Bioremediation

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ABSTRACT

Environmental degradation is a result of growing population density and industrialization. The environment is full of pollutants due to their toxicity and nonbiodegradability, including heavy metals, polychlorinated biphenyls, plastics, and various agrochemicals. A technique gaining popularity is bioremediation, which removes toxic waste from polluted environments. Different techniques, including *in* situ and *ex* situ are used in bioremediation to treat polluted sites. There are different types of factors (biological factors, oxygen availability, moisture content, nutrients availability, temperature, pH, site characterization, metal ions and also microorganisms) that determine the rate of biodegradation. Various biological systems (microbe-plant-based bioremediation) play a major role in bioremediation. The most recent developments in bioremediation techniques influence the breakdown different pollutants by microorganisms. This review could also be useful for further research in order to improvise the efficiency of degradation of different pollutants.

Keywords— bioremediation; bioaugmentation; biostimulation; phytoremediation; omics.

I. INTRODUCTION

One of the most important ancient Indian texts, along with the Vedas, Puranas, and Upanishads, is Bhagavad Gita, which is also known as the "Song of the Lord" [1]. The following words in Chapter 14, verse 3-4 expressly state that nature is the mother of all living things.

मम योनिर्महद् ब्रह्म तस्मिन्गर्भं दधाम्यहम् |
सम्भव: सर्वभूतानां ततो भवति भारत || 3||
सर्वयोनिषु कौन्तेय मूर्तय: सम्भवन्ति या: |
तासां ब्रह्म महद्योनिरहं बीजप्रद: पिता || 4||

mama yonir mahad brahma tasmin garbhaṁ dadhāmy aham
sambhavaḥ sarva-bhūtānāṁ tato bhavati bhārata
sarva-yonişhu kaunteya mūrtayaḥ sambhavanti yāḥ
tāsāṁ brahma mahad yonir ahaṁ bīja-pradaḥ pitā

Meaning: *The total material substance is Brahma, the prakṛiti. It is the womb, from where the livings are born. I impregnate it by providing distinct souls, and make the birth possible for all living beings.*
Explanation: The mother of all living things is nature. The life can only be created by nature [2].

The environment encompasses all the natural surroundings that impact our everyday lives on Earth. The presence of a safe and healthy environment is crucial for the survival of life on this planet [3]. Earlier, it was believed that we had an unlimited abundance of land and resources; however, today the resources in the world demonstrate, to a greater or lesser extent, our lack of caution and negligence in their utilization [4]. The enormous rise in the global population has resulted in the increased exploitation of natural resources and sources to meet the population's high needs for food, energy, and all other necessities [5]. Human beings have witnessed technological advancements in food production, health, infrastructure, transportation, and communications since the beginning of the 20th century. These activities require a massive amount of new materials and energy, destroying natural environmental components and generating massive amounts of trash, resulting in environmental deterioration [6]. It is estimated that approximately 1000 new chemical compounds are synthesized annually. The Third World Network (TWN) reports that toxins are emitted into the air and water on a global scale in excess of 450 million kilogram [7]. The pulp and paper industry is the sixth largest polluter (after the oil, cement, leather, textile, and steel industries), emitting a wide range of gaseous, liquid, and solid wastes into the environment [8].

Hazardous metals and metalloids in waste are generated by the industrial, residential, and agricultural sectors, which cause enormous harm to the ecosystem [9]–[11]. Water quality has deteriorated as a result of human activities such as mining and the ultimate removal of toxic metal effluents from steel mills, battery plants, and electricity generation, which are major environmental threats. Heavy metals are pollutants that naturally occur in the Earth's crust and are difficult to break down. They are found in rocks as ores and retrieved as minerals. Heavy metals are released into the environment as a result of high-level exposure. Once in an ecosystem, they may remain hazardous for a much longer [12]. Metal deposited in biological tissues is difficult to remove because of its non-biodegradability, and it has become a major concern for global health [13]. Metal pollution alters the physicochemical and biological features of soil, such as an increase in bulk density and pH as well as a decrease in soil fertility, water holding capacity, microbial diversity, and soil enzyme activity [14]–[16]. Heavy metals such As, Hg, Ni, Cr, Pb, and Cu can have various indirect and direct effects on plant growth, including chlorosis, necrosis, root injury, decreased carotenoid concentration, oxidative stress, enzyme inhibition, osmotic imbalance, decreased photosynthetic activity, and nutrient imbalance [17]–[22]. Xenobiotics are chemical compounds that are not naturally produced or are expected to be present within organisms. The term "xenobiotic" is commonly used in the context of environmental contaminants to refer to synthetic substances produced in large quantities for industrial, agricultural, and domestic uses [23]–[25]. Environmental xenobiotics such as pesticides, polycyclic aromatic hydrocarbons (PAHs), pharmaceutically active compounds (PhACs), personal care products (PCPs), phenolics, chlorinated compounds, and other industrial chemicals are potent threats to the environment. Their increasing frequency in several environmental compartments has raised concerns regarding their potential negative consequences. Its toxicity causes extraordinary health concerns and risks to environmental safety and security [3]. Pollutants are highly mobile and soluble, allowing them to bioaccumulate in the food chain and cause catastrophic damage with increasing tropic levels [26], [27]. When these pollutants enter the human body, they can cause cancer, kidney and bone diseases, cardiovascular diseases, high blood pressure, low birth weight, Alzheimer's disease, and atherosclerosis [28]–[33]. Various physicochemical approaches (such as extraction, immobilization, stabilization, coagulation, electrodialysis, vitrification, reverse osmosis, ion exchange, chemical reduction, evapotranspiration, and precipitation) have been used to degrade and detoxify heavy metals and xenobiotic compounds [34], [35]. However, these approaches are expensive, consume a lot of energy, use harsh chemicals with low removal efficiencies, and can cause secondary environmental contamination [36].

The stability of the Holocene climate supports the current growth and development of modern human society. However, the unstable Holocene climate was grossly misused by unbridled consumption, without genuine attention to the environment. Furthermore, as a result of such carelessness, the entire wilderness of the earth has been dramatically reduced to only 35% of what it once was [37]. Global climate change is influenced by factors such as global warming, polar ice meltdown, biodiversity reduction, and extinction of significant wildlife species [38]. Human activities not only damage but also destroy our ecosystem [37].

Water is undoubtedly one of the most important commodities on Earth. The surface of the earth occupies 71% of the water. There was 97% marine water and 3% freshwater. The agricultural sector consumes most of the freshwater, but the chemicals used in agriculture to promote crop productivity, such as pesticides, agrochemicals, sediments, organic matter, drug residues, and fertilizers, are extremely damaging to both surface and underground water [39], [40]. Contaminated water is dangerous to both animals and humans, resulting in diseases such as diarrhea, cholera, dysentery, typhoids, and polio [41]. Pesticides are highly spoiled contaminants in water bodies. Pesticides are chemical substances used by the agricultural industry to boost crop output [42]. Although an ecosystem tends to dilute pollutants, significant pollution in aquatic ecosystems results in changes in the flora and fauna. Small amounts of pesticides can also be fatal. The toxicity was determined based on the duration of exposure. Proper water treatment is required because the biomagnification of deadly pesticides in water results in the loss of biodiversity, animals, plants, and microbes [43]. In addition, corals die as a result of increasing ocean-water pH [44]. Corals are critical for underwater biodiversity [45]. Furthermore, ocean contamination is increasing owing to plastics and crude oils, which are not compatible with corals [46]. However, we are still heavily reliant on hydrocarbon oils [47]. As a result, calamities such as oil leaks in the middle of the oceans have become common events [48]. In addition, the air we breathe is not very good [49]–[51]. According to a recent assessment, the air quality index (AQI) in various cities is in severe condition [52], [53]. Furthermore, the release of large amounts of greenhouse gases, such as CO_2 and methane, has harmed human and animal respiratory health [54], [55].

