BIOREMEDIATION: HARNESSING NATURE'S CLEANUP CREW WITH A SUSTAINABLE APPROACH

Abstract

For the past few decades, chemicals released from industries such as petrochemicals, textiles, pharmaceuticals, agro-based industries, tanneries and other anthropogenic activities cause harmful and deleterious effects to environment as well as to human health. These contaminants can be treated, and result in nontoxic final products. A variety of aquatic and terrestrial environments that have become contaminated by rising using a variety of techniques, including advanced oxidation procedures, physical oxidation processes and chemical oxidation processes. However, these methods and technologies have their own drawbacks, and the end products are toxic and detrimental in nature. Therefore, there is a need to find and investigate environmentally friendly and sustainable processes that use less chemicals, are practical from an economic standpoint anthropogenic activity need to be treated using a variety of green technologies, which must be developed and promoted. Since the last few decades, the use of bioremediation techniques to remove environmental toxins has been practiced as a sustainable technique. This technique is based on an integrated strategy that uses basically three types of approaches including microorganisms, plants and enzymatic remediation. Numerous bacteria break down harmful substances to create carbon-dioxide or methane, water and biomass. These contaminants may undergo enzymatic modification to produce metabolites that are less harmful or harmless. Furthermore, it has been discovered that the solid waste produced by this procedure may have an impact on the

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macro- and micronutrients in the soil, indicating its use as organic manure. However, in order to be established on a wider scale with an emphasis on the effects on the environment, the bioremediation technology needed more investigation. This technology can be categorized as biostimulation (stimulating viable native microbial population), bioaugmentation (artificial introduction of viable population), bioaccumulation (live cells), biosorption (dead microbial biomass), phytoremediation (plants) and rhizoremediation (plant and microbe interaction). This chapter outlines an overview regarding the different types of bioremediation approaches, emphasising more on microbial based remediation and shall discuss the technology and its limitations in a comprehensive manner.

Keywords: Bioremediation, pollution, microorganisms, plants, sustainability.

I. INTRODUCTION

In current scenario, Earth has noticed an enhanced deterioration in the biological and geochemical cycles that sustain the functioning of the biosphere. For the last three decades, a noticeable representation is indicated by the growing levels of atmospheric $CO₂$ and other greenhouse gases ensuing global warming [1]. Furthermore, increase in global industrialization, urbanization, increased disconcerted anthropogenic activities on energy reservoirs and intensive farming due to the ever-increasing human population has left the environment exposed to numerous non-degradable pollutants which are toxic to living things. Pollutants arising from different industrial processes are major sources of pollution to the air, soil and aquatic environment [2]. The pollutants are immunologic, carcinogenic and mutagenic that depletes the energy levels within the ecological systems drastically increased health issues and environmental concerns [3]. These harmful substances include a group of organic and inorganic compounds. Most common organic compounds are polycyclic aromatic hydrocarbons, petroleum hydrocarbons, xenobiotic compounds, halogenated hydrocarbons, phenolic compounds, volatile organic compounds, nitroaromatic compounds, polychlorinated biphenyls and pesticides. Whereas, inorganic compounds consist of nitrates, phosphates and most importantly the heavy metals group, dominated by arsenic, copper, zinc, mercury, lead, cadmium, chromium, nickel, selenium and silver [4]. Once in the environment, they remain toxic for much longer [5]. Metal-contaminated soils are being remedied using chemical, biological, and physical methods. However, physicochemical methods produce a lot of waste and pollution, so they are not valued [6]. Several approaches such as incineration, excavation, and the use of chemicals have been employed to clean up polluted soils but these methods are too expensive and do not provide total cure as some just shifted the contamination from one site to other. One alternative is bioremediation, which makes use of organic biological processes to potentially eliminate or render harmless a variety of toxins [7].

