**METHODS OF BIOREMEDIATION AND THEIR RECENT ADVANCEMENT FOR ENHANCEMENT OF GREEN AND SUSTAINABLE ENVIRONMENT**

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**Abstract**

This article seeks to present an in-depth review of various bioremediation techniques and strategies used to promote the remediation process for the enhancement of sustainable development. Environmental pollution caused by xenobiotics and other associated non-biodegradable substances, which has lately been discovered as a severe hazard to both the natural environment and the health of animals including humans, is one of the main global concerns for human sustainability. Due to industrialization, a variety of pollutants, including plastics, heavy metals, and various agrochemicals, accumulate in the environment.Since these pollutants are not biodegradable,they remain in the polluted sites and either directly or indirectly create problems for human health and the environment.Toxic pollutants including both organic and inorganic must be removed from contaminated settings in order to progress sustainable development with the least amount of environmental impact. Bioremediation, which uses biological agents. Including plants, fungi, bacteria, and other creatures or the enzymes they producedto decrease pollution and restore ecosystems, is the most widely accepted, environmentally beneficial, and economically advantageous method of reducing environmental pollution.

**Keywords:** bioremediation,heavy metals, pollutants,microbes, sustainable environment

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**NTRODUCTION**

During the bioremediation process, organic wastes are biologically reduced under controlled conditions to safe levels that are below the concentration limit established by regulatory agencies (Mueller et al., 1996). The current limitations on bioremediation research and application stem from an inadequate understanding of the genetics and genome-based features of the organisms used, the role of kinetics, and the metabolic pathways. Natural bioremediation methods are difficult to use in the field since it is difficult to comprehend and predict how these processes will behave. As a method of restoring damaged habitats, bioremediation using of microbes to detoxify and break down environmental pollutants has gained increased attention in recent years (Guo *et al*., 2010). Waste including solid, liquid, or gaseous can all be harmful to one's health or significantly damage the ecosystem by contaminating the soil, water, and air. However, waste has become a major issue, so it must be disposed of in an environmentally friendly manner. Historically, nature handled the disposal of such garbage due to the low population of the human race and the abundance of natural resources. The opposite is currently applicable.Therefore,waste materials such as community sewage and others should be discarded in a sanitary and safe manner to reduce costs and promote the well-being, wealth, and cleanliness of the general population. The conversion of trash into useful items for reuse, recovery, or other means requires additional research. As contaminants are digested by microorganisms like yeast, fungi, or bacteria, bioremediation is one of the most crucial processes that may be employed to remove them from polluted soil and water (Strong *et al*., 2008). The primary goal of bioremediation approaches is to speed up the naturally occurring biodegradation process by improving the environmental conditions required for microbial activity and growth. Applications of bioremediation function by changing environmental variables to promote microbial growth and activity, which quickens the decomposition process (Margesin et al., 2000).

The industrialization-related danger chemical pollution of surface water, soil, and ground water is one of the current global concerns for human sustainability. Hazardous pollutants (both organic and inorganic), which have a negative impact on the environment, must be removed from polluted areas in order to improve sustainable development with the least negative environmental impact possible. The traditional method of treating contaminated soil, sediment, and water is discovered to be infeasible due to its high cost and creation of secondary pollutants. Bioremediation, a sustainable, cost-effective strategy, can help recover contaminated surface water, soil, and ground water by using a variety of microbes such fungi, bacteria, and other organisms or the enzymes they produced (Kumar et al., 2018). Therefore, bioremediation technology holds prominence in the research field. This review focuses on the recent research developments in bioremediation methods, how microorganisms degrade different pollutants, and how plants play an important role in remediation and also discusses the future perspectives for bioremediation in order to decrease the amount of pollution in the earth.