Bioremediation is one way to protect the environment from catastrophic damage [56]. Bioremediation is an environmentally sound technique that uses green plants, microorganisms such as fungi, bacteria, yeast, and algae, or their enzymes to assist polluted sites in restoring their original conditions [57], [58]. Bioremediation by enzyme engineering uses directed evolution and rational and semi-rational methodologies to increase the activity of microbial enzyme [59]–[61]. The late 19th century was considered the golden era of bioremediation. With further advancements, the 20th century witnessed the beginning of research in the field of microbial ecology, which involved the identification and isolation of microbes with the potential to degrade pollutants, such as *Candidatus* *accumulibacter*, which is capable of accumulating excess phosphorus as polyphosphates in their cells from sewage treatment plants [62]. The selection of microorganisms is based on the contaminated area because every microbe requires a different pH, temperature, and moisture for activation. Microbes used in this process are also known as bioremediators. This process is simple to carry out and does not disrupt human lives or the environment during conduction and transportation [63]. Several factors influence bioremediation for more effective outcomes, such as the surrounding environment temperature, aerobic or anaerobic conditions, and nutrient availability [64]. Waste management depends primarily on bioremediation. It can eliminate persistent organic contaminants that are difficult to break down and suspected to be heterologous biological compounds [65]. Bioremediation is not a new concept in the human race, but novel techniques resulting from improvements in molecular biology and process engineering are emerging [66]. Thus, implementing and enhancing these methods will result in economic and social benefits, such as reduced risks of diseases and expenses associated with waste disposal, enhanced ecological stability, and a greener environment [67].

II. PRINCIPLES OF BIOREMEDIATION

In bioremediation, there is a distinction between "bios" and "remediate", which refers to living organisms and to solve problems. The term "bioremediate" refers to the use of biological organisms to resolve environmental problems caused by contaminated soil or groundwater [68]. According to the United States Environmental Protection Agency (USEPA), bioremediation is the "use of living organisms to clean up or remove pollutants from soil, water, or wastewater; use of organisms such as nonharmful insects to remove agricultural pests or counteract diseases of trees, plants, and garden soil" [69]. By definition, bioremediation is the use of living organisms, primarily microbes, to breakdown environmental pollutants into less hazardous forms. It employs naturally occurring bacteria, fungi, and plants to degrade or detoxify substances that are dangerous to human health and/or the environment. Most bioremediation systems operate under aerobic circumstances, however anaerobic conditions can be used [70]. In the Earth's biosphere, microorganisms can be found in a wide variety of environments. They grow in soil, water, plants, animals, the deep sea, and the frozen ice environment. The sheer numbers of microorganisms and their voracious appetites for chemicals make microorganisms the perfect environmental stewards [71].

Bioremediation is an emerging and widely accepted practice for restoring heavy metal contaminated soils due to its environmental friendliness and low cost when compared to other conventional methods such as dredging, capping, and incineration, which are often very expensive and ineffective when metal concentration levels are low and frequently generate a significant amount of toxic byproducts [72], [73]. A study has been shown that cleaning metal polluted sediments and soils through landfilling and chemical treatment costs approximately 100-500 USD/ton, while bioremediation costs approximately 15-200 USD/ton and phytoremediation costs approximately 5-40 USD/ton [74]. It is estimated that bioremediation can save 50-65% of the cost of cleaning one acre of Pb-contaminated soil as compared to typical excavation and landfill [75], [76]. Furthermore, bioremediation is a non-invasive technology that can eliminate toxins permanently while leaving the ecosystem unharmed and can be combined with chemical and physical treatments [77]. The bioremediation techniques are totally based on natural biological potency. Most bioremediation techniques are dependent on soil structure, pH of polluted sites, moisture content, pollutants type, nutrient addition, microbial diversity, and temperature of the treatment site [78], [79]. Natural attenuation is a bioremediation process that naturally occurs in polluted areas [36]. The aim of bioremediation is to put microorganisms to work by providing optimal quantities of nutrients and other chemicals required for their metabolism in order for them to degrade or detoxify pollutants that are hazardous to the environment and all organisms. All metabolic reactions are mediated by enzymes. A wide variety of enzymes are involved in these reactions, including oxidoreductases, hydrolases, lyases, transferases, isomerases, and ligases. Due to their nonspecific and specific substrate affinity, several enzymes have a remarkable degradation capacity [80]. Biodegradation is the basic principle of bioremediation [81]. Putting that aside, it is important to point out that biodegradation and bioremediation are not the same thing. In bioremediation, biodegradation is only one of the mechanisms associated with or applied as part of the process. There are only some contaminants that are biodegradable, and only some microorganisms are capable of degrading them [82].

The first patent for a biological remediation agent was granted in 1974, being a strain of *Pseudomonas putida* that was able to break down petroleum. Around 70 microbial genera were found to breakdown petroleum compounds in 1991, and almost an equal number have been added to the list in the subsequent two decades. *Geobacter metallireducens* is a relatively new addition to the growing list of microbes capable of sequestering or reducing metals. This bacterium can remove uranium, a radioactive contaminant, from mine drainage waters and from polluted groundwaters. However, *Deinococcus radiodurans* is the most radiation-resistant bacteria; this organism is also being developed to assist clean up soil and water contaminated by solvents, heavy metals, and radioactive waste. A genetically engineered strain of *D. radiodurans* has been developed that can detoxify mercury (genes obtained from *Escherichia coli*) and breakdown toluene (genes obtained from *Pseudomonas putida*) in radioactive environment [83].

From the standpoint of future prospects of bioremediation, it seems that the advancement of our knowledge of microbial populations, their interactions with the natural environment and contaminants, the increase of their genetic capabilities to degrade contaminants, and long-term field studies of new economical bioremediation techniques can increase the potential for significant developments. There is no doubt that bioremediation is a current requirement that can lead to the protection and preservation of natural resources that we have depleted from the future generations [82].

III. TECHNIQUES INVOLVED IN BIOREMEDIATION

The United State Environmental Protection Agency has defined two bioremediation methods: *in situ* and *ex situ* [84]. Both *in situ* and *ex situ* remediation techniques work on the principle of biotransformation or biodegradation, involving the removal, mobilization, immobilization, or decontamination of various contaminants from the environment by the action of microorganisms (bacteria, mold or fungi, and yeast) and plants [85].

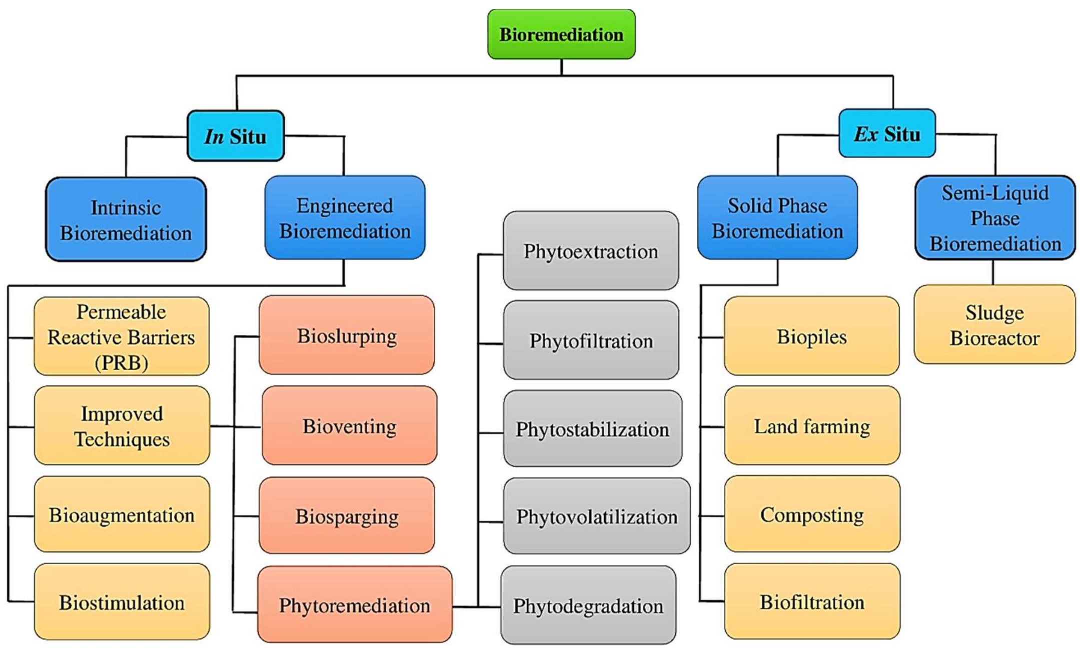

Figure 1: Different bioremediation techniques.