It can be summed up as any procedure that restores the original state of the environment damaged by toxins using bacteria, fungi, green plants, or their enzymes. In bioremediation, bacteria that can breakdown some soil pollutants, such chlorinated hydrocarbons, heavy metals etc. are utilized [8]. The majority of bioremediation research has concentrated on bacterial processes, which have many practical uses. It is well known that archaea contribute to bioremediation in many situations where bacteria are present. Bioremediation is necessary since many hostile environments have deteriorated. Microbes can also help remove contaminants from basic industrial waste or trash that contain high range parameters such as temperature, pH or salinity [9]. As a result, it employs low-tech, low-cost methods that are typically well-liked by the general population and may be used on the spot [10]. However, because of the narrow spectrum of contaminants on which it is effective, the lengthy time frames needed, and the potential inappropriateness of the achievable residual contamination levels, it may not always be suited. Despite the fact that the procedures used are not technically sophisticated, considerable knowledge and expertise may be needed to develop and implement a successful bioremediation program due to the necessity of carefully evaluating a site for suitability and optimizing circumstances to produce a satisfying result. Techniques are improving as greater knowledge and experience are gained, and there is no doubt that bioremediation has great potential for dealing with certain types of site contamination. Unfortunately, the principles, techniques, advantages and disadvantages of bioremediation are not widely known or understood, especially among those who will have to deal directly with bioremediation proposals, such as site owners and regulators. Here, we intended to assist by providing a straightforward, pragmatic view of the processes involved in bioremediation, the pros and cons of the technique with its proper and effective implications in order to manifest sustainable development.

1. Principles and mechanism of Bioremediation: The process of biologically degrading organic wastes into a reduced or neutralized state or to concentrations below those set by regulatory agencies is known as bioremediation and generally it takes place under controlled parameters [11]. The process depends on biological mechanisms to condense the available concentration of pollutants into a harmless state by various metabolic processes as degradation, detoxification, mineralization or transformation [12]. Microorganisms or plants to degrade or detoxify substances hazardous to human health and/or the environment (Figure 1). The method of pollutant removal largely relies on the type of pollutant, which can include agrochemicals, dyes, heavy metals, hydrocarbons, radioactive waste, plastics, sewage and chlorinated compounds [13]. However, recent studies in molecular biology and ecology offer opportunities for more efficient biological processes. Notable accomplishments of these studies include the clean-up of polluted water and land areas with the help of ambient microbial resources [14]. Generally, biosprospecting the enzyme within the living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms is quite economic and easy to use [15]. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated sites [16]. The hazardous and volatile compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes [12]. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate [17].

Like other technologies, bioremediation has its limitations and therefore requires specific strategies to manifest the complete process [18]. This may include contaminants, such as chlorinated organic or high aromatic hydrocarbons that are quite resistant to microbial attack. They are degraded either slowly or not at all, hence it is not easy to predict the rates of clean-up for a bioremediation exercise; there are no rules to predict if a contaminant can be degraded [20]. Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents [17]. Since bioremediation is based on natural attenuation the public considers it more acceptable than other technologies. Most bioremediation systems run under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules [21].

Figure 1: Mechanism for Bioremediation Using Biological Resources.

2. Agents of Bioremediation: The most crucial agents used in bioremediation are natural organisms, whether native or foreign. The organisms differ based on the chemical characteristics of the pollutants, and they must be carefully picked because they can only survive with a certain amount of chemical contaminants. Plants and microbes are the most essential representatives to this process and facilitate the process of phytoremediation and microbial remediation respectively. Further, mycoremediation (using fungi) and microremediation (using bacteria) techniques necessitate several subprocesses to enhance the on-site removal of the contaminants viz. bio-stimulation, bioaugmentation and biosparging, etc. Biodegradation is basically categorised into two types. The first category is called as biomineralisation. Mineralisation is the practice in which microorganisms feed on organic compounds and by a chemical process, reduces them to inorganic material such as water carbon dioxide, and other such inorganic compounds [22]. In mineralisation process total degradation of the organic matter occurs. The second category of bioremediation is called biotransformation that differs from mineralization where the organic matter is not degraded totally [23]. Some part of it is degraded and another part is converted into other smaller chain organic compounds [23]. Bioremediation and biotransformation methods are naturally occurring where the microbial catabolic diversity enables degradation, transformation or accumulation of a huge range of compounds [5] [9] [10]. This includes hydrocarbons, polychlorinated biphenyls, polyaromatic hydrocarbons, heavy metals, pesticides with endosulfan and endosulphate compounds, DDT, petroleum products, phthalate compounds, azo dyes are categorised as biodegradable pollutants [14]. Occurrence of petroleum hydrocarbons affects physical, biochemical and physiological properties of soil and the plants disposed to oil exposure due to phytotoxic nature of hydrocarbons, and immobilization of nutrients in the soil. Whereas, phytoremediation has been used effectively for the reestablishment of desolate metal-mine workings and cleaning up sites where mostly polychlorinated biphenyls have been thrown out during manufacture and mitigation of ongoing coal mine discharges [24]. Whereas, fungi have a non-specific ligninolytic and highly oxidative enzyme system that may mineralize xenobiotic chemicals to $CO₂$ and $H₂O$ as well as degrade and decolorize a variety of dyes [25] [26].

