending on the specific pH conditions and the tolerance levels of the microorganisms involved.³⁹

4.7. Site characterization and selection

Site selection involves identifying the contaminated area for remediation and employing suitable sampling and analysis techniques. This step becomes crucial to decide whether the remediation process is effective or not.⁴⁰

4.8. Metal Ions

The toxicity of heavy metals to microorganisms is influenced by their bioavailability and the absorbed dose. These metals can disrupt essential cellular processes through mechanisms such as enzyme inhibition, generation of reactive oxygen species (ROS), ion imbalance, and damage to DNA and proteins. For instance, chromium (Cr) and cadmium (Cd) cause oxidative damage and denature microorganisms, reducing their potential for bioremediation. Copper (Cu) accelerates ROS production, while aluminum (Al) stabilizes superoxide radicals, leading to DNA damage. Heavy metals also interfere with enzymatic functions and ion channels, affecting microbial growth, morphology, metabolism, and membrane integrity. Lead (Pb) and cadmium (Cd) further contribute to DNA and membrane damage by displacing essential metal ions from their binding sites.^{41,42,100}

5. Types of Bioremediations In-situ and Ex-situ Bioremediation

5.1. Ex-situ bioremediation

Ex situ methods in bioremediation include Bio pile, Windrows, Land farming and Bioreactor (**Figure 1**).

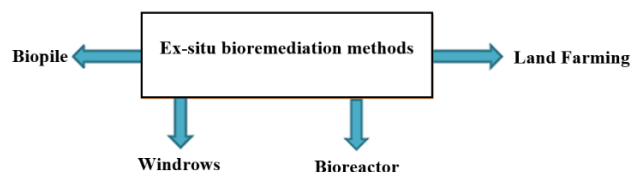


Figure 1: Ex-situ bioremediation methods

5.2. Biopile

Biopile bioremediation involves increasing microbial metabolic activities by providing appropriate percentage of oxygen and supplementation nutrients in polluted soil. It is mainly used for ex situ biodegradation. This method is particularly useful for treating volatile pollutants.^{15,45} Techniques such as land farming, biosparging, and bioventing can be used to restore air in the piled soil. Extreme air temperatures can impede bioremediation by drying the soil and increasing vaporization rather than degradation by microorganisms.^{46,47} These systems have effectively used mesophilic conditions (30°C–40°C) and low aeration rates to remove hydrocarbons.^{48,49}

5.3. Windrows

This method enhances the rate of biodegradation of hydrocarbon plastics in contaminated soils by regularly turning the heaped soils.⁵⁰ It enhances the rate of remediation process,^{15,51} windrow remediation is less effective for soils contaminated with harmful volatile chemicals.^{52,53}

5.4. Land Farming

Land farming requires minimal need for specialized equipment during the process of remediation.⁵⁴ It is more commonly used in ex situ bioremediation but can also be applied in situ under special conditions. The process involves regularly removing polluted soils. On-site treatments are classified as in situ, while ex situ methods involve treating the extracted contaminated soil.⁵⁵ Contaminated soils are typically placed on a permanent substrate layer above the surface to allow microorganisms to aerobically degrade contaminants by utilizing them as their nutrient source.⁵⁶ Land farming bioremediation is a simple and has an ecological impact, and uses very little energy without disturbing much the ecosystem.⁵⁷

5.5. Bioreactor

Bioreactors contribute in bioremediation by creating optimal conditions for growth of the microorganisms. Efficient bioreactors regulate parameters which includes pH, agitation, temperature, aeration, substrate concentration, and inoculum concentration, which in turn reduce the time required for bioremediation process.^{58,59}

5.6. In situ bioremediation techniques

In situ methods in bioremediation include bioventing, phytoremediation, and biosparging (**Figure 2**). In situ approaches have successfully treated areas contaminated

with organic and inorganic pollutants.^{60,61} These methods are categorized into intrinsic and engineered types.

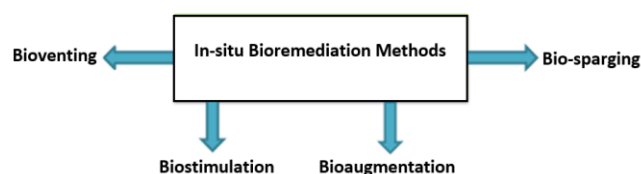


Figure 2: In situ bioremediation

5.6.1. Intrinsic in situ bioremediation

Also known as natural attenuation, intrinsic bioremediation uses existing microbial populations at polluted sites without human intervention.⁶² This method aims to stimulate naturally occurring microorganisms to degrade pollutants through aerobic and anaerobic processes. It is cost-effective due to minimal intervention.⁶³ Techniques such as aerobic treatment, biosparging, and bioslurping are usually employed.⁶⁴ Intrinsic bioremediation is also effective for decontaminating Cr (VI) in soils.^{65,66} [65,66]. Reductive reactions are promoted through interactions between Cr (VI) and Fe (II) ions.⁶⁷