A. *In situ* Bioremediation

The technique involves the implementation of a biological treatment to clean up harmful substances and has been widely used to degrade pollutants in saturated soils and groundwater [86]–[88]. It relies on the microbial activity to destroy and detoxify of pollutants present in a place. Bioremediation through *in situ* becomes more sustainable because it eliminates the need for transport, contaminated soil deposition, groundwater pumping, treatment, and discharge to recipients. Additionally, it provides many advantages such as cost effectiveness, the use of native harmless microbial species, and the ability to treat large volumes of contaminated soil or water with less release of toxic contaminants. The *in situ* bioremediation method has mostly been used to degrade anilines, chlorinated hydrocarbons, nitrobenzenes, nitriles, and plasticizers in soil and groundwater [89]. *In situ* bioremediation under anaerobic circumstances may also be aided by the addition of electron acceptors such as nitrate or sulfate [90]. The selection of one organism or a consortium of organisms with the potential ability to detoxify the targeted metals is a difficult challenge for *in situ* bioremediation. In lab-scale, it was emerged that Fe^{3+} and sulfate-reducing microbes have the enzymatic ability to biodegrade heavy metals such as U(VI), Tc (VIII), Cr (VI), and Co (III) [91]–[93]. Also, *Geobacteraceae* sp. were identified to be a dominant group during the stimulation process for decreasing $Fe^{3+,}$ and members of this group were detected during the stimulation process for reducing U(VI) of contaminated Aquifer. As a result, the *Geobacteraceae* family was treated to play a crucial role in the stabilization of pollutants and the reduction of metals within underground ecosystems [94]. *In situ* techniques can be categorized as intrinsic and engineered bioremediation [95].

1. Intrinsic Bioremediation

Intrinsic bioremediation is a natural degradation process that relies strictly on the metabolism of native microorganisms to remove harmful pollutants, with no artificial stage to boost biodegradation activity. Intrinsic bioremediation also known as passive bioremediation or natural attenuation [96], [97].

The criteria for the application of intrinsic bioremediation are the appropriate population of biodegrading microbes in the contaminated site; optimum circumstances in the environment (temperature, pH, humidity threshold, O_2 concentration); carbon and nitrogen supplies available to support microbial activity and growth; enough time for microorganisms to change contaminants into less hazardous products [97]. In microbial communities, hydrocarbon-degrading ability is common because native microorganisms are already adapted to site conditions and have evolved a relationship with hydrocarbons [96]. Intrinsic *in situ* bioremediation can be accomplished through anaerobic reductive dechlorination, aerobic treatment, amendment administration, biosparging, and bioslurping [65]. *In situ* bioremediation has been employed to remediate blocked groundwater using a stimulation-optimization methodology powered by machine learning and particle swarm optimization (ELM-PSO) techniques [98]. *In situ* remediation has also been searched for the decontamination of Cr (VI) in shallow unsaturated soil. Microorganisms may survive in soil with high concentrations of Cr (VI), and their subcellular machinery was used to interact with heavy metals. Microbial inoculants can be used for heavy metal in situ treatment [99]. Cr (VI) interacts with Fe (II) ions by redox reactions, and the release of iron in soluble forms stimulates the reductive reactions [100]. However, before implementing intrinsic bioremediation, a risk assessment should be conducted to guarantee that the time required to complete bioremediation is less than the time required by the pollutant to reach the nearest site of human and animal exposure [101].

2. Engineered Bioremediation

In the second technique, a specific microorganism is introduced into the contaminated site. *In situ* bioremediation is a technique that uses genetically engineered microbes to accelerate the degradation process [65]. Engineered bioremediation is the modification and customization of physicochemical conditions promoting the growth of introduced microorganisms and hence fastening the bioremediation process [102].

2.1. Permeable Reactive Barriers

Permeable Reactive Barriers (PRBs) are an in-situ techniques utilized to remediate groundwater contaminated through different pollutants such as chlorinated hydrocarbons and heavy metals [103]. A permanent or semipermanent reactive barrier formed mostly of iron is immersed in the contaminated groundwater stream [104]. When polluted water naturally flows across the barrier, the pollutants are retained and react, releasing cleared water [105]. PRBs should be sufficiently reactive to capture pollutants, permeable enough to allow the water flow, passive with minimal energy consumption, and affordable [106]. The efficiency of such a technique is dependent on the type of medium, which in turn is based on the type of pollutant, environment, health consequences, biogeochemistry, hydrogeology, system stability, and cost [107]. PRBs have been combined with other methods to remediate several kinds of contaminants in the past few decades [108].

2.2. Improved Techniques

2.2.1. Bioventing

Bioventing is a technology that delivers oxygen to the unsaturated zone to stimulate the activity of indigenous microorganisms for bioremediation. The addition of nutrients and moisture during the bioventing process helps the bioremediation process. Microorganisms will transform pollutants into harmless compounds as a result of this process [109]. Bioventing is a technique that utilizes aeration to stimulate the indigenous microflora in order to improve the biodegradation ability of the various bacteria and encourage the precipitation of heavy metal contaminants [110]. The proportions of nutrients and humidity are maintained to achieve pollutant transformation. This method has been utilized successfully in the remediation of oil-contaminated soils [111]. Bioventing may be more efficient in anaerobic biodegradation, and combining nitrogen with oxygen will enhance the potency of chlorinating remediation [102].

Sui and Li [112] evaluated the influence of air injection rate on the volatilization, biodegradation, and biotransformation of a toluene-contaminated site by bioventing. It was observed that there was no significant difference in pollutant (toluene) elimination at the end of the study period (200 days) at two different air injection rates (81.504 and 407.52 m^3 /d). However, at an earlier stage of the study (day 100), it was found that higher air injection rates resulted in greater toluene elimination via volatilization than lower air injection rates. In other words, increasing the airflow rate does not increase the rate of biodegradation nor make pollutant biotransformation more effective. This is due to early saturation of air in the subsurface (either a high or a low air injection rate) for oxygen demand during biodegradation. Nonetheless, the low air injection rate increased biodegradation significantly. It therefore indicates that air injection rate is one of the fundamental parameters for pollutant dispersal, redistribution, and surface loss in bioventing.

Unlike bioventing, which depends on modest air input to enhance microbial degradation at the vadose zone, soil vapour extraction (SVE) increases volatile organic compound volatilization through vapour extraction [113]. Despite the fact that both techniques use same technology, the configuration, philosophical design, and functioning differ significantly [114]. The airflow rate in SVE is higher than in bioventing. SVE may be considered a physical form of remediation because to its pollutant removal process; however, the pollutant removal mechanisms for both techniques are not mutually exclusive. During on-site field trials, achieving similar results obtained during laboratory studies is not always possible due to other environmental factors and different characteristics of the unsaturated zone to which air is injected; as a result, with bioventing, treatment time may be prolonged. Apparently, high airflow rates facilitates the transfer of volatile organic compounds to the soil vapour phase, requiring off-gas treatment of the consequent gases before to discharge into the atmosphere [115]. This particular difficulty can be overcome by combining bioventing and biotrickling filter techniques to minimize both contaminant and outlet gas emission levels, hence decreasing the treatment time associated with bioventing alone [113].

2.2.2. Bioslurping

This technology combines vacuum-enhanced pumping, soil vapour extraction, and bioventing to achieve soil and groundwater remediation by indirect oxygen provision and pollutant biodegradation stimulation [116]. This technology is intended for the recovery of free products such as light non-aqueous phase liquids (LNAPLs), thus remediating capillary, unsaturated, and saturated zones. It can also be used to remediate soils that has been contaminated with volatile and semi-volatile organic substances. The method utilizes a "slurp" that extends into the free product layer and sucks liquids (free products and soil gas) from this layer in a way similar to how a straw takes liquid from any vessel. The pumps move LNAPLs upward, where they separate from air and water [117]. During vacuum extraction of LNAPLs, the bioslurping tube begins to eliminate vapors from the unsaturated area as the fluid level in the well decreases. Steam extraction promotes the movement of soil gases, which improves aeration and aerobic decomposition. After all toxins have been removed, the facility can be used for typical bioventing to complete bioremediation. The bioslurping system should only be placed if the contaminants are no deeper than 7 m below the soil surface because the vacuum pump is inefficient in sucking LNAPLs at larger depths. The main disadvantage is excessive soil moisture inhibits air permeability and decreases oxygen transfer rate, which reduces microbial activity [109]. Although this technique is not appropriate for low permeable soil remediation, it is a cost-effective operation process since it uses less ground water and reduces storage, treatment, and disposal expenses [71].