II. BACTERIA AS EFFICIENT KEY PLAYERS IN BIOREMEDIATION

The process of breaking down organic material into basic nutrients that may be utilized and recycled by other organisms is known as biodegradation. The term "biodegradation" in the context of microbiology refers to the breakdown of all organic materials by bacteria, yeast, fungus, and perhaps other organisms. Microbes are widely dispersed throughout the biosphere, and because to their exceptional and dynamic metabolic capacity, they may easily spread under a variety of environmental conditions. The adaptability of the diet can also be used to promote the biodegradation of harmful contaminants. Microbial enzymes must be used in a bioremediation process to convert hydrocarbons into less dangerous molecules. Enzymes produced by bacteria and fungi, such as oxidoreductases, esterases, peroxidases, and hydrolases, are crucial for accelerating the biodegradation of agricultural chemicals. [14]. Several bacteria are found to be natural scavengers that have developed gradually, degraded effectively the organic pollutants and attained energy from the contaminated sites. They have been employed as a biotechnological technique to bioremediate environmental pollutants due to their capacity to repair environmental problems. Due to its effectiveness, affordability, and sustainability, the degradation of naphthalene, phenanthrene, polycyclic aromatic hydrocarbons, or PAH, and heavy metals has attracted considerable attention on a global scale [27]. Because of their persistent nature and ability to bioaccumulate, heavy metals pose another significant hazard to biodiversity and human health. [4] [5] [6]. Studies have shown that microorganisms are effective at degrading heavy metals. Detailed research is being done on the widespread usage of genetically altered microbes that can also aid in the removal of xenobiotic substances including petroleum, naphthalene, toluene and benzene where the association of microorganisms frequently produce this conversion process [28] [29]. In addition, bioremediation is only successful when the environment supports microbial activity and development; otherwise, it may be necessary to alter specific environmental factors to promote microbial growth so that breakdown may occur more quickly [17].

Most bioremediation techniques operate in a mostly aerobic environment, however in some cases, anaerobic conditions may be required to treat particular refractory molecules. Better results from bioremediation depend on a number of variables, including the temperature of the surrounding environment, aerobic or anaerobic illnesses and nutrition availability. The oxidation-reduction or redox processes continues when one chemical species provides an electron to another that absorbs an electron, are supplemented by a number of bioremediation techniques, which are roughly categorized as aerobic and anaerobic types of reactions. Bacteria found to degrade or neutralize a broad spectrum of xenobiotic chemicals include both aerobic e.g., Bacillus, Escherichia, Gordonia, Pandoraea, Pseudomonas, Moraxella, Micrococcus, Sphingobium, Rhodococcus and anaerobic types e.g., Pelatomaculum, Desulfotomaculum, Syntrophobacter, Syntrophus, Desulphovibrio, Methanospirillum, Methanosaeta [30]. Aerobic bioremediation is predominant form of oxidative bioremediation. Here oxygen acts as the terminal electron acceptor of various chemicals such as petroleum products, phenols, polyaromatic hydrocarbons (PAHs), etc. and undergo oxidation. The demand for oxygen suggests it has a larger oxidative potential and that some enzyme systems need it to start the degradative process [31]. Meanwhile, research has been done on the underlying chemical issue that limits the biodegradability of aromatic pollutants in both aerobic and anaerobic conditions.

Basically, aerobic microorganisms use oxygenase enzymes to attack aromatic molecules, but this is stopped by electron-depleting groups like azo, chlorine and nitro. The anaerobic microbes work together to get rid of the pollutants in one go, while the microorganisms use enzymes to attack the molecules. The first reductive action on the pollutants is made stronger when there's an electron-donor group, while the electron-donor groups slow down the anaerobes' ability to turn the molecules into anaerobic form [27] [28] [29]. However, the full lack of both electron donating and withdrawing groups will increase the hydrocarbon's recalcitrance in the anaerobic environment. Furthermore, it has been observed that the byproducts of complex molecule anaerobic biodegradation, such as polychlorinated and polynitroaromatic compounds, are suitable for aerobic mineralization but resist further anaerobic biodegradation [31]. Environmental pollutants, including persistent organic compounds, heavy metals, toxins and air pollutants that are synthetic or natural in origin, enter ecosystems primarily through anthropogenic processes and pose a threat to life forms such as plants, animals, and humans [10]. References to the studies where bacteria are the ample source of bioremediation of different pollutants are listed in Table 1. It has been also established that different microbial enzymes are the potential bioresources that are used in the removal of pollutants. Some of the prominent of them are enlisted in Table 2. But the biochemical pathways and the catabolic gene metabolism done by bacteria during the complete process of bioremediation is not mentioned in this chapter. The present section provides an outline regarding various ambient resources and focusses on the fundamental concept of bioremediation.