**PRINCIPLES OF BIOREMEDIATION**

Bioremediation, a crucial component of biotechnology, involves using microorganisms to restore the condition of a contaminated place. Bacteria are typically the degraders, but other creatures, like soil animals or plant roots, also contribute to the growth of bacteria by giving them nutrients and co-substrates (Romantschuk *et al*., 2000; Juwarkar *et al*., 2010). Considering that bioremediation strives to improve natural processes and is widely seen as "environmentally appropriate", it enjoys substantial public support in theory. However, compared to physical techniques like transferring the contaminated material to a securelandfill, bioremediation rates are frequently much slower (Prince,2010). The development of bioremediation relies on the creation of novel metal-sequestering peptides, rational and irrational route engineering, and enzyme design in addition to tools from molecular, genetic, microbiological, and protein engineering(Singh *et al*., 2008). The inclusion of living creatures and nutrients can promote bioremediation, which occurs naturally. The technology used in bioremediation is mostly based on biodegradation. It refers to the total elimination of organic harmful pollutants into harmless or naturally occurring components like carbon dioxide, water, and inorganic chemicals which are safe for human, animal, plant, and aquatic life (Hamer, 1993).

**METHODS OF BIOREMEDIATION AND THEIR RECENT APPROACHES**

Recently microbe assisted phytoremediation has been practiced widely by researchers and various experiments are going on in this field. It has been found that the inoculation of microorganisms such as *Bacillus subtilis* with alfalfa plant significantly decreases the amount of plant malondialdehyde (MDA) and enhances the plant’s antioxidant enzyme activity and the activity of enzymes involved in soil nutrient cycling. Dual inoculation shows the high efficiency of removal of heavy metals such as Cd (Li *et al*., 2021). In order to detoxify harmful pollutants, bioremediation uses a variety of bacteria that can act simultaneously or sequentially. To put it another way, it can be thought of as the acceleration of the normal metabolic process, in which microorganisms (such as fungi and bacteria), green plants (a process known as phytoremediation), or their enzymes break down or transform toxic contaminants into inorganic salts, microbial biomass, H2O, CO2, and other by-products (metabolites) whose toxicity is lesser as compared to the parent compounds(Chakraborty et al.,2012). Bioremediation techniques can be classified into two types:(1) In- situ Bioremediation and (2) Ex-situ Bioremediation.

1. **In-situ Bioremediation**

These methods entail handling contaminated materials right where the pollution occurred. It does not require any excavation; therefore, it is accompanied by little or no disturbance to soil structure (Christopher *et al*., 2016). The presence of pH,moisture content, electron acceptor, the availability of nutrients, and temperature are just a few of the crucial environmental factors that must be met for successful in-situ bioremediation (Philp and Atlas, 2005). The in-situ bioremediation can be of two distinct types: intrinsic and engineered.

1. **Intrinsic In-situ Bioremediation**

Enhancing the already present microbial population is the primary goal of intrinsic in situ bioremediation. This is regarded as a cost-effective technique for the biodegradation of refractory contaminating chemicals since it involves both aerobic and anaerobic processes by microorganisms (Sharma, 2019). Using this method, the particle swarm optimization (ELM–PSO) technique was implemented for the groundwater treatment which resulted in the reduction of the concentration of pollutants from 40 ppm to 5 ppm in 3 years (Cecchin *et al*., 2021). In situ bioremediation is also used in the purification of Cr (VI) that is found in shallow unsaturated soil where the microorganisms show their survival capacity and sub-cellular activity which has been considered useful for the treatment of heavy metals (Cecchin *et al*., 2021). Biosparging anaerobic reductive dechlorination, amendment delivery,aerobic treatment, and bioslurping are the example of intrinsic in situ bioremediation (Akubude *et al*., 2020).

1. **Engineered In-situ Bioremediation**

By enhancing the physicochemical features of microbial growth, this method makes use of genetically modified, particular microbes for effective breakdown and pollution cleanup (Kumar, 2018). It has been found that in arsenic bioremediation, genetically modified organisms such as bacteria and plants have played an important role (Verma *et al*., 2019).

**1.1. Bioventing**

In order to promote bioremediation by boosting the activity of indigenous bacteria, this technique involves the stimulation ofregulated airflow by supplying oxygen to the unsaturated (vadose) zone. The ultimate goal of bioventing is to achieve the microbial transformation of contaminants into a harmless condition. To enhance bioremediation bioventing amendments are made by adding moisture and nutrients and are useful for decontamination of heavy metals (da Silva *et al*., 2020).

