MODERN APPROACHES AND ADVANCEMENTS IN BIOREMEDIATION

Abstract

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, Humidity, Nutrient Availability, Temperature, pH, Site Characterization, Metal Ions, And Also Microbes) That Determine The Rate Of Biodegradation. The Polluted Soils Are Restored Using Bioremediation, Which Uses Plant-Based, Microorganism-Plant- Connected, And Other Cutting-Edge Approaches. An Overview And Critical Analysis Of The Bioremediation Abilities Of Bacteria, Fungi, And Plants Have Been Conducted. The Most Prevalent Enzymes Are Cytochrome P450s, Laccases, Hydrolases, Dehalogenases, Dehydrogenases, Proteases, And Lipasesin Bioremediation, Those That Are Promoted By A Variety Of Processes, Including Oxidation, Reduction, Elimination, And Ring-Opening. Metagenomics, Proteomics, Transcriptomics, And Metabolomics Are Just A Few Of The Cutting-Edge Techniques Currently Being Successfully Used To Characterize The Metabolic Machinery, Novel Proteins, And Catabolic Genes That Participate In The Breakdown Process Of Polluting Microbes. This Review Could Also Be Helpful For Ongoing Research In Order To Improve The Effectiveness Of Different Contaminants Being Degraded.

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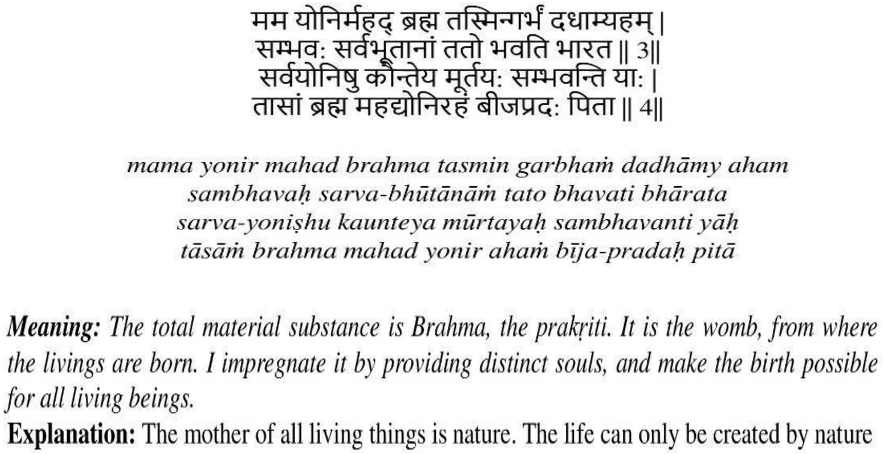
I. INTRODUCTION

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



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-yoniṣhu 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

The environment encompasses all the natural surroundings that impact our everyday lives on Earth. The presence of a secure and wholesome atmosphere is crucial to the survival of all living things in this world [3]. Earlier, it was considered that humans had an endless supply of assets and territory; however, current assets on Earth demonstrate, to a superior or inferior extent, lack of caution or carelessness in their utilization [4]. In order to fulfill the population's growing requirements for food, energy, environmental assets, materials, and all other necessities, they have been exploited more often as a result of the massive expansion of the global populace [5].Human beings have witnessed technological advancements in the fields of food engineering, the medical sector, construction operations, transit-oriented development, and telecommunications since the beginning of the 20th century. These aforementioned acts deplete the resources that constitute the environment, utilize a massive amount of modern supplies and energy, and generate massive amounts of trash, resulting in environmental deterioration [6]. Every single year, 1,000 new chemicals are synthesized roughly in the globe. Third World Network (TWN) reports that, toxins are emitted into the atmosphere and groundwater on an intercontinental level in an excess of 450 million kilograms [7]. There are a wide range of solid, liquid, organic, or inorganic gaseous air pollutants being released within the biosphere by the pulp and paper manufacturing sector (after the petroleum, quicklime, tannery, textile, and feeder sectors) [8].

The commercial, residential, and farming industries generate trash that contain hazardous metals as well as semimetals, which are extremely harmful for the environment

[9]–[11].The standards of aquatic environment have been deteriorated due to the anthropogenic activities, including excavation and the eventual elimination of hazardous substances from feeder industries, battery plants, and power generation, which represent serious ecological threats. The lithosphere contains metallic elements, comprising of contaminants that are quite sturdy to bebreak down naturally. Normally, they are found as ores within rocks and retrieved as minerals from them. High levels of exposure lead to the discharge of heavy metals into the ecosystem. Once in an ecosystem, they may remain hazardous for much longer [12].Metals deposited within the living tissues that is difficult to eliminate, because of their inability to biodegrade, which makes them a hazard for worldwide health [13].The biological and physicochemical features of soil are changed by metal pollution, including increments to bulk density and pH in addition to reductions in soil fertility, capacity to retain water, diversity of microbes, and efficiency of soil enzymes [14]–[16].Heavy metals such as Hg, Cu, As, Cr, Pb, and Ni are able to damage plant cultivation and development and have various effects, both directly and indirectly. These effects include chlorosis, root damage, necrosis, oxidative damage, decreased carotenoid concentration, inhibition of enzymes, osmotic imbalance, reduced activity of photosynthesis, and nutritional deficiencies [17]–[22].Xenobiotics are those chemical substances that are not formed organically or are not supposed to exist in living things. The expression "xenobiotic" is commonly applied in connection with the contaminants in environment referring to artificial substances manufactured in huge quantities for commercial, farming, and residential uses [23]–[25].Environmental xenobiotics such as personal care products (PCPs), chlorinated compounds, pharmaceutically active compounds (PhACs), pesticides, phenolics, polycyclic aromatic hydrocarbons (PAHs), and other commercial chemicals are potent threats to the environment. As they occur more frequently several concerns have been expressed about ecological divisions regarding their capacity for negative consequences. Its toxicity causes extraordinary health concerns and significant threats to the security and safety of the environment [3].Pollutants are highly mobile and soluble, allowing them to bioaccumulate within the food cycle and cause catastrophic consequences by raising the ecological pyramid [26], [27].While these pollutants reach the human body, they may result in conditions such as cancer, cardiovascular diseases, Alzheimer’s disease, high blood pressure, atherosclerosis, kidney and bone diseases, and low birth weight[28]–[33].Heavy metals and xenobiotic compounds have been detoxified and degraded using a variety of physicochemical approaches (including extraction, immobilization, stabilization, coagulation, electrodialysis, vitrification, reverse osmosis, ion exchange, chemical reduction, evapotranspiration, and precipitation)[34], [35].However, these approaches are expensive, consume a lot of energy, use hazardous chemicals that have low effectiveness for removal, and cause secondary ecological contamination [36].

The consistency of the Holocene climate supports the current expansion and modernization of society. However, the unstable Holocene climate was grossly misused by unbridled consumerism, lacking sincere environmental consideration. Furthermore, as a result of such carelessness, the entire backwoods of this planet has been significantly reduced to barely 35% of what it once was[37]. The transformation of the world’s climate is influenced by factors such as global warming, polar ice meltdown, biodiversity reduction, and the extinction of significant wildlife species [38]. Human activities not only damage but also destroy our ecosystem [37].