2.2.3. Biosparging

This technique is similar to bioventing in the context of delivering the air into the soil subsurface to stimulate microbial activity and increase pollutant removal from polluted soil. However, unlike bioventing, air is injected at the saturated zone, which can trigger upward migration of volatile organic compounds to the unsaturated zone, promoting biodegradation. The efficiency of biosparging is determined by two key factors: soil permeability, which determines contaminant bioavailability to microorganisms, and contaminant biodegradability [118]. Biosparing operation is closely associated technology known as in-situ air sparging (IAS) in bioventing and soil vapor extraction (SVE), which rely on high air-flow rates for pollutant volatilization, whereas biosparging encourages biodegradation [119]. Biosparging has been widely used to treat aquifers polluted by oil derivatives, primarily kerosene and diesel, which have good biodegradation of the BTEX group and naphtalenes [120]. Aerobic bacteria can be utilized to degrade mineral oils, BTEX, and naphtalenes. However, the deepest layers of soil and groundwater are primarily anaerobic. To encourage the growth of aerobic microorganisms, injection filters infuse oxygen into the soil and groundwater [109].

A study by Kao [121] reported that biosparging of a benzene, toluene, ethylbenzene, and xylene (BTEX)-contaminated aquifer plume resulted in a transition from anaerobic to aerobic conditions; this was demonstrated by increasing dissolved oxygen, redox potentials, nitrate, sulphate, and total culturable heterotrophs, with a corresponding decrease in dissolved ferrous iron, sulphide, methane, and total anaerobes and methanogens. The overall decrease in BTEX reduction (>70%) implies that biosparging can be utilized to remediate BTEX contaminated ground water. The primary limitation is forecasting the direction of airflow.

2.2.4. Phytoremediation

Phytoremediation can be used to clean up contaminated soils. This approach reduces pollutant toxicity in contaminated areas by utilizing plant interactions at the physical, biological, chemical, biochemical, and microbiological levels. Depending on the quantity and form of the contaminant, phytoremediation employs a wide range of techniques [122]. Elemental pollutants, such as heavy metals or radioactive elements, are primarily removed, transformed, and sequestered, whereas organic contaminants are primarily eliminated through rhizodegradation, biodegradation, vaporization, or stabilization [123]. Plants interact with pollutants in a variety of ways within phytoremediation [124].

2.2.4.1. Phytoextraction

Phytoextraction (also known as phytoaccumulation, phytoabsorption, or phytosequestration) is the process of removal of contaminants from soil or water by plant roots, followed by their transfer and accumulation in aboveground biomass, that is shoots, which are subsequently harvested [125], [126]. Pollutant translocation to shoots is an important biochemical step desirable for effective phytoextraction, because harvesting root biomass is often not practical [127], [128]. Continuous phytoextraction can use of plants that accumulate significant quantities of contaminants over their lifecycle [129]. In general, the phytoextraction process consists of four basic steps: pollutant mobilization in the rhizosphere, pollutant uptake by plant roots, translocation into aerial plant parts, and pollutant sequestration in plant tissue [130], [131]. Pollutant tolerance is required for the phytoremediation process because strong tolerance of plant tissues can be accompanied by little unfavorable effects on plant health. In general, cell wall metal binding, active transport of metal ions into vacuoles, chelation of metal ions with proteins and peptides, and complex formation all contribute to a plant's pollution tolerance potential [130]. The depth available for plant root growth, seasonal weather, and climatic variables are all factors that influence phytoextraction effectiveness [132]. The use of mobilizing agents such as citric acid, ethylenediaminetetraacetic acid, nitrilotriacetic acid, aminopolycarboxylic acids, and ethylenediaminedisuccinic acid can improve the efficiency of phytoextraction [133].

2.2.4.2. Phytofiltration

Phytofiltration, also known as rhizofiltration, involves the adsorption or precipitation of contaminants from solution onto plant roots, as well as absorption into the roots encompassing the root zone [134]. Its mechanism is associated with the creation of specific compounds inside the roots, which result in the adsorption of pollutants, because some plants may contain multiple phytochelatins to improve the binding capacity of pollutants such as metal ions [135]. Rhizofiltration can be easily linked to effluents, polluted streams, or groundwater frameworks. The success of rhizofiltration needs a thorough understanding of pollutant speciation and the interactions of all contaminants and nutrients. An ideal plant for rhizofiltration should have rapidly growing roots that can remove pollutants from solutions over long periods of time [69].

2.2.4.3. Phytostabilization

Phytostabilization or phytoimmobilization is the process of using plants with the ability to reduce the mobility and bioavailability of pollutants in order to prevent their leaching into ground water or entry into the food chain through various mechanisms such as adsorption by roots or the formation of insoluble compounds in the root zone [129], [134]. Phytostabilization can be defined as (a) limiting a pollutant in contaminated media through assimilation and aggregation by roots, adsorption onto roots, or precipitation within the root region of plants, and (b) deploying plants and plant roots to avoid contaminant movement through wind and water, draining, and soil dispersion [136]. The ultimate goal of phytostabilization is to stabilize pollutants rather than remove them, reducing their risk to human health and the environment, with the intention that the plants play a similar role with soil amendments. Unfortunately, phytostabilization is not a permanent solution to contamination because it reduces the contamination of neighboring media or area rather than the concentration of contaminants [137], [138]. As a result, phytostabilization has been recognized as one of the most experimental forms of phytoremediation, with potential use for various metals, particularly lead, chromium, and mercury that are stabilized in soil and decrease the interaction of these pollutants with associated biota [133], [139].

2.2.4.4. Phytovolatilization

Another phytoremediation approach, phytovolatilization, uses plant-mediated absorption of pollutants to convert them into volatile compounds, which are then released into the atmosphere in the same or altered form due to metabolic and transpiration pull [134]. Transpiration is the evaporation of water vapors from leaf surfaces into the atmosphere via stomata. Certain plant species with extensive root systems are often capable of absorbing and degrading pollutants through the development of specific enzymes or genes [140]–[142]. Pollutants are taken up from the soil or water during phytovolatilization and transformed into less hazardous vapors, which are subsequently discharged into the atmosphere via the plant’s transpiration process [138]. The method is applicable to organic contaminants and some heavy metals, such as As, Se, and Hg, which occur in the environment as gaseous species [143]. Thus, the phytovolatilization technology often utilizes genetically engineered plants to improve the ability of plants to volatilize metals [138]. Furthermore, phytovolatilization involves little erosion and no disposal of contaminated plant biomass, as well as minimal site disturbance [138]. Phytovolatilization thus is one of the most controversial phytoremediation processes [144], [145].

2.2.4.5. Phytodegradation

Phytodegradation, also known as phytotransformation, is the capture of contaminants and nutrients from water, sediment, or soil, followed by chemical modification of contaminants as a direct result of plant metabolism, often resulting in contaminant inactivation, degradation, or immobilization in plant roots and shoots [146], [147]. Some plants can convert the absorbed pollutants into less hazardous chemicals through the plant's metabolic process or enzymes [140]. Thus, phytodegradation is a metabolic method used by plants to detoxify and degrade pollutants within the plant tissues [133].

2.3. Bioaugmentation

In bioaugmentation, the autochthonous microflora of the polluted site is supplemented by adding previously selected indigenous or genetically engineered species of microorganisms to improve the remediation process. Bioaugmentation is utilized in soils and groundwater contaminated with tetrachloroethylene and trichloroethylene to ensure that *in* situ microorganisms degrade these toxins to harmless chemicals such as ethylene and chlorides [148].

2.4. Biostimulation

Biostimulation is the use of native microorganisms that are stimulated to proliferate by the addition of nutrients such as phosphorus and nitrogen, as well as oxygen or other oxidizing agents. Stimulating compounds are often applied underground via injection wells. The implementation of well-adapted autochthonous microorganisms is the main advantage of this method. Recently, it has been proposed that both of these procedures, despite categorized as *in* situ bioremediation approaches, can also be used ex situ [149], [150].