**1.2. Bioslurping**

By indirectly providing oxygen and promoting pollutant biodegradation, this technique combines soil vapour extraction, bioventing, and vacuum-enhanced pumping to remediate soil and groundwater (Gidarakos and Aivalioti, 2007).Bioslurping is useful for recovering both saturated and unsaturated zones and also light non-aqueous phase liquids (LNAPLs) which help in flammable and moderately flammable organic substance-containing soils free from contamination. From the free product layer liquid is drawn through a “slurp” that spreads into the layer and with the help of a pumping machine the LNAPLs are lifted to the surface and then separated from the surroundings (Tong, 2018).

**1.3. Biosparging**

This technique, which is very similar to bioventing, involves injecting air beneath the soil's surface to promote the activity of microbes and help remove contaminants from contaminated areas. As opposed to bioventing, air is pushed at the saturated zone, which could move volatile organic molecules upward to the unsaturated zone to promote the breakdown process. The soil permeability, which affects the pollutant's bioavailability to microorganisms, and the biodegradability of pollutants are the two main parameters that influence how effective biosparging is (Philp and Atlas, 2005). For the enhancement of the bioremediation process oxygen is supplied to the microorganisms that take part in the biodegradation process and it is commonly used for the removal of kerosene and diesel from water supplies (Maitra, 2018).

**1.4. Bioaugmentation**

One method of in situ bioremediation known as "bioaugmentation" aims to increase the ability of polluted sites to degrade organic matter by introducing native, allochthonous wide-form, or genetically modified microbial consortia with desired catabolic activities to break down resistant compounds in such environments(El Fantroussi *et al*., 2005).

**1.5. Phytoremediation**

Phytoremediation is the use of plants, either directly or indirectly, to remove pollutants from the environment (air, soil, and water). In the last few decades, phytoremediation has been a widely acceptable remediation process due to its affordability and eco-friendliness (Arthur *et al*., 2005). The chemical, physical, biological, biochemical, and microbiological levels of plant interactions have been shown in the phytoremediation process to mitigate the toxicity of the environment. Based on the concentration and the types of contaminants the approach to phytoremediation varies. For example, phytoextraction, transformation, and sequestration are some common approaches used for the removal of heavy metals while immobilization, decaying, rhizodegradation (known as rhizoremediation), and evaporation of organic pollutants such as chloro-compounds and oils can be treated by using energy crops such as willow or alfalfa (Wei *et al*., 2021 and Odoh *et al*., 2019). Recently the microbe assisted phytoremediation has been brought into light in the field of bioremediation. The nature of the plant and the contaminants can be alterable and due to these native plants can be bioaugmented by natural plants or it can be a combination of both in the contaminated sites. Phytoremediation can also be accomplished directly by the majority of hyperaccumulator plants present at the metal-polluted site (Nkrumah *et al*., 2018). To mitigate the toxicity of heavy metals such as Zn, Cu, and Ni, a number of plants have been widely studied. The process of absorbing heavy metals in the root and then replenishing them is known as phytostabilization, and it is used to immobilize heavy metals by reducing their bioavailability and preventing their transfer to other sites. *Acanthus ilicifolius* and *Virola surinamensis*, are well known for their Cd photostability and some decorative plants also take part in phytoremediation viz. *Euonymus japonicus, Osmanthus fragrans, Ligustrum vicaryi, Cinnamomum camphora, and Loropetalum chinense*. They take part in the phytostabilization of Cd (Zeng *et al*., 2018).

1. **Ex-situ Bioremediation**

Ex-situ bioremediation methods are typically assessed based on some factors such as treatment cost, depth and type of contamination, degree of contamination, geographical blockade, and geology of the contaminated site. These methods entail removing pollutants from contaminated areas and then moving them to another location for treatment.Performance requirements have been characterized as factors that influence the selection of ex-situ bioremediation methods(Philp and Atlas, 2005).