Undoubtedly, among the most precious and important assets on Earth is water. The earth surface 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 fertilizers, organic matter, drug residues, sediments, agrochemicals, and pesticides, are extremely damaging to both surface and underground water [39], [40]. Polluted water is dangerous to humans as well as animals and causes diseases such as dysentery, polio, cholera, typhoid, and diarrhea[41].Pesticides are heavy contaminants in aquatic environments. Pesticides are chemical compounds 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 of 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 toxic pesticides' biomagnification in water results in the destruction of biodiversity, including plants, animals, and microorganisms [43]. In addition, corals die as a result of increasing ocean pH [44]. Corals are critical for underwater biodiversity [45]. Furthermore, ocean contamination is increasing due 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 midst of the oceans are becoming frequent occurrences [48].In addition, the air we breathe is not very good [49]–[51].In accordance with a recent assessment, the air quality index (AQI) in various cities is in a severe situation[52], [53].Furthermore, the release of excessive amounts of greenhouse gases (GHGs), such as CH_4 and CO2,has harmed human and animal respiratory health [54], [55].

Employing bioremediation is one method to protect the ecosystem from catastrophic damage [56].The Green plants, microbe which include fungus, yeast, bacteria, and algae, or their enzymes can help contaminated places return to an earlier condition through the ecologically friendly process of bioremediation[57],[58].Bioremediation by enzyme engineering uses directed evolution and rational and semi-rational methodologies to increase the activity of microbial enzymes[59]–[61].The late 19th century was considered as the golden era of bioremediation. With further advancements, the 20th century witnessed the initial stage of research in the area of microbial ecology, which involved the identification and isolation of microorganisms that can break down contaminants, such as *Candidatus accumulibacter*, which is able to absorb additional phosphorus found in their cells as polyphosphates from wastewater treatment plants [62].The selection of microorganisms is based on the polluted area because each microorganism has a different temperature, humidity, and pH for activation. The microbes that are utilized in this method are referred to as bioremediators. This process is simple to carry out and does not disrupt human lives or the ecosystem during conduction or transportation [63]. Several factors influence bioremediation for more effective outcomes, such as the neighboring habitat temperature, anaerobic or aerobic circumstances, and nourishment availability [64]. Waste management depends primarily on bioremediation. It can eliminate persistent organic contaminants that are difficult to break down and are suspected to be heterologous biological compounds [65].Bioremediation is not a new concept in the human race; it is a novel technique resulting from improvements in the fields of molecular biology and bioengineering [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 are used to describe the biotic world and to solve problems. The word "bioremediate" is used to describe the utilization of biological organisms to resolve environmental problems caused by contaminated soil or groundwater [68]. The United States Environmental Protection Agency (USEPA) claims that the application of living things to purify or eliminate contaminants from the water, soil, or wastewater; the utilization of microbes, including non-harmful invertebrates to eradicate pests from crops or treat plants, trees, and agricultural soil diseases" refers to bioremediation. [69]. By definition, bioremediation is employing alive organisms, mainly microbes to breakdown environmental pollutants into forms that are less hazardous. It employs organically occurring plants, microbes, and molds to eliminate or detoxify compounds that are dangerous to the ecosystem or public health. Most natural reduction systems operate under aerobic circumstances; however, anaerobic conditions can be used [70]. In the ecosphere of this planet, microbes can be found in a wide variety of environments. They develop from the oceans, water, soil, living organisms, and the frozen ice ecosystem. The sheer number of microorganisms, in addition to their voracious appetites for chemicals, make microorganisms the perfect environmental stewards [71].

Bioremediation processes have become a developing and an acknowledged procedure for improving soils that have been polluted with heavy metals because of their favorable ecological impact as well as their affordable cost when compared with alternative standard techniques like capping, dredging, and combustion, which are frequently quite costly and inefficient when metallic levels of concentration are inadequate and also frequently generate significant quantities of hazardous waste [72], [73]. A study has shown that the cost of removing metal-contaminated sediments and soils by chemical treatment and landfilling ranges from $100 to $500 per ton. 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 removing an acre of Pb-polluted soil in comparison with standard excavations and garbage dumps [75], [76]. Furthermore, bioremediation is a harmless and safe technology that can eliminate toxins abidingly while leaving the ecosystem unharmed and might be combined with both chemical and physical treatments [77]. The bioremediation techniques are totally based on natural biological potency. Most bioremediation techniques are dependent on the kind of soil, contaminants types, amount of moisture, addition of nutrients, diversity of microbes, contaminant site pH, and humidity at the remediation site [78], [79]. Natural attenuation is a bioremediation process that naturally occurs in polluted areas [36]. The ultimate objective of bioremediation is to put microorganisms to work by providing optimal quantities of nutrients and other synthetics required for their metabolism in order for them to eliminate or detoxify pollutants that are dangerous to the ecosystem and all organisms. Enzymes are involved in each and every metabolic process. A wide variety of enzymes are involved in these reactions, including lyases, hydrolases, isomerases, ligases, oxidoreductases, and transferases. 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 just one of the procedures associated with or implemented as part of the technique. There are only specific pollutants that are able to be broken down, by some microbes that are capable of degrading them [82].

In 1974, *Pseudomonas putida* strain, which was capable of breaking down hydrocarbons received the first invention for a biological agent. In 1991, around 70 species of microbes were found to breakdown petroleum components; during the next 20 years, nearly a comparable number of new species were discovered. *Geobacter metallireducens* is a comparatively new collation to the growing collection of microbes capable of sequestering or reducing metals. This kind of bacteria is capable of eliminating the radionuclide contaminant uranium from polluted subsurface water and mining wastewater streams. The most radioresistant bacteria is *Deinococcus radiodurans*, which has also been developed to help cleanup water as well as soil polluted with chemicals, radionuclide byproducts, and heavy metals. *D. radiodurans* has been developed genetically to be able to eliminate mercury (using genomes of *Escherichia coli*) and degrade toluene (using genomes of *Pseudomonas putida*) in radiological environments [83]. From the standpoint of the potential for major developments in bioremediation, it appears that the advancement of our comprehension of the population of microbes, their relationships with the ecosystem and pollutants, the expansion of their genetic capacity to breakdown pollutants, and permanent experimental investigations of new affordable methods for bioremediation are able to improve this possibility. It is believed that there is no question that the bioremediation process is a current requirement that can result in the sustainable use and preservation of the resources of nature that we have depleted for future generations [82].

III. TECHNIQUES INVOLVED IN BIOREMEDIATION

Two bioremediation techniques (*in situ* and *ex situ*) have been defined by the United States Environmental Protection Agency [84]. The fundamentals of biological degradation and biological transformation support both *in situ* and *ex situ* bioremediation techniques, which involve the elimination, immobilization, mobilization, and detoxification of different contaminants in the ecosystem by the activity of plants and microbes [85].