Target Compounds	Bacteria linked with degradation	References
Endosulfan compounds	Mycobacterium sp., Bacillus sp. and Staphylococcus sp.	[32] [33]
DDT	Dehalospirilum multivorans, Streptomyces sp.	[34] [35]
$2,4-D$	Cupriavidus necator JMP134	[36]
HCH	Sphingobium ummariense sp. nov.	$[37]$
Napthalene	Pseudomonas putida, Bacillus licheniformis JUG GS2 $(MK106145)$ and Bacillus sonorensis	$[38]$ [39]
Halogenated compounds	Dehalococcoides sp.	[40] [41]
Dibenzofurans	Bacillus pumilus and Staphylococcus warneri	[42]
Phenolic compounds	Alcaligenes odorans, Bacillus subtilis, Corynebacterium propinquum, Pseudomonas aeruginosa	[43]

Table 1: List of Toxic and Recalcitrant Compounds and Degrading Bacterial Genera.

Table 2: Target Compounds and the Microorganisms with their Enzymes.

III. MECHANISM OF BIOREMEDIATION

 Bioremediation is an economical and environmentally friendly process employing microorganisms or their enzymes to efficiently clean and eradicate pollutants from contaminated environmental sites. This biological mechanism is a promising solution for combatting environmental pollution, leveraging the power of microorganisms to detoxify and remove contaminants effectively [17]. Bioremediation plays a crucial role either in the breaking down of complex molecules or abolition of toxic compounds or immobilization, or purification of different chemical and physical hazardous materials, with a primary principle involving the complete or partial breakdown of components by enzymatic activity and finally lead to the conversion of pollutants, such as hydrocarbons, oil, heavy metals, pesticides, dyes into non-toxic forms. This process, known as metabolization, contributes to resolving numerous environmental problems [61]. Two types of factors, biotic (related to living organisms) and abiotic (pertaining to non-living factors), influence the rate of degradation during bioremediation. Bioremediation reduces them to harmless levels by biologically degrading organic in the degradation process, a practice known as bioaugmentation. The transformation of contaminant compounds occurs through the metabolic processes of these living organisms, often involving a collaborative effort among multiple wastes under controlled conditions in accordance with regulatory standards [62]. Utilizing living organisms, mainly microorganisms, this approach transforms environmental pollutants into less harmful forms as it harnesses the natural abilities of bacteria, fungi, or plants to detoxify hazardous substances, safeguarding both human health and the environment. These microorganisms can be either naturally occurring in the contaminated area or introduced from external sources to assist organisms.

- **1. Factors Affecting Bioremediation:** This depends on the interactions between four main components: pollutants, organisms, nutrients and the environment. The interactions between these four components also influence biodegradation, bioavailability, and overall physiological requirements, all of which play a role in determining the bioremediation possibility [12] [20] [22]. Some of the significant factors that affect biodegradation processes are:
	- **Nature and Concentration of Contaminant:** Microbial degradation of organic pollutants is dependent on the bioavailability of the contaminant and the catabolism of the microorganisms. Toxins can interact with their immediate environment to alter their availability to microorganisms that are able to degrade them. Bioavailability varies from species to species, and can thus be defined as the degree to which a pollutant is able to pass into or onto an organism. Microbial degradation of pollutants requires the enzyme activity of the contaminant to be catabolized in order for it to be successfully bioremediated. In some cases, environmental conditions may necessitate manipulation to maximize microbial growth and rate of degradation [52]. The success of the bioremediation process relies on the availability of nutrients and the presence of other essential factors that support biological functions [10]. These factors include the concentration of contaminants which directly impacts microbial activity. High concentrations may have toxic effects on bacteria, while low concentrations may hinder the induction of bacterial degradation enzymes.
- **Availability of Nutrients:** The optimal result of bioremediation depends on the presence of vital nutrients needed for the growth and activity of bacteria. The soil or water environment where bioremediation is to take place must have nitrates, phosphates, and diverse electron transport sources. The nutritional requisite C: N (carbon to-nitrogen ratio) should be 10:1 and C: P (carbon-to-phosphorous ratio) should be 30:1 during degradation [63].
- **Physical Parameters:** Physical parameters include optimum pH, optimum temperature, oxygen availability and proper moisture content. While most bioremediation systems operate under aerobic conditions to create an oxygen-rich environment, running a system under anaerobic conditions may offer opportunities for microbial organisms to break down otherwise stubborn molecules. The effectiveness of bioremediation relies on favorable environmental conditions that support microbial growth and activity [27]. Microbial growth requires an optimal presence of water in the environmental matrix, for ideal growth and proliferation, microorganisms typically need 12% to 25% moisture [63]. The effectiveness of any bioremediation strategy is significantly influenced by site environmental conditions, including pH with an optimum in the range of 6-8, directly proportional to temperature, optimum moisture content, proper nutrient availability with recommended amounts of trace elements and factors affecting redox potential [64].

