BIOREMEDIATION TECHNIQUES: HARNESING THE POWER OF MICROORGANISMS FOR ENVIRONMENTAL RESTORATION

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ABSTRACT

Bioremediation, a cost-effective and eco-friendly approach, utilizes the inherent capability of microorganisms to degrade or transform contaminants into less harmful forms. This review article provides a comprehensive overview of bioremediation techniques, focusing on their efficacy, limitations, and potential applications in various environmental scenarios. The article highlights recent advancements in molecular tools and genetic engineering that enhance bioremediation efficiency. Additionally, it discusses emerging trends in bioremediation research and emphasizes the need for interdisciplinary collaboration to address complex environmental challenges.

Keywords: Bioremediation, Microorganisms, Environmental Pollution, Biology, Applications, Molecular Tools.

I. INTRODUCTION

Bioremediation is a highly specialized process that uses microorganisms to their full potential to completely eliminate, immobilize, or detoxify Pollutants and contaminants to less harmful forms and combat environmental degradation [1] [2]. Microorganisms are found throughout the biosphere, due to their remarkable metabolic capacity and ease of growth in a variety of environmental conditions. As a result, environmental contaminants can be easily bioremediated [2]. Numerous intricate components work together to optimize and regulate bioremediation processes. These elements include the availability of pollutants to the microbial population, the existence of a microbial population capable of degrading the pollutants, and environmental factors such as soil type, pH, temperature, and the presence of oxygen or other electron acceptors and nutrients [3]. In-depth discussion of bioremediation methods and their potential for reducing contamination problems is provided in this review. We will investigate the essential role that microbes perform as nature’s cleanup team. On this voyage, we will explore the suitability of different methods in various environmental circumstances and talk about their limitations as well as their success. We will highlight new advances in genetic engineering and molecular techniques that make bioremediation more effective in our pursuit of sustainability. (Figure: 1)
II. TYPES OF CONTAMINANTS

2.1 Organic Contaminants

2.1.1 Total Petroleum Hydrocarbons

TPH or Total petroleum hydrocarbons, are difficult-to-solve hydrophobic pollutants made up of intricate blends of asphaltenes, aromatics, aliphatics, and resins [5] [6]. Because of oil exploration, refinery operations, oil spills, underground storage tank leaks, and industrial runoffs and discharges, TPH-contaminated sites are prevalent around the world [5] [6] [7]. It was believed that contamination from such spills would cause environmental damage that could be irrevocable and permanent [9]. Treatment techniques for soil contaminated with TPH are essential and have garnered significant scientific interest in the past two decades [5] [6]. Some microbes have the ability to break down petroleum hydrocarbons and utilize them as their only source of carbon and energy for growth [8]. Compared to chemical and physical approaches, biological methods are more cost-effective and efficient [7] [8].

2.1.2 Chlorinated Solvents

Probably half of the problems with organic pollutants in the environment that exist now are caused by chlorinated organic compounds. The site of enzymatic attack is often blocked by chlorine, which limits the capacity of aerobic bacteria to break down these compounds. As a result, a portion of these substances remain in the environment. The last ten years have seen a greater understanding of anaerobic microbes’ capacity to reductively dechlorinate several of these substances [10]. The majority of this kind of pollution is found in groundwater and aquifer sediments, where chlorinated solvents, including chlorinated alkenes and alkanes, are present. The most prevalent of these contaminants are Perchloroethene (PCE), trichloroethene (TCE),
dichloroethene (DCE), vinyl chloride (VC), carbon tetrachloride (CT), chloroform (CF), dichloromethane (DCM), and chloromethane (CM) [11].

2.1.3 Polychlorinated Biphenyls (PCBs)

The synthetic chemical compounds known as polychlorinated biphenyls (PCBs) are made of biphenyl carbon and one to ten chlorine atoms. 20 to 60 congeners are found in commercial products, out of a possible theoretical total of 209 congeners [12]. PCBs are among the most well-known persistent organic pollutants (POPs) [13]. Because of their physicochemical properties, PCBs are persistent in the environment and can easily affect nearby habitats through intimate interactions with a number of environmental matrices, including as sediments, soils, groundwater, surface water, and the food chain [14] [15]. The breakdown of PCBs by microorganisms occurs in two distinct metabolic modes. The first is that, in the absence of other carbon and energy sources, microorganisms can use PCBs as their sole source of carbon and energy, breaking them down through their own metabolic processes. The second is that, in the event that microorganisms are growing in the presence of external carbon and energy sources, PCBs will be broken down by co-metabolism [16]. In many anaerobic settings, such as freshwater (ponds, lakes, and rivers), estuaries, and marine sediments, microbial PCB dechlorination is common [17] [18]. The addition of PCBs to the sediments, dechlorination activity of PCBs in uncontaminated sediments was also seen in the laboratory. Microbial communities in PCB-contaminated sites are more suited for PCB dechlorination than those from settings with no or very little PCB contamination, presumably as a result of environmental choices [19] [20].