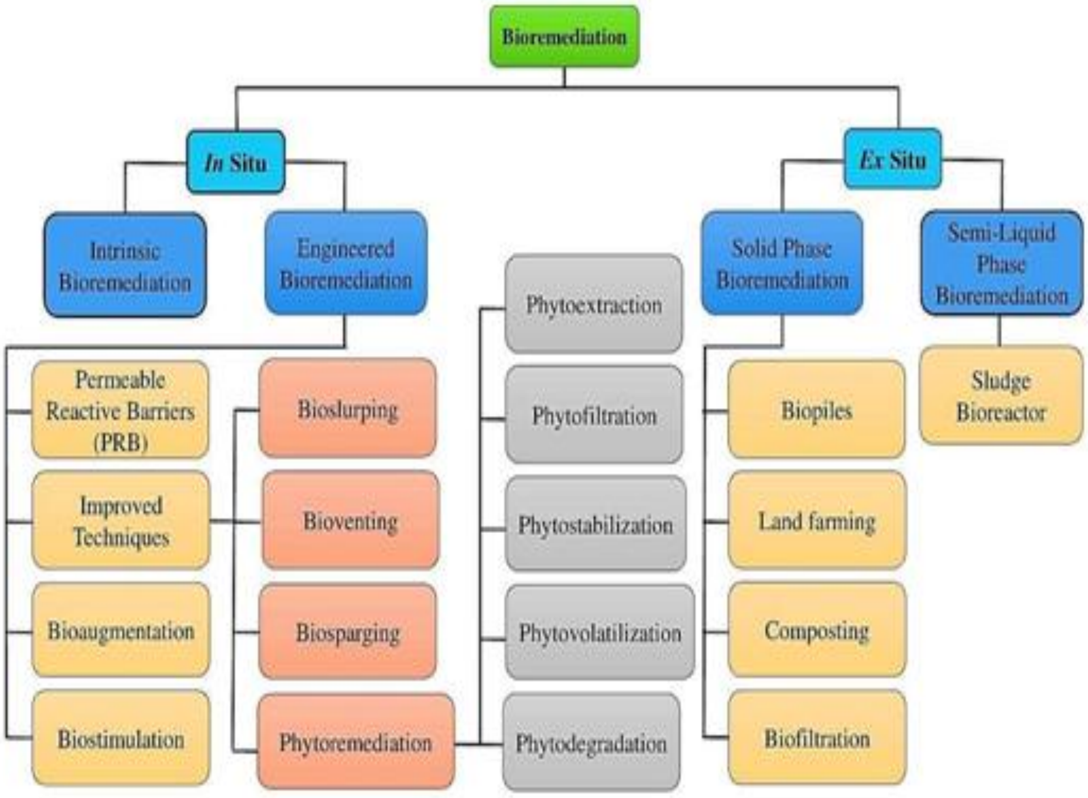


Figure 1: Different bioremediation techniques.

1. ***In situ* Bioremediation:** The method includes the implementation of biological techniques to remediate harmful substances and is widely used to degrade pollutants in underground water and moist soils [86]–[88]. It is dependent on the activity of microbes to destroy or detoxify pollutants existing on an impure site. Bioremediation *in situ* becomes more sustainable because it eliminates the need for transport, polluted soil deposition, pumping of underground water, treatment, and release to recipients. Additionally, it provides several benefits, including low costs, the use of environmentally friendly microbes, and the ability to treat large volumes of polluted water and soil that release smaller amounts of dangerous elements. The *in situ* bioremediation method has mostly been used to degrade nitriles, chlorinated hydrocarbons (CHC), nitrobenzenes, aminobenzene, and plasticizers in soil and groundwater [89]. The addition of electron acceptors, including sulfate or nitrate, can assist with *in situ* bioremediation under anaerobic circumstances [90]. The choice of a single organism or group of organisms with the capacity to eliminate the desired metals is a difficult challenge for *in situ* bioremediation. It was discovered that both Fe^{3+} and sulfate-reducing microorganisms (SRM) had the catalytic capacity to degrade heavy metals like Cr (VI), Co (III), Tc (VIII), and U (VI) [91]–[93]. Also,

Geobacteraceae sp. were identified during the activation process for decreasing Fe^{3+} and U(VI) of polluted water-bearing strata. As a result, the *Geobacteraceae* family was considered as of utmost importance in the stabilization of pollutants and the reduction of metals within underground ecosystems [94]. Both intrinsic and engineered bioremediation are types of *in situ* techniques [95].

- **Intrinsic Bioremediation:** Intrinsic bioremediation is a form of organic degradation that doesn't use a synthetic step to boost the biodegradation cycle; instead, it solely depends on the metabolic processes of native microorganisms to remove hazardous contaminants. Intrinsic bioremediation is also known as passive bioremediation or natural attenuation [96], [97].

  Certain requirements must be met before using intrinsic bioremediation: the appropriate community of decomposing microbes within the contaminated location; the most favorable circumstances in the environment (temperature, pH, humidity threshold, concentration of oxygen); nitrogen and carbon supplies available to support the development and activity of microbes; and microbes' ability to change contaminants into non-hazardous products.[97]. In microbial communities, hydrocarbon-degrading ability is common because native microorganisms have already acclimated to the site's circumstances and have evolved a correlation with hydrocarbons [96]. There are some methods, including amendment administration, dehalorespiration, aerobic septic, bioslurping, and biosparging, for intrinsic *in situ* bioremediation [65]. *In situ* bioremediation has been employed to remediate blocked groundwater utilizing a stimulation-optimization approach that is supported by machine learning and particle swarm optimization (ELM-PSO) methods [98]. The removal of Cr (VI) in shallow unsaturated dirt was additionally investigated using *in situ* treatment. Strong Cr (VI) soil concentrations may not be fatal to microbes, and even subcellular systems have been shown to cooperate with heavy metals. The removal of heavy metals can be accomplished with microbiological inoculants [99]. Redox interactions between Cr (VI) and Fe (II) ions induce reductive processes when iron is released in soluble forms [100]. To ensure that the duration required to carry out the bioremediation is shorter compared to the duration required for the pollutant to get to the nearest site of both animal and human contact, an evaluation of risk is required to be conducted before using intrinsic bioremediation [101].

- **Engineered Bioremediation:** The following technique is to introduce a particular microbe to the contaminated site. *In situ* bioremediation is a method that utilizes genetically engineered microbes to accelerate the degradation process [65]. The modification and customization of both chemical and physical circumstances promotes the growth of inserted microbes, thereby fastening the process of bioremediation, which is called engineered bioremediation [102].

- **Permeable Reactive Barriers:** The Sub-surface water polluted by different contaminants, including chlorinated hydrocarbons (CHC) along with heavy metals, can be remediated using permeable reactive barriers [103]. The polluted sub-surface water stream is surrounded by an eternal or temporary reactive barrier formed mostly of iron [104]. The pollutants are retained or released by discharging cleared water when polluted water normally crosses the line of separation [105]. PRBs should be sensitive enough to remove contaminants, completely transparent to enable water to flow, inactive with minimal power requirements, and affordable [106]. The effectiveness associated with such a technique depends on the kind of medium, and that in turn is based upon the level of contaminants, health consequences, geohydrology, system stability, biogeochemistry, circumstance, and expense [107]. PRBs have been combined with other methods to remediate several kinds of contaminants in the past few decades [108].