B. *Ex situ* Bioremediation

This method involves digging contaminants from polluted areas then transporting them to another area for treatment. *Ex* situ bioremediation procedures are used when the depth of contamination, kind of pollutant, treatment cost, and geographical location of the polluted site are all taken into consideration [71]. The technology is further classified into solid-phase and slurry-phase systems based on the state of the pollutant to be eliminated [151]

1. Solid Phase Bioremediation

This technique consists of four steps: excavation of the soil, piling of the soil (which may contain municipal, agricultural, and organic wastes), stimulation of the biodegradation process by supplying oxygen through a network of pipes to enhance microbial respiration or subsequently microbial activity, and the use of microbial stripping columns used to treat air emissions, followed by biofiltration. Solid-phase bioremediation needs a huge amount of space and time to be finished [152]

1.1. Biopiles

Aeration and nutrient supplementation are employed in bioremediation to boost microbial metabolic activity in piled-up toxic soil above ground. This technique includes aeration, nutrients, irrigation, leachate collection, and treatment bed systems. *Ex* situ biodegradation is becoming increasingly popular due to its low cost and useful features such as pH and nutrient management. The biopile has the potential to be used to clean up polluted cold environments and cure low-molecular-weight volatile contaminants [153], [154]**.** The adaptability of the biopile allows for a reduction in remediation time by increasing microbial activity and pollutant availability while simultaneously enhancing biodegradation rate. Bioremediation is improved when warm air is supplied into the biopile system by providing both air and heat at the same time. The inclusion of bulking agents such as straw, sawdust, or wood chips has aided the biopile's cleanup process. Ex situ bioremediation techniques such as land farming, biosparging, and bioventing can be used to refill the air supply to contaminated piled soil in biopiles [65]. These techniques, are costly to adopt and require a power supply in remote locations. Extreme air temperatures may impede bioremediation by drying soil and making it more prone to be evaporated rather than broken down by living organisms [155]. Bio-available organic carbon (BOC) plays an essential role in bioremediation using the biopile method. Petroleum-contaminated soil was bioremediated with alpha, beta, and gamma proteobacteria under mesophilic conditions (30°C-40°C) with a modest aeration rate [156].

Gomez and Sartaj [157] used response surface methodology (RSM) based on factorial design of experiment (DoE) tone to investigate the effects of different application rates (3 and 6 ml/m^3) of microbial consortia and mature compost (5 and 10%) on total petroleum hydrocarbon (TPH) reduction in field-scale biopiles at low temperature conditions. At the end of the 94-day trial period, the bioaugmented and biostimulated setups had 90.7% TPH decrease compared to the control setups, which had 48% average TPH elimination. TPH reduction was ascribed to a significant proportion.

Although biopile systems preserve space when compared to other field *ex* situ bioremediation techniques such as land farming, robust engineering, maintenance and operation costs, and a lack of power supply, particularly at remote sites, which would enable uniform distribution of air in contaminated piled soil via air pump are some of the limitations of biopiles. Furthermore, excessive air heating can cause drying of bioremediation soil, which inhibits microbial activity and promotes volatilization rather than biodegradation [158].

1.2. Landfarming or Prepared Bed Bioreactors

Land farming is the most significant and easy bioremediation method because of its cheap operating expenses and absence of specialist equipment [159]. Ex situ bioremediation is the most typical approach, however in situ bioremediation can also occur. This is due to the location of the treatment. It is normal practice in land farming to remove and till polluted soils on a regular basis, and the type of bioremediation used is determined by the site of treatment. In situ treatment refers to on-site treatment, whereas ex situ bioremediation procedures are utilized to treat contaminated soil [160]. Extracted contaminated soils are typically deposited on a permanent layer of substrate much above the Earth's surface to allow native microorganisms to breakdown pollutants aerobically. Land bioremediation of dirty soil utilizing land farming bioremediation technology is a relatively simple procedure that requires little money, has a small ecological footprint, and utilizes very little energy [161]. It has been stated that when a pollutant is located <1 m below ground surface, bioremediation may proceed without excavation, however pollutant located >1.7 m below ground surface must be delivered to the ground surface for bioremediation to be effectively enhanced [162]. Excavated polluted soils are often carefully put above the ground surface on a fixed layer support to promote aerobic biodegradation of pollutant by autochthonous microorganisms. Tillage, which causes aeration, fertilizer addition (nitrogen, phosphorous, and potassium), and irrigation are the key operations that encourage the activity of autochthonous microorganisms to enhance bioremediation during land farming. Nonetheless, it was reported that tillage and irrigation without nutrient addition in a soil with appropriate biological activity increased heterotrophic and diesel-degrading bacterial counts, thereby accelerating bioremediation; dehydrogenase activity was also found to be a good indicator of biostimulation treatment and could be used as a biological parameter in land farming technology [119].

1.3. Composting

Composting bioremediation is similar to landfarming bioremediation in the context of excavating contaminated soil to the surface and stimulates indigenous microorganisms by feeding nutrients and injecting air, but it differs in the context of supplementing the soil with a large amount of additives such as corncobs, straw, and hay, which aids in oxygen distribution through the soil, maintaining a constant moisture content, and turning frequency [163]. Composting is a process through which organic wastes are decomposed by microorganisms at high temperatures. Compost temperatures typically vary between 55-65°C. The higher temperatures are caused by the heat produced by microbes during the breakdown of organic material in garbage. The following basic steps have been used to demonstrate windrow composting. First, contaminated soils are dug and screened to eliminate large rocks and debris [164].

The soil is moved to a composting pad with an interim structure to supply containment and weather protection. Amendments (straw, alfalfa, manure, agricultural wastes, and wood chips) are utilized as bulking agents and as a source of additional carbon. Windrows are lengthy mounds of soil and additives. The windrow is completely blended by spinning it with a commercially available windrow turning equipment. Moisture, pH, temperature, and the concentration of explosives are all measured. The windrows would be disassembled at the end of the composting time, and the compost would be transported to the final disposal area [82].

1.4. Biofiltration

Biofilters are often used in semi-closed recirculating systems to treat and reuse aquaculture waste water. Water is recirculated between a culture facility and a water treatment facility containing the biofilter in the recirculating systems. The waste is collected in concentrated effluents, thickened to sludge, and subsequently digested by microorganisms in the biofilter. The bioremediation effectiveness of biofilters makes recirculating aquaculture systems an efficient technique of minimizing aquaculture waste water contamination in marine aquaculture systems. Microbial mats, activated sludge, trickling filters, rotating biological contactors, and denitrifying filters are the most often utilized types of biofilters.

- **Microbial mats:** Microbial mats are multi-layered sheets formed by laminated-cohesive microbe communities that develop embedded on a polymeric gel matrix around moist submerged surfaces.
- **Activated sludge:** Activated sludge is a type of aerated suspension that stimulates microbial growth, adsorption, and agglomeration of suspended colloidal particles into microbial flocs, and so breaks down organic waste.
- **Trickling filter:** A trickling filter is a basic stationary bed of stones and gravels built to enhance the surface area available for microbial adhesion.
- **Rotating biological contactor:** Rotating biological contactors are biological filters constructed of fixed film disks or film flow bioreactors that enhance the surface area for microorganisms to attach, proliferate, and eventually degrade organic matter.

- **Denitrifying filters:** Denitrifying filters promote the growth of anaerobic bacteria by establishing anaerobic zones, which increases the conversion of nitrate to nitrogen gas [165].

2. Semi-liquid Phase Bioremediation

This method involves excavating polluted soil, mixing it with water, and transporting the mixture to a bioreactor, followed by the removal of stones and rubble. The amount of water required is determined by the type and concentration of the pollutant, the composition of the soil, and the rate of biodegradation. Following this, the soil is separated by flotation or centrifugation, the soil is dried and retransferred to its original site, and the fluids are subjected to additional treatment [102]

2.1. Sludge Bioreactor

The utilization of biological processes in a contained space or reactor for the biological treatment of relatively modest volumes of waste. This procedure is used to treat slurries or liquids. Slurry reactors or aqueous reactors are used for ex situ treatment of polluted soil and water pumped up from a contaminated plume. In reactor bioremediation, polluted solid material (soil, sediment, sludge) or water is processed through a designed containment system. A slurry bioreactor is a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition in order to increase the bioremediation rate of soil-bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass capable of degrading target contaminants. Bioreactors have been used to treat petroleum-contaminated soil and other materials [88].