Moreover, effective biodegradation is facilitated by the enriched catabolic activities of microbes through genetic alteration, fabrication of specific enzymes and selective supplementation for organisms so that they transform the target molecules The extent to which contaminants are adsorbed to solids or sequestered by molecules in contaminated media, their diffusion in macropores of soil or sediment and whether they exist in non-aqueous phase liquid form determines the bioavailability of contaminant [65]. Contaminants with stronger sorption to solids, enclosed in matrices, widely diffused in macropores, or in NAPL form exhibit lower bioavailability for microbial reactions [66].

2. Advantages of Bioremediation: Bioremediation is a natural, highly specific, targeted and eco-friendly approach to environmental cleanup, as it relies on the activity of microorganisms and plants to degrade or transform pollutants [3] [5]. It is cost-effective method for remediating contaminated sites, especially when compared to more expensive and invasive techniques like excavation or incineration [6]. Once established, bioremediation can provide long-term treatment, as microorganisms continue to degrade pollutants over time, reducing the risk of contaminant migration. Bioremediation can be applied to a wide range of contaminants, including hydrocarbons, heavy metals, pesticides, and organic compounds, making it a versatile solution for different types of pollution [12]. It can work in synergy with natural attenuation processes, enhancing the degradation of contaminants and promoting a more sustainable remediation strategy [61]. In many cases, bioremediation requires minimal energy inputs and can address contaminants that are challenging to treat using traditional methods, such as certain chlorinated compounds and recalcitrant organic pollutants, as it utilizes naturally occurring microbial and plant processes [9] [12] [13]. Bioremediation is generally well-

received by the public and communities, as it employs natural processes and poses fewer perceived risks compared to some chemical or physical treatment methods.

3. Limitations of Bioremediation: Bioremediation has gained popularity as a viable alternative to traditional waste treatment methods. It is restricted to compounds that are biodegradable. Not all pollutants can be rapidly and completely degraded by biological processes. There are concerns that the by-products generated during biodegradation may be more persistent or even more toxic than the original contaminants. Successful bioremediation requires specific site factors, including the presence of suitable microbial populations, appropriate environmental conditions for microbial growth, and adequate levels of nutrients and contaminants [11]. Therefore, it can be challenging to extrapolate results from small-scale bench and pilot studies to full-scale field operations.

IV. BIOREMEDIATION TECHNIQUES AND APPLICATIONS

 Bioremediation technologies need to be developed and engineered to address sites with complex mixtures of contaminants that may not be evenly dispersed in the environment. Bioremediation often takes longer to achieve desired cleanup levels compared to other treatment options like soil excavation or incineration. There is uncertainty regarding acceptable performance criteria for bioremediation and the definition of "clean" can be challenging to establish and evaluate in the context of bioremediation processes. This approach harnesses the power of natural microbial activity, facilitated by diverse consortia of microbial strains, to degrade contaminants in waste compounds and media. Numerous studies have explored bioremediation, and the scientific literature highlights the continuous development of various bioremediation techniques over time (Figure-2).

1. In-Situ Bioremediation : In-situ bioremediation can be categorized into two types: intrinsic bioremediation and engineered bioremediation. Intrinsic bioremediation, also known as natural reduction, is a passive in-situ bioremediation technique used to remediate polluted sites without human intervention. This method relies on stimulating the growth of naturally occurring microbial populations that can biodegrade the pollutants present, including those that are difficult to break down. Both aerobic and anaerobic microbial processes are utilized in this approach. Since it does not require external forces or human intervention, intrinsic bioremediation is a cost-effective method compared to other in-situ techniques. Whereas, engineered in-situ bioremediation involves the intentional introduction of specific microorganisms to the contaminated site. Genetically engineered microorganisms are employed to accelerate the degradation process by improving the physicochemical conditions, thus promoting the growth and activity of the targeted microorganisms. This technique aims to enhance the bioremediation efficiency and speed up the cleanup process at the contaminated location.