- **Improved Techniques**

- **Bioventing:** Bioventing is a technology that delivers oxygen to the unsaturated zone to encourage the activity of indigenous microbes for bioremediation. In bioventing technique, nutrients as well as water are added, helping the bioremediation process. Microorganisms will transform pollutants into harmless compounds as a result of this process [109]. By using aeration to stimulate the surrounding microflora, the biodegradation capacity of diverse bacteria is increased, and contaminants containing heavy metals are more likely to precipitate; this process is known as "bioventing"[110]. For successful pollutant transformation, the ratios between moisture and nutrients must remain unchanged. Oil-contaminated soil restoration has been accomplished with success utilizing this method [111]. Bioventing could be utilized more efficiently in anaerobic biodegradation, and combining oxygen and nitrogen can enhance the effectiveness of chlorinating treatment [102].

Sui and Li [112] evaluated the influence of air infusion frequency upon bioventing's ability to biodegrade, volatilize, and biotransform a toluene-polluted area. At the conclusion of the research phase (200 days), it was found that the removal of the pollutant (toluene) at the two distinct air infusion rates (81.504 and 407.52 m^3 /d) did not differ significantly from one another. On the other hand, it was found that higher atmospheric infusion rates led to greater toluene elimination by agitation than lower air infusion rates at the beginning of the experiment (day 100). To put it another way, increasing the airflow rate had no influence on the rate of biodegradation or the efficiency of contaminant metabolic transformation. This was because the oxygen requirement in biological degradation had caused the air in the subsurface to quickly become saturated (either at an increased or decreased air infusion rate). However, the low air infusion rate increased biodegradation significantly. It therefore indicates that air infusion rate is one of the fundamental parameters for contaminant dispersion, redistribution, and surface loss in bioventing.

Unlike bioventing, which depends on modest air input to enhance the degradation of microbes at soil vapor extraction (SVE), the vadose zone increases the volatilization of volatile organic compounds (VOCs) through vapor extraction [113]. Despite the fact that both methods use the same technology, there are philosophical design, configuration, and functional differences [114]. SVE has a greater airflow rate compared to bioventing. SVE's pollution elimination process makes it possible to classify it as a physical kind of remediation, although the mechanisms for removing contaminants from both methods cannot be considered. Due to the additional surroundings and unique properties of the unsaturated area into which air is injected, it may not always be feasible to produce equivalent findings to those seen in laboratory research during on-site field experiments. As a result, treatment times with bioventing may be extended. High airflow rates, it seems, make it easier for volatile organic substances to move from the soil vapor stage, necessitating off-gas treatment of the consequent gases before discharge into the environment [115]. The specific difficulty may be overcome through the combination of biotrickling and bioventing filter methods to minimize the amounts of output gas emissions and pollutants, thereby decreasing the duration of treatment required by bioventing separately [113].

- **Bioslurping:** This technology integrates soil vapor extraction, bioventing, and vacuum-enhanced pumping to achieve groundwater and soil remediation through indirect oxygen provision and contaminant biological degradation stimulation [116]. It is designed to clean up unsaturated and saturated zones and capillaries by recovering free substances that include non-aqueous phase liquids (LNAPLs). Additionally, it can be utilized for cleaning up soil that has been polluted by semi-volatile and volatile organic substances. The method utilizes a slurp, which reaches into the free product layer and suckers fluids (free products and soil gas) from this layer in a manner equal to the way a straw takes fluid out of a container. The pumps move LNAPLs upward, where they separate from air and water [117]. The bioslurping tube starts to remove vapors from the unsaturated zone when the fluid level in the well decreases during the vacuum evacuation of LNAPLs. The flow of soil gases is facilitated by vapor evacuation, which improves the processes of aerobic decomposition and aerification. The facility may be utilized for typical bioventing to conclude the bioremediation once all toxins have been removed. The vacuum pump is ineffective in extracting LNAPLs at depths larger than 7 m; hence, the bioslurping mechanism can only be placed when the pollutants are below that level. The main disadvantage is that excessive moisture in the soil inhibits air permeability and reduces the transfer of oxygen, which reduces the activity of the microbes [109]. Despite the fact that this technique is not appropriate for low permeability soil remediation, it is an affordable operation method because it consumes minimal groundwater and disposes of waste.[71].

- **Biosparging:** This is a method that introduces air into the soil's subsurface in order to increase the activity of microbes and increase the elimination of contaminants from contaminated soil. The approach resembles bioventing. In contrast to bioventing, air is introduced in the saturated area, which might cause a higher-level of migration of volatile organic compounds to the unsaturated area, also promoting biological degradation. Two important aspects affect the efficiency of biosparging: the permeability of the soil, which affects how accessible contaminants interact with microorganisms, and contaminant biodegradability [118]. *In situ* air sparging (IAS) or biosparging is a very similar technology used in soil vapor extraction (SVE) and bioventing, which rely on high airflow rates for contaminant volatilization, while biosparging promotes biological degradation [119]. Biosparging is currently extensively used in order to clean up aquifers contaminated with oil derivatives, primarily diesel and kerosene, which result in strong biodegradation of the BTEX group and naphtalenes [120]. It is possible to utilize aerobic bacteria to degrade naphtalenes, mineral oils, and BTEX. However, anaerobic circumstances are dominant in the deepest layers of subsurface water as well as soil. To encourage the growth of aerobic microbes, injection filters infuse oxygen into the subsurface water and soil[109].

  According to a study by Kao [121],the biosparging of a toluene, benzene, ethylbenzene, and xylene (BTEX)-polluted aquifer effluent caused an aerobic transition in the environment; increases in dissolved oxygen, nitrate, sulfate, redox potentials, and total culturable heterotrophs were shown to be indicative of this, whereas reductions in dissolve ferrous iron, methane, sulfide and entire anaerobes and methanogens were indicated of the opposite. The overall reduction in BTEX elimination (>70%) implies that biosparging is able to be utilized for cleaning up BTEX-polluted subsurface water. The primary problem is forecasting the path of airflow.

- **Phytoremediation:** Phytoremediation is able to clean up polluted soils. This approach reduces pollutant toxicity in contaminated areas by utilizing relations between plants on biological, biochemical, physical, chemical, and microscopic levels. Phytoremediation uses a wide range of techniques that vary according to the type and nature of the contaminants [122]. Elemental pollutants, such as radionuclide substances or heavy metals, are primarily removed, converted, and sequestered, whereas biological pollutants are primarily removed by biological degradation, rhizodegradation, stabilization, or vaporization[123]. Plants interact with pollutants in a variety of ways within phytoremediation [124].