5.6.2. Engineered in situ bioremediation

Engineered in situ bioremediation employs genetically modified microorganisms to enhance decomposition thereby improving the physicochemical conditions which favours growth.⁶⁸

5.7. Bioventing

Bioventing increases microbial activity by providing controlled airflow to the unsaturated zone, promoting bioremediation. The addition of nutrients and moisture during bioventing aids in the microbial transformation of pollutants into harmless substances. This technique has gained popularity due to its effectiveness.⁶⁹ Bioventing stimulates indigenous microbes through adequate aeration, enhancing biodegradation and promoting the precipitation of heavy metal.⁷⁰

5.8. Bioslurping

Bioslurping combines direct oxygen supply with vacuum-assisted pumping.^{71,72} While it reduces microbial activity by using soil moisture to decrease air permeability and oxygen transfer rate, it is cost-effective for treating low-permeable soils. Bioslurping requires excavation, where contaminants are removed from the water and subsequently treated.⁷³ This method combines bioventing and vacuum-enhanced systems to recover free products and enhance soil aeration and microbial degradation.⁷⁴

5.9. Biosparging

Biosparging method involves injecting of air into the soil directly to stimulate microbial activity and remove pollutants. The technique, used in saturated zones, which encourages the

upward movement of organic chemicals to an unsaturated zone for further treatment.⁷⁵ The effectiveness of biosparging depends on soil porosity and the biodegradability of contaminants. High air-flow rates are used to volatilize contaminants, while biosparging promotes microbial degradation.⁷⁶ Biosparging influences the process of bioremediation by supplying appropriate amount of oxygen to microorganisms so as to activate its metabolic activity.^{77,78}

5.10. Phytoremediation

Phytoremediation is considered as one of the technique that uses plants to reduce the harmful effects of pollutants in contaminated soil and the surrounding ecosystems, which help naturally clean the environment.^{79,80} This method depends on selecting specific plant qualities or traits that enhance a plant's ability to and break down pollutants. Choosing the correct plant species or by modifying them genetically, the remediation process becomes more effective.^{81,82}

In contrast, Phyto-mining, is the process where plants are used to extract metals from contaminated soils, providing an eco-friendly alternative approach to mining practices. Certain plants, known to possess the unique ability to absorb specific amounts of metals like nickel, copper, and zinc through their roots. These metals accumulate in the plants' tissues which are later harvested. Phytomining is a sustainable method of metal recovery, especially in areas with moderate metal contamination.⁸³

Another approach is phytostabilization, a bioremediation technique that uses plants and microorganisms to reduce the mobility and toxicity of heavy metals in contaminated soil. Specific plant species are planted in polluted areas, where they work with soil microorganisms to stabilize metals and prevent their spread to nearby environments. Phytostabilization is particularly useful for managing metal contamination in soils, as it minimizes the risk of metals leaching into groundwater or being absorbed by living organisms.^{84,85}

6. Recent Advancements and Challenges in Bioremediation

6.1. Golden biotechnology insights in bioremediation

Bioinformatics is a branch of biotechnology which will help to understand the degradation mechanisms employed by specific organisms.^{86,87} By using bioinformatics, researchers have understood different pathways and mechanisms of bioremediation.⁸⁸ Proteomics studies are also crucial for understanding bioremediation methods.⁸⁹ These research areas allow computer science and biology, to study DNA, RNA, and protein information.^{86,89}

6.2. Omics and bioremediation

The ability of genomics, and proteomics plays significant role in bioremediation studies. These technologies help to

understand in situ bioremediation processes by correlating DNA sequences with, protein, and mRNA.^{89,90}

6.3. Genomics

To enhance understanding of mechanisms in biodegradation process various genomic tools such as PCR, DNA hybridization, metabolic footprinting, and metabolic engineering are used. PCR-based techniques, are used for genotypic fingerprinting.⁹² RAPD can assess related bacterial species in soil microbial communities, while LH-PCR detects natural length variations of SSU rRNA genes. T-RFLP allows profiling of multiple microbial taxonomic groups simultaneously.^{93,94} PCR-based quantitative analysis helps determine the abundance and appearance of taxonomic and operational gene markers in soil.⁹⁵

6.4. Role of transcriptomics and meta-transcriptomics in bioremediation

Transcriptomics provides enlarged view of gene expression across the genome, with DNA microarray analysis being a powerful tool for assessing mRNA expression levels.⁹⁶ The process involves isolating and enriching total mRNA, synthesizing cDNA, and sequencing the cDNA transcriptome. DNA microarrays enable examination of almost every gene in an organism's mRNA expression.⁹⁷ Metatranscriptomics, studies transcriptional mRNA profiles, which access the activities of environmental microbial communities.⁹⁸ By combining these methods researchers can discover pathways employed during the process of biodegradation.⁹⁹