2.2 Inorganic Contaminants

2.2.1 Heavy Metals

Metals with atomic weights (more than 50), atomic numbers, and densities (higher than 5gm/cm3) that are considered significantly high are referred to as heavy metals [21]. Heavy metal contamination can have dangerous effects even at extremely low quantities, it is a major problem. In addition to being bioaccumulative and biomagnified with the trophic levels, heavy metals are not biodegradable in tissues [22]. Agricultural fields, sewage sludge, waste treatment facilities, and industry (tannery, electroplating, dyeing, and mining) are the main sources of heavy metal pollution [23]. Large volumes of industrial effluent produced during the processing of raw materials are being released into water reservoirs, severely polluting them. Both soil and water bodies have been linked to contamination by untreated effluent from industry that is dumped into the environment [24]. These effluents contain a variety of harmful and poisonous compounds, including heavy metals like nickel (Ni), mercury (Hg), arsenic (As), cadmium (Cd), lead (Pb), and chromium (Cr). As a result, they pose a risk to human health and the lives of aquatic species when dumped into land or water [25]. Microorganisms metabolize the toxic heavy metals, releasing essential components and bio-products that are less toxic and easily degradable. Microbes such as bacteria, fungi, and algae have cellular machinery that allows them to metabolize and eliminate heavy metals from the environment [26].

2.2.2 Radionuclides

Radionuclides are atoms that have enough nuclear energy to disrupt them. This extra nuclear energy can be released in three ways: alpha, beta, and gamma radiation [27]. Radiation in the environment is produced by both natural and manmade sources [28]. The main contributors to nuclear waste include reactor operations, mining, fuel manufacture, fuel reprocessing, and military actions. The existence of a large number of fission products, as well as several oxidation state long-lived radionuclides like as neptunium (237Np), plutonium (239Pu), americium (241/243Am), and curium (245Cm), makes the waste streams a possible radioactive threat to the environment. Nuclear waste commonly contains significant amounts of cesium (137Cs) and strontium (90Sr). These radionuclides pose a risk to health due to their long half-lives and ease of translocation into the human body [29]. Microbes can influence radionuclide solubility in a number of ways, directly by interacting with the biological system (enzymatic degradation) or indirectly by causing changes in the environment's chemistry when they are present (biosorption, bioaccumulation, or biostimulation) [30].
III. BIOREMEDIATION APPROACHES

3.1 Bioaugmentation

Bioaugmentation is a technique used to strengthen the microbial populations in organically contaminated soils by adding microorganisms (bacteria, fungi, and their secreted enzymes) or biological additives (biosurfactants), which effectively reduce the contaminant load by transforming into less dangerous compounds [31]. Many bioaugmentation techniques have been brought up, such as inoculating strains carrying mobile genetic elements (MGE) [32], rhizosphere bioaugmentation [33], biofilm architecture [34], bacteria chemotaxis [35], and the use of heirloom species [36]. The following factors affect bioaugmentation: physicochemical pollutant characteristics (such as temperature, humidity, and ionic strength) can limit the mass transfer of the contaminants to microorganisms (such as the amount of clay and organic matter); microbial ecology (which includes energy flux, indigenous activity, predators, competitors, co-substrates, genetics of relevant microorganisms, enzyme stability and activity); and methodology (which includes constraints on the selection, concentration, and methods of inoculation, as well as inoculum heterogeneity) [37].

3.2 Biostimulation

Biostimulation involves the modification of the environment to stimulate existing bacteria capable of bioremediation [38] [39] [40]. Biostimulation is the process of adding nutrients [41], humic substances [42], or other chemicals that may have an impact on the bacterial state in order to raise the bacterial activity of different strains present in the contaminated soil [43]. Biostimulation is one of the most widely used methods for bioremediation of hydrocarbons, recent developments in molecular microbiology, stable isotope analysis, and geophysics promise to significantly expand the scope, depth, and throughput of biostimulation strategies [44]. Because biostimulation depends on indigenous organisms, it necessitates their presence as well as the ability to modify the environment in a way that will result in the desired bioremediation effect [45].