- **Phytoextraction:** Phytoextraction, often referred to as phytoabsorption, phytosequestration, or phytoaccumulation, is a technique by which plant roots remove contaminants from the water or soil, transfer those contaminants to outside biomass, that is, shoots, where they accumulate and are then collected [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]. Plants that produce significant quantities of contaminants throughout their lifecycle can be used in continuous phytoextraction [129]. In general, the phytoextraction process consists of four basic processes: pollution transfer into aerial plant parts, contaminant activation in the rhizosphere, contaminant absorption through the roots of plants, and contaminant sequestration in plant tissue [130], [131]. Whereas high sensitivity of plant tissues is often associated with few unfavorable impacts on plant health, contaminant sensitivity is required to complete the phytoremediation process. In general, the ability of a plant to tolerate pollution is influenced by several processes, including metal binding to the cell wall, active movement of metal ions into vacuoles, chelation of metal ions with proteins and peptides, and complex formation [130]. The depth at which plant roots can develop, seasonal weather, and climatic variables are all factors that influence phytoextraction effectiveness [132]. The use of mobilizing agents including nitrilotriacetic acid, aminopolycarboxylic acids, citric acid, ethylenediaminetetraacetic acid, and ethylenediaminedisuccinic acid can improve the efficiency of phytoextraction [133].

- **Phytofiltration:** Rhizofiltration, or phytofiltration, is the process of removing contaminants from a solution by adsorbing them to the surface of plant roots or precipitating them [134]. Certain plants may include multiple phytochelatins that improve the binding capacity of contaminants like metal ions; the process is associated with the creation of specific compounds inside the root system, which result in the adsorption of contaminants [135]. Rhizofiltration is easily linked to effluents, polluted streams, or subsurface water frameworks. A complete comprehension of contaminant differentiation and the connections between all pollutants and nutrients is necessary for rhizofiltration to be successful. Rhizofiltration is best performed by plants with roots that develop rapidly and can extract contaminants from solutions over an extended period of time [69].

- **Phytostabilization:** The method of employing plants having the capacity to reduce contaminant mobility and bioavailability is known as phytoimmobilization or phytostabilization. This technique witness’s an avoidance in the adsorption via roots or the development of insoluble substances in the root region, such as their leaching into subsurface water or their implementation into the food chain through various methods[129], [134].The term "phytostabilization" refers to the processes of (a) restricting contaminants in polluted media through aggregation and assimilation by roots, adsorption onto roots, or precipitation inside the root system of plants, and (b) employing plants and plant roots to prevent pollutant activity through wind and water, draining, and dispersion of soil [136]. Phytostabilization's ultimate objective is to stabilize contaminants instead of removing them hence reducing their risk for both human health and the ecosystem, with the intention that the plants will play a similar role with soil amendments. Unfortunately, phytostabilization reduces the harmful effects of surrounding media; hence, it cannot serve as a long-term fix for pollution or an area rather than the concentration of contaminants [137], [138]. As a result, phytostabilization is currently recognized as one of the most innovative types of phytoremediation, with a possible application for a variety of metals, particularly chromium, mercury, and lead, which are fixed in soil and decrease the connection of such pollutants associated with biological systems [133], [139].

- **Phytovolatilization:** Another phytoremediation approach is phytovolatilization, which utilizes plant-mediated absorption of pollutants to change them to volatile substances, which are then released into the ecosystem in their original form or altered form due to metabolic and transpiration pull [134]. The water vapors from the leaf's surface are released into the atmosphere via stomata during transpiration. Through the development of specific genes or biocatalysts, several species of plants have extensive root systems and are frequently able to metabolize and eliminate contaminants [140]– [142]. During phytovolatilization, contaminants are taken up through the water or soil and converted into less hazardous vapors that are then released into the atmosphere via the plant's evaporation process [138]. The method is applicable to natural pollutants as well as some heavy metals that are present in the ecosystem as gaseous species, including Hg, Se, and As[143]. As a result, phytovolatilization technology often utilizes genetically engineered plants to improve their capacity to volatilize metals [138]. Furthermore, phytovolatilization causes minimal site disturbance, negligible erosion, and no removal of polluted plant biomass [138]. Phytovolatilization is thus one of the most controversial phytoremediation processes [144], [145].

- **Phytodegradation:** The process of pollutant gathering from sediment, water, or soil, followed by the chemical alteration of pollutants as an immediate consequence of plant metabolism, which frequently results in pollutant elimination, decomposition, or immobilization inside plant shoots and roots, is commonly referred to as phytodegradation, also known as phytotransformation [146], [147]. Some plants can convert the absorbed pollutants into less hazardous chemicals through their metabolisms [140]. Consequently, phytodegradation is a method of metabolism employed in plants to purify and degrade contaminants within the tissues of plant [133].

- **Bioaugmentation:** The autochthonous microbiota of a contaminated site is supplemented by the addition of previously selected native or transgenic species of microorganisms, which improve the process of remediation and thus this technique is referred to as bioaugmentataion. Tetrachloroethylene- and trichloroethylene-polluted soils and subsurface water are able to benefit from bioaugmentation, which helps *in situ* microorganisms break down these toxins into harmless chemicals, including chlorides and ethylene [148].

- **Biostimulation:** Utilizes indigenous microbes to proliferate their growth through the inclusion of minerals including nitrogen and phosphorus as well as oxygen or other substances that oxidize. Through injection wells, stimulant compounds are often delivered underground. The main benefit of this method is the implementation of adaptable autochthonous microbes. Recently, it has been proposed that, while each of these procedures is categorized as an *in situ* bioremediation approach, they may also be used *ex situ* [149], [150].

2. ***Ex situ* Bioremediation:** With this method, contaminants are dug out of contaminated areas and delivered to another area for remediation. *Ex situ* bioremediation procedures are used when the depth of contamination, kind of contaminant, the expense of treatment, and geolocation of the contaminated site are all taken into consideration [71]. The technology is additionally classified into slurry-phase and solid-phase techniques based on the condition of the contaminant to be eliminated [151].

- **Solid Phase Bioremediation:** This technique consists of four steps: excavation of the soil, piling of the soil (which may include agricultural, municipal, and natural wastes), encouragement of the biological degradation process by oxygen supply via a network of pipes that increases microbial respiration or afterwards activity of microbes, and the utilization of microbial stripping columns for the purpose of treating pollutants from the air, followed by biofiltration. Solid-phase bioremediation needs an enormous amount of space and time to be finished [152].

- **Biopiles:** Aerification and additional nutrients are employed in bioremediation to boost the metabolic activity of microbes in piled-up toxic soil above the surface. The technique includes leachate collection, aerification, nutrients, irrigation, and treatment bed systems. *Ex situ* biodegradation is gaining popularity due to the fact that it is cheap and has functional characteristics like 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 enables it to reduce the remediation time by increasing the activity of microbes and pollutant accessibility while simultaneously enhancing the biodegradation rate. Bioremediation is improved when warm air is supplied into the biopile system by providing both heat and air at the same time. The inclusion of bulking substances such as straw, sawdust, or wood chips has aided the biopile's cleanup process. *Ex situ* bioremediation methods, including bioventing, biosparging, and land farming,can be applied to replenish the air supply to polluted piled soil in biopiles [65]. These methods are costly to adopt and require a power supply in remote areas. Extreme air temperatures may impede bioremediation by drying soil and making it more likely to evaporate rather than be broken down by living organisms [155]. The biopile technique of bioremediation relies heavily on bio-available organic carbon (BOC). Alpha, beta, and gamma proteobacteria are used for bioremediation of petroleum- polluted soil under mesophilic (30°C–40°C) circumstances with a modest rate of aerification [156].