• **Engineered Bioremediation**

- **Biosparging:** In biosparging, air is injected under pressure below the ground water to raise the concentration of oxygen for microbial pollutant breakdown. The aerobic decomposition and volatilization are accelerated by biosparging [67]. In this method, which is very similar to bioventing, air is injected beneath the soil's surface to encourage microbial activity and aid in the removal of pollutants from polluted regions. Unlike bioventing, which involves injecting air at the saturated zone, biodegradation may be aided by the upward migration of volatile organic chemicals to the unsaturated zone. The efficiency of biosparging depends on two key elements: soil permeability, which affects the pollutant's bioavailability to microorganisms, and pollutant biodegradability [21].
- **Biostimulation** techniques for heavy metals rely on the provision of essential nutrients (carbon, nitrogen and phosphorus), appropriate temperature, oxygen levels, pH, redox potential, and the concentration and type of organic pollutants to accelerate microbial degradation of chemical pollutants. The importance of these parameters in fostering biostimulation has been underlined in several research [12] [21] [68]. Adding biodegradable substances as main substrates, which then allow contaminants to degrade as secondary substrates at manageable rates, is another successful technique. Temperature regulation is essential since it has a favorable impact on biomass activity and, as a result, the biostimulation process. Temperature regulation is possible in engineered systems like slurry bioreactors and enclosed vessel composting. Studies indicated that metals as iron, copper, chromium and cadmium are removed utilizing heterogeneous populations of bacteria isolated from contaminated areas [69].
- **Bioaugmentation** is the process of adding enriched consortia or strains of microorganisms to a system when the necessary microbial populations are insufficient to break down the target chemicals [70]. Mixed cultures made up of various microorganisms are frequently used to achieve positive results [71]. Studies suggested that biotic and abiotic variables are necessary for effective

bioaugmentation. Enrichment of cadmium contaminated soil along with removal of heavy metals has been processed with the use of effective strains of Bacillus sp. and Streptomyces sp. as well as the removal of heavy metals as nickel, lead and zinc using a group of filamentous fungi and mediated through bioaugmentation [72]. However, a number of unfavorable biotic and abiotic conditions that affect autochthonous bacteria and may use them to release organic chemicals that may have an impact on the efficacy of the process. Nonetheless, the success of bioaugmentation can be increased by choosing the right operational procedures designed to increase the survival and long-term effectiveness of the imported microbial species.

- **Bioventing** is an in-situ bioremediation technique with the primary objective of achieving microbial transformation of pollutants into harmless compounds through the addition of nutrients and moisture, thereby enhancing bioremediation [73]. Two key requirements must be met for bioventing to be successful: preserving aerobic conditions and guaranteeing appropriate biodegradation rates. The air injection rate is a critical factor in optimal pollution dispersion because sufficient quantities of naturally occurring microorganisms that break down hydrocarbons must be present. Typically, oxygen and nutrients are introduced into the soil via bioventing or biostimulation techniques for "in situ" remediation of heavy metals [74]. In the unsaturated zone of Earth, soil vapor extraction increases the volatilization of volatile organic compounds, whereas bioventing achieves microbial decomposition by mildly infusing air [76]. Notably, compared to bioventing, soil vapor extraction uses a higher air flow rate [73]. Techniques known as "bioventing" include the purposeful stimulation of airflow to the unsaturated (vadose) zone with the goal of enhancing native microorganisms' normal bioremediation activities. The method encourages microbial transformation of contaminants into benign forms by giving oxygen to the subsoil. In addition, modifications like moisture and nutrients are added to improve the effectiveness of bioremediation. Bioventing has become one of the most widely used in-situ bioremediation techniques due to its efficiency [76].
- **Intrinsic Bioremediation:** This is a process where local microbiota and environmental factors allow a contaminant to naturally attenuate to safe levels over a reasonable period of time. Due to the fact that it requires no intervention, this is typically the initial option for biotreatment [11]. Additionally, there is a lack of explicit recommendations on acceptable sampling and analytical techniques to guarantee that measures of intrinsic bioremediation produce reliable results. The accurate identification of site characteristics will have a significant impact on how widely intrinsic bioremediation is eventually adopted. This strategy is frequently referred to as monitored natural attenuation since the key to its effectiveness lies in careful monitoring of biodegradation over time [73].
- **Advantages of In-Situ Bioremediation:** There is no need for excavation or the removal of contaminated materials because in-situ bioremediation takes place right in the contaminated environment. Because of this, there will be less need for comprehensive site repair. As a result of avoiding the costs of excavation,