**2.1. Biopiling**

Biopile-mediated bioremediation involves stacking excavated contaminated soil above ground, followed by nutrient replenishment and occasionally aeration, to increase bioremediation by essentially increasing microbial activity. This strategy comprises of a treatment bed, irrigation, nutrient-collecting systems, and aeration. The use of this particular ex-situ technique is being researched more frequently due to its advantageous properties, such as cost-effectiveness, which enables effective biodegradation provided that temperature, nutrients, and aeration are adequately maintained. (Dias *et al*., 2015; Ding *et al.,* 2017). In the biopile system, warm air is introduced to provide heat and air simultaneously for the improvement of bioremediation methods, biopile’s adaptability increases microbial activity, contaminant availability, and the rate of biodegradation, while decreasing the remediation time. The addition of wood chips, sawdust, or straw helps to enhance the remediation and the ex-situ bioremediation techniques viz. land farming, bioventing, and biosparging can be applicable for the refill of air supply to the contaminated piled soil in biopiles (Arora et al., 2022). Bio-available organic carbon (BOC) plays a crucial role in this method of bioremediation. Petroleum-contaminated soil has been remediated using alpha, beta, and gamma proteobacteria by removing total petroleum hydrocarbon (TPH) which is possible in mesophilic conditions 30˚C-40˚C and a low aeration rate (Naeem and Qazi, 2020).

**2.2. Windrows**

Windrows is one of the bioremediation methods which depend on the regular rotation of the piled contaminated soil to increase the functioning of the hydrocarbonoclastic bacteria present in the contaminated soil which reduces hydrocarbons. Bioremediation by biotransformation, mineralization, and assimilation can be achieved. Uniform distribution of pollutants, aeration, nutrients, and microbial degradation activities were promoted by periodic turning of contaminated soil and the addition of water (Baar, 2002). Due to the growth of an anaerobic zone within heaped dirty soil, which often happens after reduced aeration, the application of windrow treatment has been linked to the production of greenhouse gas (CH4) (Hobson *et al*., 2005). This method was applied at Gurugram–Faridabad dumpsite in Bandhwari, India which in turn shows a reduction of garbage (Fortin Faubert *et al*., 2021).

**2.3. Land Farming**

Land farming is an ex-situ remediation process that is considered the most common and cost-friendly method. Land farming can also occur with in-situ bioremediation due to its site of treatment. It is a simple regular basis method for the removal of polluted soils from the treatment site. On the basis of the site of treatment, the type of bioremediation is categorized, when the treatment is on-site it is categorized as in-situ and when the polluted matters like contaminated soil, sediments, etc. are transported to the location of land-farming it is called ex-situ bioremediation. It is mostly applicable to the treatment of contaminated soil (Guerin, 2021). In this method, a permanent layer of substrate is prepared where the contaminated soils are disposed of as layers with variable thickness and allows the native microorganisms to degrade the contaminants aerobically (Patel *et al*., 2022).

**2.4. Composting**

 In the biological disintegration process known as composting, bacteria convert organic wastes into humus-like substances, a stable organic byproduct (compost). Composting involves removing the contaminated soil and blending it with an organic substance (such as animal dung, wood chips, or plant waste, for example) and a bulking agent (Kumar *et al*., 2018). As contaminated soils are dug and screened to remove large boulders and debris, windrow composting has been constructed (Zucchi *et al*., 2004). Composting is carried out by three main types of microbes: psychrophiles, mesophiles, and thermophiles. For composting to be successful, microbes require nutrients, moisture, temperature, and oxygen. Bacteria use the breakdown of organic materials in composting to get the nutrients (N, P, and K) they need to survive over the long term as well as energy for their metabolic operations. The most crucial components for microbial breakdown among the numerous other elements are C and N (Kumar *et al*., 2018).

**2.5.Bioreactor**

Bioreactors provide the ideal conditions for the growth of microorganisms. These conditions include manageable regulation of pH, agitation, temperature, aeration, substrate concentration, and inoculum concentration. The process has several merits regarding contaminated soil remediation treatment. Bioreactor-based remediation approaches significantly require less time and designs to maximize microbial degradation while having the minimum abiotic loss (Davoodi *et al*., 2020).

 These all methods are widely accepted and applied to accomplish bioremediation. There are various methods and tools of bioinformatics and molecular biology present to date for the advancement of these techniques in the field of bioremediation. There are various research going on in bioinformatics approaches that are based on omics and nanotechnology.

