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

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 produced to 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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I. INTRODUCTION

In regulated circumstances, organic wastes are biologically reduced throughout the bioremediation process to safe levels below the limit of concentration set by regulatory bodies (Mueller *et al*., 1996). Bioremediation research and application are now constrained by a lack of proper understanding of genome-based traits and genetics of the organism that is used, the involvement 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). Enhancement of the environmental conditions necessary for microbial activity and growth, the main focus of bioremediation techniques is to facilitate the biodegradation process that naturally occurs. The applications of bioremediation work by manipulating the different parameters of the environment to enable microbial activity and growth that fasten the degradation process (Margesin *et al.*, 2000).

One of the current worldwide worries for human sustainability is the contamination of surface water, soil, and groundwater with toxic chemicals as a result of industrialization. In order to advance sustainable development with minimal effect on the environment, it is imperative that toxic organic and inorganic pollutants be eliminated from contaminated environments. Due to its high expense and generation of secondary pollutants, the conventional way of treating contaminated soil, sediment, and water is found to be infeasible. With the aid of biological agents like bacteria, fungus, and other organisms or their enzymes, contaminated soil, surface water, and groundwater can now be restored using bioremediation, a natural, economical, and sustainable method (Kumar *et al*., 2018).

Therefore, bioremediation technology holds prominence in the research field. This review focused on the most recent research on bioremediation techniques, the ways in which bacteria break down various toxins, the significance of plants in remediation, and the potential of bioremediation to reduce global pollution.

II. PRINCIPLES OF BIOREMEDIATION

Using microorganisms to clean up a polluted area is known as bioremediation, an essential aspect of biotechnology. Although bacteria are primarily the degraders, other organisms, such as soil, animals or plant roots, also aid in the growth of bacteria by providing them with nutrients and co-substrates. (Romantschuk *et al*., 2000; Juwarkar *et al*., 2010**).**

Considering that bioremediation strives to improve natural processes and is widely known as "environmentally appropriate", it enjoys substantial public support in theory. However, compared to physical techniques like transferring the contaminated material to a secure landfill, bioremediation rates are frequently much slower (Prince,2010). Along with methods from molecular, genetic, microbiological, and protein engineering, the development of bioremediation depends on the development of novel metal-sequestering peptides, both rational and irrational route engineering, and enzyme design. (Singh *et al*., 2008). The inclusion of living creatures and nutrients can promote bioremediation, which occurs naturally. Biodegradation is the primary technological backbone of bioremediation. It describes the complete conversion of dangerous organic pollutants into innocuous occurring substances, such as water, inorganic componds and carbon dioxide, which are safe for aquatic life, terrestrial life, and human beings. (Hamer, 1993).

III. 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 discovered that inoculating bacteria like Bacillus subtilis with alfalfa plants considerably reduces the quantity of malondialdehyde (MDA) produced by the plant and increases the activity of its antioxidant enzymes and enzymes involved in the cycling of nutrients in the soil. Dual inoculation demonstrates the effectiveness of removing heavy elements like cadmium. (Li *et al*., 2021). A variety of microorganisms that can work concurrently or sequentially are used in bioremediation to detoxify hazardous contaminants. To put it another way, think of it as speeding up the natural metabolic process, whereby microorganisms (like fungi and bacteria), green plants (a process known as phytoremediation), or their enzymes break down or transform toxic contaminants into inorganic minerals, biomass from microbial sources, H2O, CO2, and other by-products (metabolites) whose toxicity is lower than the parent compounds. (Chakraborty et al.,2012). Bioremediation techniques can be differentiated 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. Since there is no need for excavation, the soil structure is not significantly disturbed. (Christopher *et al*., 2016). For in-situ bioremediation to be successful, a number of critical environmental conditions must be satisfied, including pH, moisture content, the presence of an electron acceptor, the availability of nutrients, and temperature. (Philp and Atlas, 2005). The in-situ bioremediation can be of two distinct types: intrinsic and engineered.

   - **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). With the aid of this technology, the particle swarm optimization (ELM-PSO) technique, the concentration of contaminants in groundwater was reduced from 40 parts per million to 5 ppm in three years. (Cecchin *et al*., 2021). Additionally, in shallow unsaturated soil, where microorganisms demonstrate their ability to survive and engage in subcellular activity, in situ bioremediation is utilized to remove Chromium (VI), which has been thought to be beneficial for the treatment of heavy metals. (Cecchin *et al*., 2021). Biosparginganaerobic reductive dechlorination, amendment delivery,aerobic treatment, and bioslurping are the example of intrinsic in situ bioremediation (Akubude*et al*., 2020).

- **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(GMO) such as bacteria and plants have played an important role (Verma *et al*., 2019).