Sartaj and Gomez [157] utilized response surface methodology (RSM) based on the factorial design of experiment (DoE) tone to investigate the impacts of various 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 temperatures. The bioaugmented and biostimulated setups had a 90.7% TPH decrease at the conclusion of the 94-day trial period as opposed to the conventional setups, which had a 48% average TPH elimination. TPH reduction was ascribed to a significant proportion.

The disadvantages of biopilemechanims include robust engineering, maintenance and operation costs, and a shortage of an electricity supply, particularly at remote sites that would allow for a consistent flow of air in polluted piled soil by an air pump. Biopile techniques do, however, preserve space in comparison to other field *ex situ* bioremediation methods, such as land farming. The drying effect of high-air heating on bioremediation soil can also inhibit the activity of microbes and encourage volatilization instead of biodegradation [158].

- **Land Farming or Prepared Bed Bioreactors:** Land farming is a highly effective and easy bioremediation technique as a result of its cheap operating expenses and absence of specialist machinery [159].Ex situ bioremediation is the typical approach; however, *in situ* bioremediation can also occur. The type of bioremediation used depends on the site of the treatment. It is standard practice in land farming to regularly eliminate and till contaminated soils. *In situ* treatment refers to on-site treatment, whereas *ex situ* bioremediation procedures are utilized to treat polluted soil [160]. Polluted soils are typically deposited on a permanent layer of substrate much above the Earth's surface to allow indigenous microbes to breakdown pollutants aerobically.

  Land bioremediation of dirty soil utilizing land farming bioremediation techniques is a relatively simple procedure that has a small ecological foot print and utilizes very little energy [161]. It has been stated that when a contaminant is located <1 m below ground surface, bioremediation may proceed without excavation; a contaminant located >1.7 m below ground surface must be transferred 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 the aerobic biodegradation of pollutant by autochthonous microorganisms. Till age, 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 has been documented that cultivation and watering with no addition of nutrients in a soil that had sufficient biological activity enhanced heterotrophic and diesel-degrading bacterial counts, thereby accelerating bioremediation. Dehydrogenase action was also found to be an excellent indicator of biostimulation treatment and could be utilized as a biological parameter in land farming technological developments [119].

- **Composting:** Composting bioremediation is comparable to landfarming bioremediation in the context that it excavates polluted soil to the surface and stimulates native microbes by supplying nutrients and incorporating air, yet it varies in the context that it supplements the soil with a large amount of ingredients, including straw, hay, and corncobs, which aid in transferring oxygen via the soil and sustaining a constant humidity level [163]. Composting is a method, through which natural wastes are decomposed by microbes 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 natural substances in garbage. The following basic steps have been used to demonstrate windrow composting. Initially, big boulders and debris are eliminated from polluted soils by digging and screening [164].

  The soil is moved to a composting pad with an interim structure to provide pollutant and weather protection. Amendments, including wood chips, agricultural wastes, manure, alfalfa, and straw, 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 easily accessible windrow-turning equipment. Temperature, pH, humidity, 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].

- **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 into sludge, and subsequently digested by microorganisms in the biofilter. The bioremediation effectiveness of biofilters makes recirculating aquaculture systems an efficient technique for minimizing aquaculture waste water contamination in marine aquaculture systems. Microbial mats, activated sludge, trickling filters, rotating biological contactors, and denitrifying filters are the most commonly 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 surface.
  - **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 increase the conversion of nitrate to nitrogen gas [165].

- **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].

- **Sludge Bioreactor:** The utilization of biological methods in a closed environment or reactor for the biological elimination of relatively modest waste quantities. Liquids or slurries are treated using this approach. *Ex situ* remediation of polluted soil and water pumped up from a polluted plume is done using aqueous reactors or slurry reactors. Polluted water or solid material (soil, sediment, or sludge) is treated by a specific confinement method in reactor bioremediation. A slurry bioreactor is a confinement vessel and apparatus utilized to develop a three-phase (solid, liquid, and gas) combining circumstance in order to increase the bioremediation rate of soil-bound and water-soluble contaminants as a water slurry of the polluted soil and biomass capable of breaking down target pollutants. Petroleum-contaminated soil and other components have been treated using bioreactors [88].

IV. FACTORS AFFECTING BIOREMEDIATION

1. **Biological Factors:** The competition of soil microbes for carbon sources, as well as the mutual predation of protozoa and phages, can all impact the breakdown of organic compounds. Contaminants and catalyst levels have an impact on derivatization rates. The breakdown process of pollutants is able to be accelerated or decreased by enzymes. For the contaminant to have affinity and availability, enzymes are additionally engaged in the pollutant's metabolism. The primary biological factors include interactions (competition, predation, and succession), the number of individuals, and diversity [166], [167].

2. **Oxygen Availability:** Utilizing microorganisms that do not need oxygen can speed up the biodegradation process. Oxygen availability is also one of the significant

factors influencing the biodegradation process. In most conditions, the addition of oxygen is able to speed up the metabolism of hydrocarbons [65]. Most biodegradations require aerobic conditions operating under the influence of oxygen.

3. **Water Content:** An adequate supply of water is necessary for microorganisms to proliferate. The biodegradation agents are less effective when the soil is too damp [168].

4. **Nutrients Availability:** The rate and efficiency of biodegradation, as well as the proliferation and reproduction of microorganisms, can all be affected by nutrients. Optimizing the bacterial C:N:P ratio can increase biodegradation efficiency, particularly when important nutrients including P and N are present. Microbes need a range of minerals to live, including carbon, nitrogen, and phosphorus. 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].

5. **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 helps 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 accelerate or retard bioremediation. Higher temperatures stimulate microbial activity. It proceeded to drop rapidly as the temperature increased or decreased and then gradually stopped [170], [171].

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

7. **Site Characterization and Selection:** Before proposing a bioremediation solution, 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].

8. **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].