**APPROACHES OF BIOINFORMATICS IN BIOREMEDIATION**

In recent years, the use of bioinformatics tools and techniques has attracted much attention in the research field of bioremediation. Various omics-based technologies such as genomics, proteomics, metabolomics, and transcriptomics which need to be analyzed by a variety of bioinformatics tools have been used in bioremediation to understand the degradation mechanism of individual organisms for specific contaminants (Yergeau *et al*., 2012). Bioinformatics tools have been proven to be very helpful in interpreting the genomic and structural profile of microorganisms necessitating the development of efficient remediation technology. For structural elucidation of proteins that are capable of degrading pollutants extracted from microbes, the proteomic approaches (such as microarray, gel electrophoresis, and mass spectrometry) play a crucial role in the development of remediation technologies (Vega-Páez *et al*., 2019). Some genome-based techniques like PCR, DNA hybridization, exometabolomics, molecular connectivity, metabolic engineering, etc. are employed for a better understanding of the biodegradation process. Some molecular techniques which are PCR-based namely, randomly amplified polymorphic DNA analysis (RAPD), amplified fragment length polymorphisms (AFLP), automated ribosomal intergenic spacer analysis (ARISA), amplified ribosomal DNA restriction analysis (ARDRA), single strand conformation polymorphism (SSCP), length heterogeneity and terminal-restriction fragment length polymorphism (T-RFLP), are quite useful for genotypic fingerprinting (Hakeem *et al*., 2020).

**LIMITATION OF BIOREMEDIATION**

Though there are so many techniques present already and new techniques are emerging with time, they have some limitations as well. Bioremediation is a slow process. Usually, treatment takes more time than other remediation procedures. It does not completely purge the polluted site of all toxins. Not all organic compounds or inorganic pollutants can be treated using bioremediation. An in-situ bioremediation site needs very permeable soil. Because there is no set standard for what constitutes a "clean" location, performance evaluations are challenging and performance criteria regulations are unclear. It is challenging to tell whether pollutants have been eliminated. Certain substances may undergo microbial metabolism and degrade into more harmful metabolites or by-products, such as vinyl chloride when TCE is broken down, or less biodegradable PAHs when PAHs are broken down (carcinogens). If not regulated, these might be mobilized to groundwater. Controlling volatile organic compounds may be challenging if an ex-situ technique is used. Certain substances, such as heavy metals, radionuclides, and some chlorinated compounds, are not biodegradable (Kumar *et al*., 2018). The biopiling method depends on the degree of weathering because it can make the materials more hydrophobic which can cause changes in the chemical composition that ultimately lowers the efficacy of the biopiling method for biodegradation (Oualha *et al*., 2019). Some approaches of phytoremediation can only remediate the contaminants present in the topsoil, for example, phytoextraction and rhizodegradation.It is not possible to remove all the pollutants by adopting the phytoremediation approachand also it could be time-consuming (Capuana, 2020).

**CONCLUSIONS AND PERSPECTIVE FOR FUTURE RESEARCH**

Bioremediation is an environmentally friendly and cost-effective approach that employs biological agents to eliminate hazardous environmental pollutants. Bioremediation has been found to be a very fruitful technique for the enhancement of the natural biodegradation process. With emerging times, the bioremediation technique become a very effective and attractive option for remediating, cleaning, managing, and recovering techniques for solving environmental pollution through the utilization of microbial activity. Nowadays Omics has gained popularity among researchers in the microbial remediation field of the textile industry, dairy industry, wood industry,pulp and paper industry, food industry, fisheries, soil and water treatment industry, cleaning of solid waste, cleaning of heavy metal pollution, and hydrocarbon remediation. Therefore, tools of bioinformatics are very important to better analyze microbial degradation pathways, the data of bioremediation must be computerized for the formulation of new algorithms that can be applied in mathematical modeling, data assemblage, repositioning, exploration, and transmission which entail standard protocols. It can be useful to study new biomarkers for a better understanding of the bioremediation process and the microbial remediation process could be more precise if the omics data and the genetically engineered tools are combined with each other (Bala *et al.,* 2022). The phytoremediation process is a promising approach for remediation having a number of advantages like cost-effectiveness, greater public acceptance, and eco-friendliness which need to be further studied for better improvement in this area (Lee *et al*., 2021). Due to the growing environmental pollution which has been a threat to human beings and other organisms, bioremediation has gained prominence in recent days because of its harmlessness compared to other physicochemical techniques; hence more research needs to be done to bioremediation technology which can lead to the green and sustainable environment to make the world better for life.

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