3.3 Phytoremediation

The process of using plants and the microbes they contain to clean up the environment is known as phytoremediation [46] [47] [48]. This method utilizes the organic and inorganic pollution-degrading and sequestering processes that occur naturally in plants and their microbial rhizosphere flora. For a range of organic and inorganic contaminants, phytoremediation is an effective remediation technique [49]. The popularity of phytoremediation can be due to its long-term applicability, cost-effectiveness, and aesthetic advantages [50]. Phytoextraction, Phytovolatilization, Phytostabilization, Phytodegradation, Hydraulic Control, Rhizofiltration, and Rhizodegradation are the seven mechanisms of phytoremediation that can impact the mass of contaminants in soil, sediments, and water. As in the application of phytoremediation, each of these methods will impact the amount, mobility, or toxicity of pollutants [51]. There are some inherent technological limitations with phytoremediation: The limited habitat range or size of plants exhibiting remediation potential, as well as the inadequate capacity of native plants to withstand, detoxify, and collect pollutants, may pose obstacles to the widespread application of phytoremediation [52]. Actively growing plants' root zones must contain the pollutant or be attracted to it. Phytoremediation limitations are associated to water, depth, nutrients, atmosphere, physical, and chemical nature. Furthermore, the area needs to be big enough for the farming methods to be used. It must not pose a serious risk to public health or cause additional environmental damage [53].

3.4 Mycoremediation

Mycoremediation is a bioremediation method that uses fungi to remove harmful substances; it can be done with or without the presence of macrofungi (Mushrooms) [54] [55], and filamentous fungi (Molds) [56]. Both types have enzymes that can break down a wide range of contaminants [57] [58]. Mycoremediation has advantages due to is a natural, chemical-free method that is safe for both organic and inorganic substances. It is also cost-effective and produces quick, noticeable improvements on contaminated sites [59]. This is another advantage of mycoremediation; the treated soil is biologically sound and active. However, this technique has not yet evolved into a reliable environmental biotechnology because the degradation may not always be successful. The removal of contaminants is rarely close to 100% and the process is typically slow. The ultimate outcome of the treatment is influenced by the soil matrix and the bioavailability of the specific pollutant [60].
3.5 Nanobioremediation

Nanobioremediation has the potential to improve the environment by eliminating pollutants and using cleanup techniques. Nano bioremediation will be a workable solution to remove pollutants from the environment that combines traditional bioremediation with a nano-biotechnological approach or employ direct nanoremediation techniques. Heavy metals, pesticides, herbicides, and insecticides organic and inorganic pollutants are broken down or sequestered by nanoparticles in the contaminated environment [61]. When compared to separate techniques, nanobioremediation aids in achieving a remediation that is more effective, quicker, and environmentally benign. An integrated strategy has the potential to improve cleanup outcomes by overcoming the disadvantages of individual technologies. Two methods have been identified to carry out the integrated Nano-Bioremediation process within a treatment system. The initial technique involves exposing the pollutant to nanoparticles initially, and then adding a bioagent to continue the process. The second approach adds a biological agent and nanoparticle to the system at the same time. It is also known as the concurrent or integrated method [62]. Remarkable results have been observed when using nanoparticles or zero-valent metals in nanoscale form, such as iron, nickel, and palladium, to sites contaminated with different kinds of toxic substances. The main purposes of these applications are to dehalogenate persistent organic compounds and stabilize transitional metals like arsenic and chromium [63] [64].

Table 1: Bioremediation Approaches using microbes and cultured stains

<table>
<thead>
<tr>
<th>S. No</th>
<th>Approaches</th>
<th>Microbes</th>
<th>Stains</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Bioaugmentation</td>
<td>Bacteria, Protozoa, Nematodes, Rotifers, and Fungi</td>
<td>Stearothermophilus, Penicillium sp., Aspergillus sp., Flavobacterium, Arthrobacter, Pseudomonas, Streptomyces, and Saccharomyces</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
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<td>Phytoremediation</td>
<td>Bacteria, Endophytes and Arbuscular mycorrhizal fungi (AMF)</td>
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<td>Mycoremediation</td>
<td>Fungus</td>
<td>Moulds</td>
<td>56</td>
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<tr>
<td>5</td>
<td>Nanobioremediation</td>
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IV. GENETIC ENGINEERING IN BIOREMEDIATION