9. **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

1. **Plant-Based Bioremediation:** Instead of relying solely on bacteria and their effectiveness in bioremediating any polluted medium, plants are utilized for bioremediation, either by themselves or in conjunction with microorganisms. The use of green plants to clean up any polluted medium or surface is not a new idea. Plants for wastewater treatment were conceived over 300 years ago. A wide range of plant species, including *Prosopis juliflora*, *Lantana camara*, *Parthenium hysterophorus*, *Fagopyrum esculentum*, *Odontarrhena chalcidica*, *Tagetes patula*, *T. erecta*, *Amaranthus spinosus*, *A. hypochondriacus*, *Chrysopogon zizanioides*, *Brassica juncea*, *Ricinus communis*, Chromolaena odorata, Ageratum conyzoides, Ipomoea carnea, and *Odontarrhena chalcidica* have been identified as an aid in the remediation of HM-polluted soil. Furthermore, plants such as *Beta vulgaris*, *Nicotiana tabacum*, *Arabidopsis thaliana*, and *Sedum alfredii*, have been genetically modified with appropriate bacterial genes from *Saccharomyces cerevisiae*, *Streptococcus thermophilus*, *Caenorhabditis elegans* and *Pseudomonas fluorescens* 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 natural amendments (such as different plant biochars, biosolids, and litter) are utilized as biostimulants in this plant-based bioremediation. Metal sorption is controlled by the utilization of chelators, including ethylenediamine-di-o-hydroxyphenylacetic acid (EDDHA), diethylenetriaminepentaacetic acid (DTPA), ethylene glycol tetraacetic acid (AGTA), nhydroxyethylenediaminetriacetic acid (HEDTA), citric acid, ethylene diamine tetraacetic acid (EDTA), [S,S]- ethylenediaminedisuccinic acid (EDDS), fulvic acids, tartaric acid and salicyclic acid precipitate via the creation of metal chelate complexes that improve the bioavailability of these metals as well as the effectiveness of phytoextraction. Plant-based bioremediation has been determined to be a successful technique for the transformation, immobilization,and accumulation of low-level pollutants. Plant-based bioremediation has various advantages, including public acceptance, economic efficiency, and the capacity to eliminate organic andinorganic toxins simultaneously. The simultaneous expression of GST and CYP2E1 has a significant synergistic impact that increases the accumulation and resistance of heavy metal-organic complex contaminants [36].

2. **Microorganism-Based Bioremediation:** Microbes, including fungus and bacteria, 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, such as *Sporosarcina soli* B-22, *Viridibacillus arenosi* B-21, *E.cloacae* KJ-47 and *Enterobacter cloacae* KJ-46 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 the bioremediation of polluted environments. Bacteria produce siderophores, which reduce metal bioavailability and are subsequently removed from polluted surfaces. Bacterial cells have been observed to change their shape in order to boost the synthesis of siderophores, thereby 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 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 extracellular and intracellular precipitation. Furthermore, fungi operate as a promising biocatalyst in the bioremediation process, absorbing hazardous substances into their spores and mycelium [36].

3. **Plant–Microbe Associated Remediation:** Microorganism-plant-based remediation is gaining popularity because of 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 help plants survive in contaminated environments. The plant-microbe-based bioremediation approach has two aspects. To begin with, microbes help the host plant survive under difficult environmental conditions by providing nutrients. Second, the plant serves an important function in sustaining favorable environmental conditions by increasing soil organic matter, making P, K, and N accessible, 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	Via reducing or oxidizing iron porphyrin complex, accomplishes electron transport processes and catalysis. When pyridine nucleotides are utilized as electron donors, carbon substrates and oxidized products are produced.	Within cells, the synthesis and metabolism of numerous compounds and substances xenobiotics, fatty acids, and oxidized steroids.	[176]
Laccase	Degradation of the O_2 molecule, containing one electron oxidation with various kinds of aromatic chemicals.	A single oxygen molecule in water is reduced, and free radicals are produced when the rings of aromatic compounds are broken.	[177]
Dehalogenase	This happened mostly through three processes: (1) Hydrolytic process: in the SN reaction, the hydroxyl group takes on the role of the halogen substituent, while the water particle acts as a cofactor. (2) Oxygenlytic process: mono- or dioxygenase, which incorporates either one or two molecular oxygen atoms into the substrate and also catalyzes the reaction. (3) Reductive process: it is 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 for transferring two hydrogen atoms from organic molecules to electron acceptors.	Creating energy by oxidizing organic molecules.	[180]
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. CURRENT TECHNIQUES USED IN BIOREMEDIATION

Innovative advanced molecular techniques like genomics, proteomics, metagenomics, metabolomics, and transcriptomics provide greater understanding of the actions of microbes with regard to various processes of metabolism, the expression of mRNA phases, genes, along with enzymes in response to dynamic environments. The "omics technique," which refers to the combination of these various technological advances in the bioremediation field, is used to characterize biological macromolecules inside a collection of microbes and microbial communities without deviating, as well as their distinct mechanisms influencing genetic and molecular function and structures [3].

1. **Bioinformatics Approaches in Bioremediation:** The purpose of bioremediation is to

use information from several biological data sources, including chemical structure databases, to explain the fundamental process of breakdown performed through a particular organism for a particular contaminant and composition, expression of proteins or RNA, organic substances, catalytic enzymes, processes of microbial decomposition, 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 technologies. Due to insufficient data on the variables regulating the development and metabolic processes 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 using 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 improves the fundamental differentiation of proteins produced by microbes with contaminant- degradable characteristics [186].

2. **Omics-Based Bioremediation Techniques:** Genomic, transcriptomic, metabolomic, and proteomic approaches can be useful in bioremediation research. This technology assists among in the evaluation of *in situ* bioremediation methods by correlating genetic sequences to provide information on the quantity of proteins, mRNA, and metabolites [187], [188].

3. **Genomics:** The study of bioremediation bacteria is a new area of study in genomics. This approach is predicated on microorganisms' capacity to completely comprehend their ancestry data inside the cell. A spacious range of microbes are used in bioremediation [189]. The biodegradation process is studied utilizing genomic methods like PCR, DNA hybridization, isotopic dispersion analysis, molecular interaction, exometabolomics, and engineering of metabolism. Numerous PCR-based methods are available for genotypic fingerprinting, such as automated ribosomal intergenic spacer analysis (ARISA), amplified ribosomal DNA restriction analysis (ARDRA), terminal-restriction fragment length polymorphism (T-RFLP), amplified fragment length polymorphisms (AFLP), single strand conformation polymorphism (SSCP), randomly amplified polymorphic DNA analysis (RAPD), 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]. The natural length differences of several SSU rRNA genes in microbial populations may be found using LH-PCR.T-RFLP allows for the continuous identification of several microbial taxonomic groups [191]. The presence and distribution of taxonomic and functional genetic 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].

4. **Transcriptomics and Metatranscriptomics:** The transcriptome, which acts as a crucial connection between the cellular phenotype, proteome, interactome, and

genome, represents the collection of genes that are being transcribed at a certain moment and situation. In order to adapt to environmental changes and hence ensure survival, the capacity to modulate gene expression is essential. Transcriptomics provides comprehensive knowledge of this process throughout the whole human genome. DNA microarray evaluation is a potent method in transcriptomics for quantifying mRNA expression levels [65]. A transcriptomic analysis requires the isolation and improvement of total cDNA, mRNA 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].

5. **Proteomics and Metabolomics:** In contrast to metabolomics, which is focused on all of the metabolites generated by an organism over time or in certain circumstances proteomics is concerned with the entirety of proteins produced by a cell at a specific time and location [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 processes is required. This method allows for the identification and recovery of a large number of metabolites from the sample, producing an enormous quantity 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, foot printing, metabolite profiling,and target analysis are just a few that are useful for identifying and measuring the plethora of biological byproducts found in living organisms [196]. The metabolome and proteome data will be helpful for cell-free bioremediation [65].