7. Discussion

This review discusses the growing concern over environmental pollution by oil spillages in soil. A promising solution to this problem is microbial remediation, a natural process in which microorganisms, such as bacteria and fungi, break down these contaminants in the environment. In-situ bioremediation refers to treating the contaminated soil on-site without harming the properties of soil, making it a cost-effective and sustainable solution. Microorganisms are naturally present or introduced to break down or immobilize contaminants. The success of this method depends on various factors, including the choice of microbial species, the type of contaminants present, and the environmental conditions at the polluted site.

Organic pollutants, such as solvents, and petroleum hydrocarbons, are another major concern due to their long-lasting environmental impact. These pollutants can be toxic to human, animal, and plant life, and they do not easily break down in the environment. Microbial biofilms have been shown to be an effective tool for addressing organic contaminants as well. By harnessing the ability of microorganisms to degrade these harmful substances, biofilm-based bioremediation offers an eco-friendly and cost-effective solution. However, to completely understand and

optimize biofilm-based remediation technologies, researchers need to develop better mechanisms by which microbes form biofilms and how these biofilms interact with pollutants.

Microbial enzymes play a crucial role in the bioremediation process. These enzymes are produced by microorganisms and help break down pollutants. Enzymes fall into six major categories: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Each of these enzyme classes is responsible for breaking down different types of contaminants. Research into microbial enzymes is key to developing more targeted and effective remediation technologies.

The potential of genetically engineered microorganisms (GEMs) in bioremediation is also discussed in the review. Genetic engineering allows scientists to modify the genetic makeup of microbes, making them more efficient at degrading specific pollutants. By introducing or enhancing particular metabolic pathways, GEMs can be designed to target certain contaminants more effectively. This may improve the efficiency of bioremediation processes, especially for pollutants that are difficult to degrade naturally.

In addition to genetic engineering, omics technologies, such as genomics, proteomics, and metabolomics, are becoming increasingly important in bioremediation research. These technologies provide a deeper understanding of the microbial processes involved in contaminant degradation. By analyzing the genes, proteins, and metabolic pathways of microorganisms, researchers can identify which microbes are most effective at breaking down specific pollutants. This information can be used to optimize bioremediation strategies and even develop new technologies for environmental cleanup.

The review concludes by emphasizing the need for continued research in the field of bioremediation. Understanding the factors that affect the success of bioremediation, such as microbial species selection, environmental conditions, and the nature of the pollutants, is crucial for developing more effective strategies. Additionally, the integration of new technologies, such as genetic engineering and omics tools, will play a key role in advancing bioremediation methods.

Ultimately, bioremediation represents a promising, cost-effective, and eco-friendly solution to the growing problem of environmental pollution. By harnessing the natural abilities of microorganisms to degrade contaminants, we can help restore polluted environments and protect human health. As research continues, bioremediation technologies are likely to become even more effective, ensuring a cleaner and healthier planet for future generations.

8. Conclusion

Bioremediation has become an essential method for addressing pollution across various industries, including water and soil treatment and solid waste management. This process uses living organisms, such as microbes and plants, to break down or neutralize pollutants, making it an eco-friendly solution to environmental contamination. However, to improve its effectiveness, study of different metabolic pathways as how microorganisms degrade pollutants; will help to know how bioremediation work.

Also, analyzing natural processes, integrating advanced scientific methods like omics (genomics, proteomics, and metabolomics) and genetic engineering can significantly enhance bioremediation. Omics technologies help to study organisms at a molecular level, identifying genes, proteins, and metabolic pathways responsible for breaking down pollutants. By using genetic engineering, microbes or plants can be modified to improve their ability to degrade specific contaminants, leading to faster and more efficient remediation.

Genetically engineered plants are another promising area of research. These plants could be designed to target and remove specific pollutants using well-defined metabolic processes. For example, a plant could be engineered to absorb heavy metals from soil or break down harmful chemicals in water.

Despite advancements, the next big challenge in bioremediation is identifying and comparing the most effective genes and proteins involved in pollutant degradation. Moreover, combining bioremediation with physical and chemical methods, such as filtration or chemical neutralization, could provide a more comprehensive and effective cleanup strategy.

Ultimately, bioremediation represents a powerful, sustainable approach to addressing pollution, but ongoing research is vital to refine these methods, integrate new technologies, and ensure they meet the growing demand for environmental cleanup.

9. Source of Funding

None.

10. Conflict of Interest

None.

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