IV. FACTORS AFFECTING MICROBIAL BIOREMEDIATION

A. Biological factors

Soil microorganisms combat for carbon sources, and bacteriophages and protozoa prey on one other, all of which can have an impact on organic compound breakdown. Contaminants and catalyst levels have an impact on derivatization rates. Expressed enzymes can either accelerate or decrease contaminant breakdown. Enzymes must also be involved in contaminant metabolism in order for the contaminant to have affinity and availability. Interaction (competition, predation, and succession), population size, and composition are the primary biological factors [166], [167].

B. Oxygen availability

Biodegradation rates can be increased by utilizing organisms that do not require oxygen. Anaerobic decomposition occurs because the majority of living organisms require oxygen to exist. In most circumstances, the addition of oxygen can increase hydrocarbon metabolism [65]. Most of the biodegradation require aerobic condition operating under the influence of oxygen.

C. Moisture content

Microorganisms require a sufficient amount of water in order to grow. The biodegradation agents are less effective when the soil is too damp [168]

D. Nutrients availability

Nutrients can influence microbial growth and reproduction, as well as the rate and effectiveness of biodegradation. Optimizing the bacterial C:N:P ratio can increase biodegradation efficiency, particularly when important nutrients like N and P are present. Microorganisms require a variety of nutrients to live, including carbon, phosphorus, and nitrogen. Hydrocarbon decomposition is likewise inhibited at low concentrations. Adding nutrients to cold conditions can boost the metabolic activity of microorganisms and consequently the pace of biodegradation. The availability of nutrients limits aquatic biodegradation. Microbes that consume oil require resources to grow. These important elements can only be obtained in small amounts in nature [169].

E. Temperature

Temperature is the most essential physical element regulating microbe life and hydrocarbon composition. Natural oil deterioration is slow in cold areas like the Arctic, putting extra strain on microbes to clean up spilled oil. The sub-zero water freezes the microbial transport channels, preventing them from performing their metabolic activities. The metabolic turnover of enzymes involved in degradation is affected by temperature. Furthermore, each compound's breakdown necessitates a specific temperature. Temperature influences microbial physiological parameters, which either accelerates or retards bioremediation. Higher temperatures stimulate microbial activity. It proceeded to drop rapidly as the temperature increased or decreased and then gradually stopped [170], [171].

F. pH

The acidity and alkalinity, affect microbial metabolism and the subsequent elimination process. The pH of the soil can predict microbial development. Even slight pH changes have a big impact on metabolic activities [65].

G. Site characterization and selection

Before proposing a bioremediation solution, an adequate remedial study work is required to characterize the extent of the pollution. Determining the horizontal and vertical extent of contamination, defining parameters and sample locations, and describing sample and analysis methodologies are all part of the site selection procedures [172].

H. Metal ions

Metals are required by bacteria and fungi, but excessive levels prevent cell metabolism. Metal compounds influence the rates of degradation both directly and indirectly [173].

I. Microorganisms

High concentrations of some hazardous substances can kill microorganisms and impede the remediation process. The toxicant, concentration, and bacteria exposed all influence toxicity [174].

V. MICROBE-PLANT-BASED BIOREMEDIATION

A. Plant-Based Bioremediation

Plants are utilized for bioremediation, either alone or in combination with microorganisms, rather than relying solely on bacteria and their efficacy in bioremediating any contaminated medium. The use of green plants to clean up any contaminated medium or surface is not a new idea. Plants for wastewater treatment were conceived over 300 years ago. A number of plant species, including *Amaranthus spinosus*, *A. hypochondriacus*,*Chrysopogon zizanioides*, *Brassica juncea*, *Ricinus communis*, *Chromolaena odorata*, *Ageratum conyzoides*, *Ipomoea carnea*, *Prosopis juliflora*, *Lantana camara*, *Parthenium hysterophorus*, *Fagopyrum esculentum*, *Odontarrhena chalcidica*, *Tagetes patula*, *T. erecta*, and *Odontarrhena chalcidica*, have been identified that aid in the remediation of HM-contaminated soil. Furthermore, plants such as *Nicotiana tabacum*, *Arabidopsis thaliana*, *Beta vulgaris*, and *Sedum alfredii* have been genetically modified with appropriate bacterial genes from *Caenorhabditis elegans*, *Saccharomyces cerevisiae*, *Streptococcus thermophilus*, and *Pseudomonas fuorescens* and used for contaminant remediation. For example, mercury (Hg) reductase bacterial genes such as merA and merB have been used in plants to detoxify methyl-Hg. Furthermore, manure and organic amendments (e.g., different plant biochar, biosolids, and litter) are utilized as biostimulants in this plant-based bioremediation. Metal sorption is controlled by the use of chelators such as citric acid, ethylene diamine tetraacetic acid (EDTA), [S,S]-ethylenediaminedisuccinic acid (EDDS), ethylenediamine-di-o-hydroxyphenylacetic acid (EDDHA), diethylenetriaminepentaacetic acid (DTPA), ethylene glycol tetraacetic acid (AGTA), nhydroxyethylenediaminetriacetic acid (HEDTA), fulvic acids, salicyclic acid and tartaric acid and precipitate via the creation of metal chelate complexes, which improves the bioavailability of these metals as well as the effectiveness of phytoextraction. Plant-based bioremediation has been determined to be an effective technique for the accumulation, transformation, and immobilization of low-level pollutants. Plant-based bioremediation has various advantages, including cost effectiveness, public acceptance, and the capacity to remove inorganic and organic toxins simultaneously. A significant synergistic impact generated by the simultaneous expression of CYP2E1 and GST leads to increased accumulation and resistance of heavy metal-organic complex contaminants [36]

B. Microorganisms-Based Bioremediation

Microorganisms (such as bacteria and fungus) are essential in the microbial bioremediation process. Furthermore, microorganisms have numerous genes encoded by heavy metal resistance proteins and transporters that are found in transposons and plasmids. Kang [175] recently discovered that four bacterial strains, *Enterobacter cloacae* KJ-46, E. *cloacae* KJ-47, *Sporosarcina soli* B-22, and *Viridibacillus arenosi* B-21, showed synergistic effects on Cd, Pb, and Cu remediation from polluted soil. Furthermore, after 48 hours of experiments, the combination of bacteria strains exhibits stronger resilience and efficacy for metal bioremediation than a single strain. Microbes secrete a number of compounds that are important in bioremediation of polluted environments 3. Bacteria produce siderophores, which reduce metal bioavailability and are subsequently removed from contaminated surface. Bacterial cells have been observed to change their shape in order to boost the synthesis of siderophores, hence promoting the intercellular accumulation of metals. Microbial cell wall biomolecules contain negatively charged functional groups such as phosphate, hydroxyl, and carbonyl, which attach readily to harmful metal ions and aid in bioremediation. Furthermore, bacteria can grow and thrive in any controlled and harsh environmental circumstances, making them an ideal bioremediation agent. Similarly, fungi may be grown in hostile environments and detoxify metal ions by accumulation, valence change, and extra and intracellular precipitation. Furthermore, fungi operate as a promising biocatalyst in the bioremediation process, absorbing hazardous substances into their spores and mycelium [36]

C. Plant–Microbe Associated Remediation

Microorganism-plant-based remediation is gaining popularity because to its better removal efficiency as compared to plant-based remediation. These microorganisms participate in a variety of biochemical processes, including carbon and nitrogen mineralization, nitrogen fixation, and organic matter decomposition, all of which contribute to soil formation, nutrient cycling, and energy transmission. In contaminated locations, HMs also damage soil microbes. They tend to tolerate and develop distinct traits with a few specific microbial populations when exposed continuously. These specialized bacteria can be used to remediate harmful metals from damaged areas. Furthermore, the most successful species in the soil reclamation process are soil microbes that create a symbiotic relationship with host plants. Mycorrhizal fungi create intimate symbiotic relationships with host plants, which have been used in many bioremediation applications. Because of their abundance in soil, arbuscular mycorrhizae, the most well-known symbiotic fungus, are commonly used in phytoremediation. They can evolve numerous methods to survive high metal concentrations in soils, encouraging plant development. Furthermore, plant growth promoting bacteria (PGPB) can promote plant growth and assist plants survive with a contaminated ecology. The plant-microbe-based bioremediation approach has two aspects. To begin with, microbes assist the host plant survive under difficult environmental conditions by giving nutrients. Second, the plant serves an important function in sustaining favorable environmental conditions by increasing soil organic matter, accessible P, K, and N, allowing soil microbes to thrive, and therefore enhancing the reclamation process. Planting Salix in Cd-contaminated soil improved the diversity of beneficial microbes, including *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Niastella*, *Novosphingobium*, *Niabella*, *Anaeromyxobacter*, *Rmlibacter*, *Solitalea*, etc. [36].