6. **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 environment-friendly methods [196].

7. **Microbes and Nanotechnology:** The effective microbes (EM) technique can be used to treat wastewater, and the cleaned water is able to be applied to crops as irrigation [197]. Nanotechnology and EM technologies are useful for water purification. Recalcitrant naturally occurring contaminants, including 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 could be effective.

8. **Engineered Polymeric Nanoparticles for Hydrophobic Contaminant Bioremediation:** Organic contaminants, including 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 elimination from polluted groundwater materials. Polymeric nanoparticles are produced using precursor chains of poly-(ethylene) glycol-modified urethane acrylate (PMUA). PMUA nanoparticles are made to retain their properties when exposed to various bacterial populations [65].

9. **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, has this unique ability as a type of virus resistance. In the CRISPR-Cas system, guide RNA participates alongside 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 *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 Rhodococcusruber 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 have 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 are 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

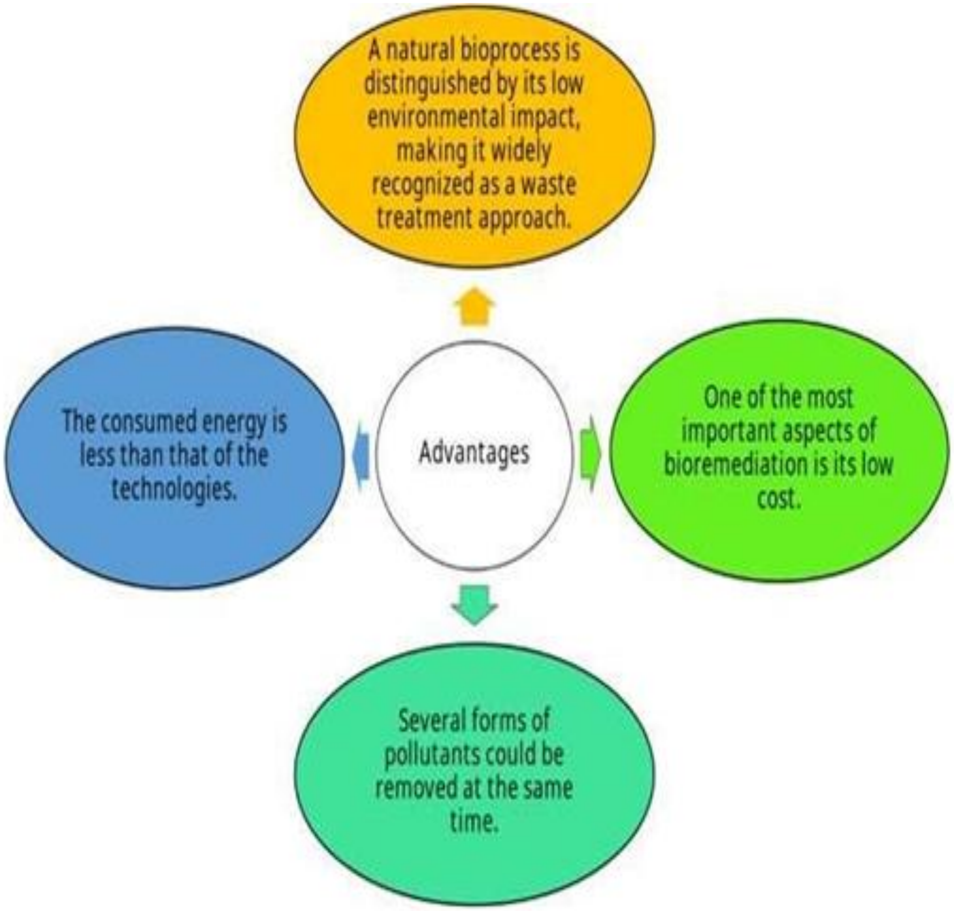


Figure 2: Advantages of bioremediation.

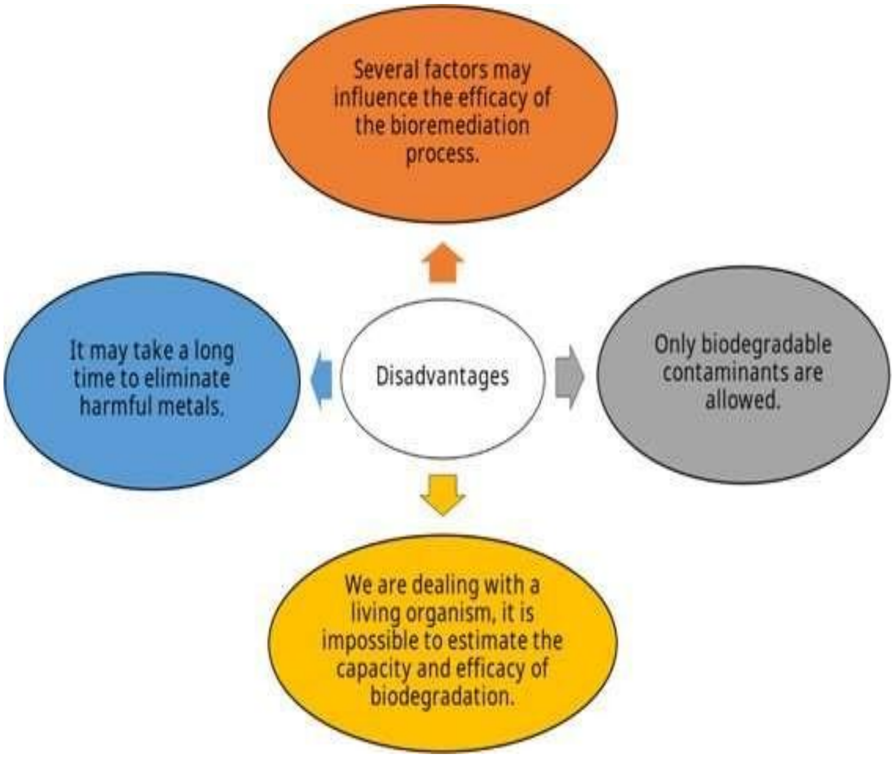


Figure 3: Disadvantages of bioremediation [209].

IX. CONCLUSION

Bioremediation is a method of removing pollutants by increasing natural biodegradation processes. The natural world can be cleaned up, managed, and recovered from pollution through biodegradation. Therefore, these possibilities offer the chance to make significant advancements by better understanding microbial populations and their reactions to the surroundings and contaminants, increasing our understanding of microbial genetics to increase their capabilities in terms of how well they are able to breakdown contaminants, testing new affordable bioremediation methods in the field, and dedicating sites for ongoing investigations. Bioremediation is undoubtedly a trendsetter for creating better lands. Regardless of the element of the bioremediation employed, this approach offers a quick and affordable way to clean up polluted soil and groundwater. Its advantages often outweigh the disadvantages, as seen by the growing number of sites that utilize it and its growing popularity. The contaminated environment has once again been cleaned up utilizing bioremediation technology, which can now be employed as a management tool.

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