4.1 Genetically Modified Organisms (GMOs)

Microorganisms in the ecosystem struggle to break down many toxic pollutants due to slow degradation. Chemical elements resistant to biodegradation hinder their effectiveness. To address this, enhancing microbe capabilities through genetic engineering is crucial. This involves constructing novel microbe strains with unique characteristics for broad bioremediation potential [65]. Genetically engineered microorganisms are created to improve their biodegradation abilities, offering a solution to detoxify specific contaminants. Genetically modified microorganisms (GMOs), are crucial in the remediation of industrial waste. They reduce the toxicity of some hazardous substances and helps in the remediation of pollutants caused by petroleum and hydrocarbon discharges [66]. The primary methods for creating genetically modified organisms (GMOs) include modifying the affinity and specificity of enzymes, developing bioprocesses, creating and regulating pathways, developing bioactive bioreporter sensors for chemical sensing, end-point analysis, and toxicity reduction. Developing genetically modified organisms for bioremediation applications typically involves the following strategies: directed evolution, metabolic engineering, saturation mutagenesis, and rational designing [67]. Several bacterial strains, including Bacillus idriensis,Ralstonia eutropha, Sphingomonas desicabilis, Pseudomonas putida, Escherichia coli, Mycobacterium marinum, and others, have been employed for the creation of genetically modified microorganisms that can bioremediate contaminated environments by means of inserting functional
when using a gene-centric metagenomics: This method entails examining individual genes that have been extracted from environmental samples’ metagenomes. It is impossible to determine which genes originated in which creatures.

sites often include adjustments of nutrients, carbon sources, pH, temperature, oxygen, and water content to handle. In such cases, little synthetic biology genome modifications may enable futuristic organisms to respond to changes in a variety of environmental factors because treatment scenarios applied to contaminated bacteria are left in the environment. Thus, the ability of the strains to survive should be restricted by the development of unique containment mechanisms to reduce the negative effects on the environment.

4.2 Synthetic Biology Applications

The creation of new genetic components, tools, and systems, as well as the redesign of existing biological systems for improved performance, is known as synthetic biology. The field of synthetic biology has rapidly developed, combining engineering and biological concepts with the goal of reorganizing living things, mostly through genetic reprogramming [76] [77]. A variety of cloning techniques, such as genome assembly and synthesis, cell-free protein synthesis, clustered regularly interspaced short palindromic repeats with associated proteins (CRISPR Cas), and synthetic devices, such as small noncoding RNAs like riboregulators, riboswitches, synthetic oscillator, toggle switch, and biologic gates, are included in synthetic biology [78] [79] [80]. Synthetic biology could provide a framework for the development of high throughput sensors in microbes, giving them the capacity to react to, detect, and distinguish between highly identical or closely related substances at very low concentrations [76] [77]. The transition toward the application of synthetic biology in remediation would enhance the bioremediation procedures by incorporating strong [81], specific pollutants that can dissipate [82]. Cellular modulations are mediated by synthetic biological systems to ensure the smooth operation of current activities [83]. The application of synthetic biology approaches in the field of bioremediation is still concentrated on the creation of microbially based systems. Plants play a significant part in bioremediation; yet current technologies only allow for the expression of one or a few transgenes; true synthetic biology methods have not yet been developed [84]. Microbes that have been logically created by advanced genetic engineering techniques in the field of synthetic biology, metabolic engineering, and computational systems biology approaches are known as synthetic microbial scavengers [85] [86] [87]. Even though bioremediation proves to be an effective approach for managing waste, organic compounds frequently manage to evade the standard treatment process because of their toxicity or poor bioavailability, which can be difficult for wild-type microbes to handle. In such cases, little synthetic biology genome modifications may enable futuristic organisms to withstand toxic compounds and release enzymes that will solubilize and degrade the same [88].