transportation, and off-site treatment of contaminated materials, it is frequently more affordable than ex-situ techniques. Since in-situ bioremediation may be conducted on a broad scale, it may be utilized to clean up huge, heavily contaminated areas without requiring the transportation of a lot of resources. It leverages microbial degradation's inherent natural processes, making it a sustainable and environmentally responsible method of environmental remediation [15] [16] [19]. Once established, in-situ bioremediation can offer long-term contamination treatment because microbial activity keeps contaminant breakdown running for a long time. By encouraging microbial activity and fostering conditions for breakdown, it may enhance the bioavailability of pollutants. Techniques for in-situ bioremediation can be modified to remediate a variety of pollutants, including as organic substances, hydrocarbons, and some heavy metals. Since it relies on naturally occurring microbial activity, in-situ bioremediation frequently requires little energy inputs. By reducing the need for excavation, transportation, and the production of extra trash, in-situ bioremediation can result in a smaller overall environmental impact. When combined with natural attenuation processes, in-situ bioremediation can sometimes further accelerate the breakdown of pollutants.

• **Limitations and Challenges of In-Situ Bioremediation:** In-situ bioremediation might take some time, especially for pollutants with complicated chemical structures or limited bioavailability. The effective breakdown of such contaminants by microbes may take a long time. When pollutants are strongly attached to soil particles or trapped in low-permeability zones, they are less accessible to microorganisms, which reduce the efficiency of in-situ bioremediation. In order to stimulate microbial growth and breakdown, in-situ bioremediation frequently needs the input of nutrients. However, it can be difficult to provide the necessary nutrients in the right quantities, and low nutrient availability may restrict microbial activity. Favorable environmental factors, such as temperature, pH, and moisture, are crucial for the effectiveness of insitu bioremediation [10]. The repair process might be slowed down by unfavorable conditions that prevent microbial activity. Different microorganisms compete for nutrients in natural habitats, and certain native bacteria may outcompete the remedial microbes that are added, decreasing the efficacy of in-situ bioremediation.

In some situations, there may be a rebound effect after in-situ bioremediation, in which the concentration of contaminants rises once more as a result of the dispersal of pollutants that were previously held in the soil or sediment. Not all pollutants or site circumstances will respond well to in-situ bioremediation. It's possible that some contaminants can't be biodegraded by local microorganisms, or perhaps the site's characteristics will make it challenging to apply in-situ bioremediation effectively. Stakeholders might be concerned about the potential risks connected with introducing microorganisms into the environment and regulatory authorization for the use of insitu bioremediation techniques may be necessary. Assessing the distribution of microorganisms, their activity, and the breakdown of pollutants in the subsurface environment is essential for accurate monitoring and validation of in-situ bioremediation processes. In order to maintain the remediation process' effectiveness over time, successful in-situ bioremediation may need constant upkeep and supervision.

2. Ex-Situ Bioremediation: Ex-situ bioremediation techniques require the removal of contaminants from polluted areas and their transportation to another location for processing. Ex-situ bioremediation procedures are frequently chosen based on variables such as the degree of pollution, the kind of pollutant, the depth of contamination, treatment costs, and the location of the contaminated site.

The following steps are usually included in the ex-situ bioremediation process: To ascertain the level and kind of contamination, the polluted location is evaluated. Understanding the types of contaminants present, how they are distributed, and if ex-situ bioremediation is feasible in general all depend on site characterization. Excavation and transportation of the contaminated soil, silt, or other material to a designated treatment area are required. After that, the substance is adequately contained to stop any further contamination spread. The infected material is cleaned up and ready for bioremediation in the treatment area. To expand surface area and improve microbiological access to the contaminants, this might involve grinding, shredding, or screening. To improve the conditions for biodegradation, contaminants may also be combined with additives or amendments. In order to break down or change contaminants, bioremediation involves adding microbes, nutrients, or other biological agents. Throughout the bioremediation procedure, the contaminated material is observed in order to gauge the process's success and efficacy. Following the conclusion of the bioremediation procedure, the treated material is examined to determine whether the pollutant levels have been sufficiently decreased to meet legal requirements and the cleaning objectives particular to the site. Depending on the degree of decontamination attained, the treated material may be dumped at a suitable landfill or repurposed for innocuous uses like fill material in construction. The appropriate ex-situ bioremediation strategy is also dependent on compliance with performance standards [67].