- **Bioventing:** This technique involves stimulating regulated airflow by giving oxygen to the unsaturated (vadose) zone in order to enhance bioremediation by increasing the activity of indigenous bacteria. To achieve the microbial transformation of pollutants into a harmless state is the final objective of bioventing. Bioventing amendments are made by adding moisture and nutrients to improve bioremediation and are helpful for heavy metal decontamination. (da Silva *et al*., 2020).

- **Bioslurping:** This method uses a combination of vacuum-enhanced pumping, bioventing, and soil vapour extraction to treat soil and groundwater by indirectly supplying oxygen and encouraging pollutant biodegradation. (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).

- **Biosparging:** By injecting air below the surface of the ground, this method, which is very similar to bioventing, encourages the activity of microbes and aids in the removal of toxins from polluted areas. Unlike bioventing, air is forced at the saturated zone, which may drive volatile organic compounds upward to the unsaturated zone to speed up the breakdown process. The two main factors that govern how successful biosparging is the soil permeability, which influences the pollutant's bioavailability to microorganisms, and the pollutants' biodegradability. (Philp and Atlas, 2005). For the enhancement of the bioremediation process The microbes receives oxygen that take part in the biodegradation process and it is commonly used for the removal of kerosene and diesel from water supplies (Maitra, 2018).

- **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).

- **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, Evaporation of organic contaminants and rhizodegradation, also referred to as rhizoremediation 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. Due to these native plants, natural plants can be bioaugmented by native plants, or it can be a combination of both, in the contaminated sites, changing the nature of the plant and the contaminants. The bulk of the hyperaccumulator plants at the metal-polluted site can also perform phytoremediation directly. (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 Loropetalumchinense*. They take part in the phytostabilization of Cd (Zeng *et al*., 2018).

2. **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).

   - **Biopiling:** In order to accelerate bioremediation by effectively boosting microbial activity, biopile-mediated biological remediation requires piling up excavated contaminated soil above ground, followed by nutrient replacement and occasionally aeration. This plan includes an irrigation system, nutrient-collection devices, aeration, and a treatment bed. Due to its beneficial qualities, such as the effectiveness of costs, which enables effective biodegradation given that temperature, nutrients, and aeration are appropriately maintained, the application of this specific ex-situ approach is being investigated more frequently. (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. Wood chips, sawdust, or straw can be added to improve cleanup, while ex-situ bioremediation processes including land farming, bioventing, and biosparging can be used to resupply the contaminated soil biopiles with air. (Arora et al., 2022). Bio-available organic carbon 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 circumstances Low oxygen rate, 30°–40°C, and temperatures (Naeem and Qazi, 2020).

- **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. Continuous turning of the polluted soil and the addition of water enhanced uniform distribution of contaminants, aeration, nutrients, and microbial degradation activities. (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).

- **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. The type of biological remediation is divided into in-situ and ex-situ categories depending on where the treatment takes place. In-situ bioremediation occurs on-site, whereas ex-situ bioremediation occurs when polluted materials, such as contaminated soil and sediments, are carried to the site of land farming.. 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).

- **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).

- **Bioreactor:** Bioreactors provide the ideal conditions for the growth of microorganisms. These conditions include manageable regulation of agitation, pH temperature, inoculums concentration, aeration, and substrate 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.

IV. APPROACHES OF BIOINFORMATICS IN BIOREMEDIATION

In recent years, the use of bioinformatics tools and techniques has attracted much attention in the research ares of bioremediation. Various omics-based technologies such as proteomics, genomics, 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 techniqies 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 clear understanding of the biodegradation process. Some molecular techniques which are PCR-based namely, Randomly Amplified Polymorphic DNA (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).

V. 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).

VI. CONCLUSIONS AND PERSPECTIVE FOR FUTURE RESEARCH

In order to remove dangerous environmental pollutants, bioremediation uses biological agents in an economical and environmental friendly manner. Bioremediation has been found to be a very fruitful technique for the enhancement of the natural biodegradation process. The bioremediation technology, which uses microbial activity to remediate, clean, manage, and recover methods for resolving environmental contamination, has grown increasingly popular and effective in recent years. Omics has recently grown in popularity among scientists working in the field of microbial remediation for the textile, dairy, wood, pulp and paper, food, fisheries, soil and water treatment, cleaning of solid waste, cleaning of heavy metal pollution, and hydrocarbon remediation industries. As a result, bioinformatics tools are crucial for improving the analysis of microbial degradation pathways. Data from bioremediation also needs to be computerized so that new algorithms can be developed for data collection , repositioning, exploration, and transmission that follow set standards. If the omics information and the genetically modified tools are coupled, it can be advantageous to investigate new biomarkers for a better understanding of the bioremediation process and the microbial remediation process could be more exact. (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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