VI. MICROBIAL ENZYMES ASSOCIATED IN BIOREMEDIATION

Table 1: Microbial enzymes associated in bioremediation and their function

Enzymes	Mechanism	Function	Reference
Cytochrome P450	Performs electron transfer processes and catalysis by reducing or oxidizing heme iron. Pyridine nucleotides are used as electron donors, resulting in carbon substrates and oxidized products. $NAD(P)H + O_2 + R \longrightarrow NAD(P)^+ + RO + H_2O$	Within cells, the synthesis and metabolism of numerous compounds and substances oxidize steroids, fatty acids, and xenobiotics.	[176]
Laccase	Reduction of the O_2 molecule, including one electron oxidation with a wide variety of aromatic chemicals.	Ring breakage in aromatic compounds reduces one oxygen molecule in water and produces free radicals.	[177]
Dehalogenase	This happened mostly through three mechanisms: (1) Hydrolytic mechanism: the water molecule acts as a cofactor; the halogen substituent is replaced by the hydroxyl group in the SN reaction. (2) Oxygenlytic mechanism: catalyzed by mono- or dioxygenase, which incorporates one or two molecular oxygen atoms into the substrate. 3) Reductive mechanism: it is associated with carbamide. Under aerobic conditions, halogen is substituted by hydrogen in this course, with organohalides serving as terminal electron acceptors.	The carbon-halogen bond breaks down and the halogens are removed.	[178], [179]
Dehydrogenase	As an electron acceptor, use coenzymes such as NAD^+ or $NADP^+$ or flavins such as FAD and FMN to catalyze the reactions. It is responsible	Creating energy by oxidizing organic molecules.	[180]

	for transferring two hydrogen atoms from organic molecules to electron acceptors.		
Hydrolase	Triglyceride hydrolysis occurs when one mole of triglyceride (T) interacts with three moles of water (W) to create one mole of glycerol (G) and three moles of fatty acids (P).	Fat and protein degradation.	[181]
Protease	Catalyze the breaking of protein peptide linkages.	Degradation of proteins such as keratin and casein, as well as leather dehairing and wastewater treatment.	[182]
Lipase	The carbonyl group of the substrate is attacked by the transfer of a proton between the aspartate, histidine, and serine residues of the lipase and the hydroxyl residue of the serine. A nucleophile assaults the enzyme during the deacylation process, renewing it and liberating the product.	produces fatty acids and glycerol by hydrolyzing mono, di, and triglycerides. Facilitate the esterification and transesterification processes as well.	[183]

VII. RECENT ADVANCED TECHNOLOGIES EMPLOYED IN BIOREMEDIATION

Innovative advanced molecular techniques like genomics, metagenomics, proteomics, transcriptomics, and metabolomics provide deeper insights into microbial activities with regard to their genes, proteins, mRNA expression levels, enzymes, and metabolic pathways in response to changing environments. The "omics approach," which refers to the combination of these various technologies in the field of bioremediation, is used to characterize biological macromolecules in a set of microorganisms and microbial communities without deviating, as well as their distinct genetic and molecular structures and function mechanisms [3].

A. Bioinformatics Approaches in Bioremediation

The purpose of bioremediation is to interpret the underlying degradation mechanism carried out by a specific organism for a specific pollutant by using data from various biological databases, such as databases of chemical structure and composition, RNA/protein expression, organic compounds, catalytic enzymes, microbial degradation pathways, and comparative genomics [184]. All of these sources are analyzed using a range of bioinformatics technologies in order to explore bioremediation and create more potent environmental cleaning technology. Due to a lack of information on the variables regulating the growth and metabolism of bacteria with the potential for bioremediation, there are only a few applications for bioremediation [185]. The mineralization pathways and processes of these bacteria with bioremediation capacities have been mapped utilizing bioinformatics. Investigating bioremediation techniques and technologies requires the use of proteomic tools such as mass spectrometry, microarrays, and two-dimensional polyacrylamide gel electrophoresis. The scientists claim that it greatly enhances the structural characterization of microbial proteins with contaminant-degradable characteristics [186].

1. Bioremediation Tools Based on Omics

Genomic, transcriptomic, metabolomic, and proteomic approaches can be useful in bioremediation research. This technology assists in the assessment of the *in* situ bioremediation process by correlating DNA sequences with the amount of metabolites, proteins, and mRNA [187], [188].

2. Genomics

The study of bioremediation bacteria is a new area of study in genomics. This approach is predicated on microorganisms' capacity to fully comprehend their genetic data inside the cell. A wide range of microorganisms are used in bioremediation [189]. Genomic methods like PCR, isotope distribution analysis, DNA hybridization, molecular connectivity, metabolic footprinting, and metabolic engineering are utilized to better understand the biodegradation process. Numerous PCR-based methods are available for genotypic fingerprinting, such as amplified fragment length polymorphisms (AFLP), amplified ribosomal DNA restriction analysis (ARDRA), automated ribosomal intergenic spacer analysis (ARISA), terminal-restriction fragment length polymorphism (T-RFLP), randomly amplified polymorphic DNA analysis (RAPD), single strand conformation polymorphism (SSCP), and length heterogeneity [190]. Using RAPD, one can create functional structural models, identify genetic fingerprints, and evaluate bacterial species that are naturally connected to one another [65]. LH-PCR can be used to identify the natural length variations of different SSU rRNA genes in microbial communities. T-RFLP allows for the simultaneous profiling of several microbial taxonomic groups [191]. The presence and distribution of taxonomic and functional gene markers in the soil can be evaluated using a quantitative PCR-based investigation of the soil microbial communities. Amplified PCR results provide a starting point for the direct investigation of particular molecular biomarker genes in DNA analysis procedures [65].

3. Transcriptomics and Metatranscriptomics

The set of genes that are being transcribed at a certain moment and condition is represented by the transcriptome, which serves as a vital link between the cellular phenotype, interactome, genome, and proteome. In order to adapt to environmental changes and hence ensure survival, the capacity to modulate gene expression is essential. A thorough understanding of this procedure across the entire human genome is provided by transcriptomics. DNA microarray analysis is a potent method in transcriptomics for quantifying mRNA expression levels [65]. A transcriptomic analysis requires the isolation and enrichment of total mRNA, cDNA synthesis, and sequencing of the cDNA transcriptome. Almost every gene's mRNA expression in an organism can be evaluated and investigated using a DNA microarray as a transcriptomics tool [192]. Transcriptomics, sometimes referred to as metatranscriptomics, is the study of transcriptional mRNA patterns and is essential for getting functional insights into the operations of environmental microbial communities [65]. Scientists can utilize metatranscriptomics to study the expression of genes [193].

4. Proteomics and Metabolomics

Proteomics focuses on the total proteins expressed in a cell at a specific location and time, as opposed to metabolomics, which is focused on the total metabolites produced by an organism in a certain period of time or environment [194]. Proteomics has been used to analyze protein abundance and composition changes, as well as to identify important microbe-related proteins [65]. Metabolomics investigations can be applied to biological system analysis in two main ways. To carry out the first kind of study, no prior understanding of the biological system's metabolic pathways is required. This method allows for the identification and recovery of a large number of metabolites from the sample, producing an enormous amount of information that can be utilized to show how different samples are connected by certain metabolic pathways. An additional choice is to perform a focused investigation to identify particular metabolic pathways or metabolites based on previous studies [195]. Among the various technologies in the microbial metabolomics toolbox, metabolite profiling, foot printing, and target analysis are just a few that can be used to identify and quantify the plethora of biological byproducts found in live organisms [196]. The metabolome and proteome data will be helpful for cell-free bioremediation [65].

B. Bioremediation Using Nanotechnological Methods

Nanotechnology uses the nanometer as the smallest unit of measurement. Because of their unique abilities against numerous resistant pollutants, they can assist in the removal of many harmful compounds. Nanotechnology has altered how we view various technologies, including water treatment. The term "nanofiltration" currently refers to environmental-friendly methods [196].