V. ENHANCING BIOREMEDIATION EFFICIENCY THROUGH MOLECULAR TOOLS

5.1 Metagenomics

Metagenomics is the study of the metagenome, or the entire genetic material present in the environmental sample, directly extracted from the environmental samples [89] [90] [91]. Metagenomics contributes to our understanding of how organic and inorganic contaminants are broken down and detoxified by microorganisms at the contaminated site. The application of metagenomics techniques could be helpful in identifying a possible microbial degrader for the bioremediation of a particular pollutant or the catabolic gene accountable for the degradation and detoxification of that particular pollutant. Metagenomics is also employed to compare the functional diversity of microorganisms at several contaminated sites affected by a particular contaminant [92].Metagenomic studies of bioremediation will also provide information on how microbial communities respond to changes in a variety of environmental factors because treatment scenarios applied to contaminated sites often include adjustments of nutrients, carbon sources, pH, temperature, oxygen, and water content [93].Based on its techniques, two major categories can be used to categorize metagenomics [94]. 1) Gene-centric metagenomics: This method entails examining individual genes that have been extracted from environmental samples’ metagenomes. It is impossible to determine which genes originated in which creatures when using a gene-centric approach. Consequently, reconstructing the metabolic pathways or establishing a
Functional metatranscriptomics is an effective approach to obtaining whole genomes by single-cell genome sequencing or by assembling individual genes [96] [97].

5.2 Metatranscriptomics

A subset of metagenomics called metatranscriptomics offers important insights into the whole gene expression profile for complex microbial communities within an environment. The diversity of the active genes within this community, their expression patterns, and how these levels change in response to environmental changes are all provided by metatranscriptomics. Applications of metatranscriptomics include the study of human microbiomes as well as those found in plants, animals, soils, and aquatic systems. Utilizing mRNA extracted from environmental samples, metatranscriptomics is a viable method for searching the eukaryotic gene pool for genes with biotechnological significance. If applied to the soil environment, and functional metatranscriptomics involves the extraction and analysis of mRNA, which provides information on the expression profiles and regulation of complex communities [98]. Therefore, functional metatranscriptomics is an effective approach that enables the direct characterization of genes expressed by various eukaryotic microorganisms (such as fungus, protists, etc.) in their natural environment. This method provides a great deal of potential in biotechnology for finding new genes that are interesting for the bioindustry, bioremediation, and biomarkers. The technique allows the characterization of genes associated to the degradation of organic matter or to the adaptability to stressful situations. When applied to the soil environment, functional metatranscriptomics involves the extraction and study of mRNA, which offers information on the expression profiles and regulation of complex communities. It is predicated on the extraction of environmental RNA rather than DNA and the separation of the polyadenylated mRNA specific to eukaryotes using affinity chromatography from the mixture of environmental RNAs as a whole [99]. Metatranscriptomics has been employed to assess the physiology of pure and mixed microbial cultures that have been separated from their environment [100].

VI. CONCLUSION

Although bioremediation methods have demonstrated significant promise, there are still issues that need to be resolved. The efficacy of bioremediation techniques can be impacted by variables such pollutant kinds, site-specific circumstances, and regulatory considerations. These issues will be covered in the article along with some possible fixes. It will also highlight new research fields and technologies that have the potential to improve the effectiveness and practicality of bioremediation methods.

In conclusion, bioremediation methods present a viable approach to restoring the environment through the utilization of microorganisms. The applications and difficulties of biostimulation, bioaugmentation, and phytoremediation have been briefly discussed in this review paper. Researchers and practitioners can choose the best bioremediation strategy for a specific contaminated site by being aware of the advantages and disadvantages of each technique.

6.1 Recommendations for Future Research

Future avenues for future study in bioremediation are promising. In order to increase the range of contaminants that can be effectively remedied, it is first necessary to explore the unexplored microbial diversity by isolating and characterizing novel microorganisms. In order to improve microbial metabolic pathways and maybe create genetically modified strains with better stress tolerance or substrate specificity, genetic engineering approaches should be investigated further. Furthermore, incorporating nanotechnology into bioremediation techniques may have synergistic benefits that enhance enzymatic activity, microbial adhesion, and the elimination of pollutants from complicated matrices. Developing accurate, long-term monitoring systems is crucial to evaluating the sustainability and ecological impact of bioremediation techniques. The use of bioremediation in harsh environments, like the Polar Regions or deep-sea environments, may open up new avenues for tackling contamination problems in unusual contexts. Designing efficient and robust remediation solutions requires examining the dynamics of microbial communities and promoting the creation of bioinformatics tools for analysis and prediction. In order to ensure the responsible and informed use of bioremediation technologies, policy frameworks and communication strategies must be influenced by studies on public perception and acceptance of these technologies. As a result of interdisciplinary cooperation and a comprehensive strategy,
these guidelines seek to advance bioremediation research into new areas and promote creativity and long-term solutions for environmental restoration.

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