1. Microbe and Nanotechnology

Wastewater can be treated using effective microbes utilizing the effective microbes (EM) technique, and the treated water can subsequently be used for irrigation [197]. Nanotechnology and EM technologies are useful for water purification. Recalcitrant organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs) containing numerous benzene rings, cause a plethora of significant environmental problems that are pervasive and innumerable. The mutagenic compounds Polycyclic aromatic hydrocarbons (PAHs) are nonbiodegradable and also mutagenic [198]. A study by Ramos [199] reported that silver nanoparticles using entire Trichoderma spp. fungal cells.

2. Engineered Polymeric Nanoparticles for Hydrophobic Contaminant Bioremediation

Organic contaminants, such as petroleum hydrocarbons and PAHs, have less solubility and mobility after being absorbed by soil, which lessens their environmental impact. Polymeric nano-network particles enhance both phenanthrene solubility and phenanthrene release from contaminated groundwater material. Polymeric nanoparticles are produced using precursor chains of poly-(ethylene) glycol-modified urethane acrylate (PMUA). PMUA nanoparticles are made to maintain their characteristics in the presence of various bacterial populations [65].

C. Genetic and Metabolic Engineering

The term "gene editing" describes scientific and technological advancements that allow rational genetically generated fragments at the genome level to give precise addition, deletion, or replacement of DNA molecule fragments. Transcription activators are used in a number of widely used gene editing techniques, including as TALENs, ZFNs, and CRISPRs. The most effective and simple gene editing technology is CRISPR-Cas, according to experts [200]. The sequence of the host DNA is complementary to a DNA-binding site in

TALEN. Double-stranded breaks (DSBs) are produced when TALEN binds to DNA and exposes sticky ends for stabilization. ZFNs also have a DNA-binding domain made up of 30 amino acids. The Fok1 cleavage domain generates DSBs in the target site of the host DNA. To overcome molecular difficulties, a new perspective on composite endonucleases comprised of TALENs and ZFN nucleases was necessary [201]. The CRISPR-Cas system is characterized by sequence similarity complementarity and simultaneous gene editing. *Streptococcus pyogenes*, the bacteria, gives this unique ability as a type of virus resistance. In the CRISPR-Cas system, guide RNA participates crisper-derived RNA (crRNA) and trans-acting antisense RNA (trcRNA). The Cas9 enzyme is able to perform the required DSB when gRNA detects the target DNA sequence. The knock-in and knock-out impacts of these gene editing tools are being evaluated for use in bioremediation research [202]. The CRISPR-Cas system has been extensively recognized by researchers in model organisms like as *Pseudomonas* and *Escherichia coli* [203]. Bioremediation is also investigating new insights into CRISPR toolkits and the synthesis of gRNA for the creation of remediation-specific genes in non-model species (such as *Rhodococcus ruber* TH, *Achromobacter* sp. HZ01, and *Comamonas testosteroni*) [204]

Bioremediation of hexachlorocyclohexane and methyl parathion has been demonstrated using genetically engineered bacteria [205], [206]. *P. putida* KT2440 was genetically engineered and utilized for organophosphate and pyrethroid bioremediation investigations [207]. Since the advent of metabolic engineering, the breakdown and catabolism of a wide range of persistent substances has been reported. *Sphingobium japonicum* and *Pseudomonas* sp. WBC-3 demonstrated methyl parathion and -hexachlorocyclohexane degradation pathway bioremediation [208]. When three enzymes from two different bacteria integrated in *E. coli*, a persistent fumigant known as 1-, 2-, 3-trichloropropane is released into the environment through heterologous catabolism [65].

VIII. ADVANTAGES AND DISADVANTAGES OF BIOREMEDIATION

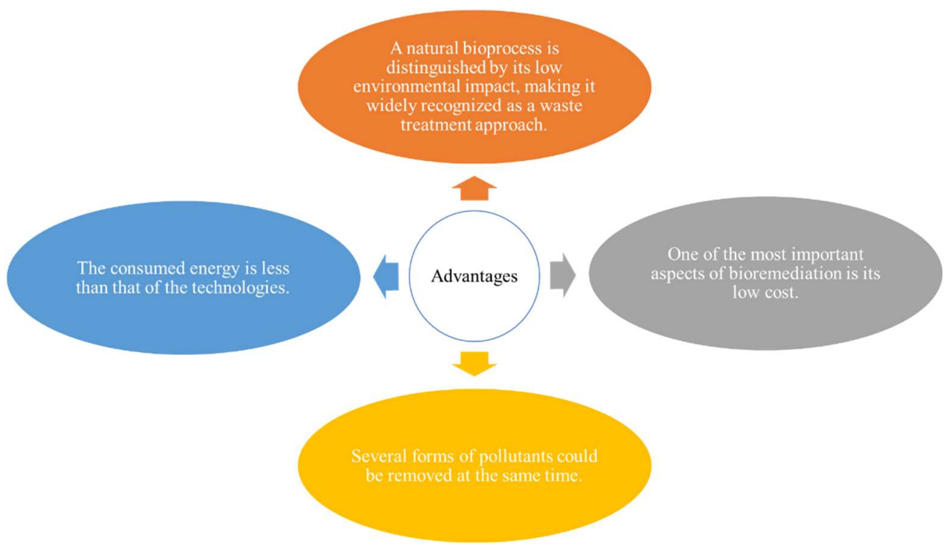

Figure 2: Advantages of Bioremediation

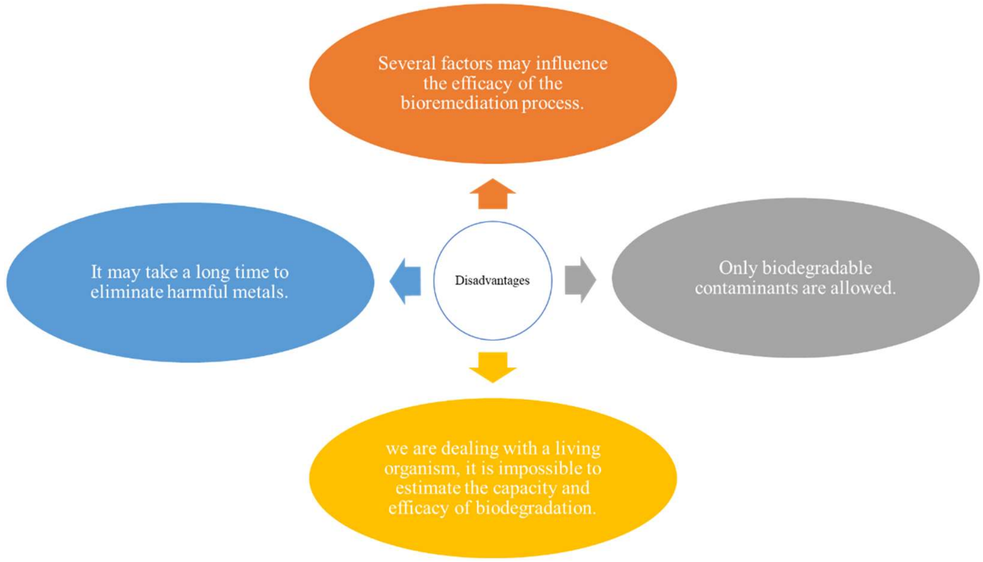

Figure 3: Disadvantages of Bioremediation [209]

IX. CONCLUSION

Bioremediation is a method of removing pollutants by increasing natural biodegradation processes. Biodegradation is a useful option for remediating, cleaning, managing, and recovering the environment from pollution. So, by developing an understanding of microbial communities and their responses to the natural environment and pollutants, expanding knowledge of microbe genetics to increase capabilities to degrade pollutants, conducting field trials of new cost-effective bioremediation techniques, and dedicating sites for long-term research, these opportunities offer the potential for significant advances. There is no doubt that bioremediation is leading the path to greener pastures. Regardless of the element of bioremediation employed, this technology provides an efficient and cost-effective method of treating contaminated ground water and soil. Its advantages often outweigh the disadvantages, as seen by the growing number of sites that utilize it and its growing popularity. Once again, bioremediation technology has been utilized to clean up the polluted environment and may thus be used as a management tool.

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