Ex-situ bioremediation procedure known as "solid-phase treatment" entails excavating contaminated soil and piling it up. Organic waste, including leaves, animal manure, agricultural trash, household, industrial, and municipal waste, is also added to the piles using this technique. A network of pipes positioned throughout the piles promotes bacterial development by allowing for appropriate ventilation and microbial respiration. In contrast to slurry-phase techniques, this method needs a lot of room, and the cleanup procedure takes longer. Solid-phase treatment includes a number of procedures, including biopiles, windrows, land farming and composting. Slurry-phase bioremediation is a process that moves forward a bit more quickly than other forms of treatment. In this method, contaminated soil is combined with water, nutrients, and oxygen in a bioreactor to provide microorganisms with the best possible habitat in which to break down the pollutants contained in the soil. The polluted soil is removed from the stones and debris during this procedure. The amount of additional water is determined by the concentration of pollutants, the rate of biodegradation, and the physicochemical characteristics of the soil. After the procedure is finished, the dirt is collected and dried using centrifuges, vacuum filters, or pressure filters. The next steps involve disposal of the soil and treating the fluids that are produced in an efficient way.

V. EMERGING TECHNOLOGIES IN BIOREMEDIATION USING AMBIENT BIORESOURCES

 The elimination of toxins from the environment can be accomplished with the help of developing bioremediation methods. These innovations are socially and economically feasible as well as environmentally friendly [76]. The following are some of the burgeoning bioremediation technologies:

- **1. Role of Genetically Engineered Bacteria in Bioremediation:** Management of environmental pollution by microorganisms is a promising technology. Different genetic approaches have been developed and used to optimize the enzymes, metabolic pathways and organisms relevant for biodegradation. Novel facts on the inter connections between the metabolic routes and bottlenecks of degradation is still accumulating and amplifying the available toolbox to decipher probable processes to harness the cleanup process with sustainability [28]. In this section, a brief outline has been mentioned regarding the new concept of bioremediation facilitated by bacteria using some modern and sustainable methodologies.
	- **Microbial bioremediation:** This method makes use of microbes to break down or change pollutants into less dangerous chemicals. This has been already discussed in section II of this chapter. Hydrocarbons, heavy metals, and pesticides, insectides, synthetic dyes, bird feathers, recalcitrant xenobiotic compounds are some of the prominent type of contaminants that bacteria degrade very efficiently [17] [25] [46] [51] [62]. Moreover, a comprehensive and systematic understanding of interactions between the metabolic and genetic diversity along with the abiotic and other biotic factors are therefore required to suggest promising projections in the future.
		- **Enzymatic Bioremediation:** Enzymes are essential for bioremediation because they catalyze the degradation of pollutants and the process is known as enzymatic bioremediation [52] [53] [58] [59]. The list of key enzymes and their microbial resources has been enlisted in Table 2. But the vast discussion regarding the function has not been done. This is a separate area and needs more data and research. Recent developments in synthetic biology and enzyme reengineering have created new opportunities for improving enzymatic bioremediation procedures.
		- **Microbial Fuel Cells:** A type of bioremediation technology known as microbial fuel cells that usually combines the oxidation of pollutants with the generation of power. Microorganisms are used in these type of cells are used to decompose organic material while also producing electrical energy [77].
		- **Bioelectrochemical Systems:** To remove toxins from wastewater or soil, bioelectrochemical systems that rely on interactions between microorganisms and electrodes [78]. Heavy metals and organic pollutants, among other contaminants, can be treated using this particular system. Therefore, the right application of this technology for the treatment of recalcitrant xenobiotic chemicals and other

pollutants is a sustainable and energy-efficient technical solution with the potential for concurrent energy plus values recovery.

2. Role of Plants in Bioremediation: This is also very commonly used technique used from a long time back to clean up the contamination locations or areas with the help of plants [79]. Using plants to absorb, gather, and detoxify contaminants from the soil or water is a technique known as phytoremediation. The ability of various plants to remove particular pollutants varies. Phytoremediation is a multidisciplinary field that aims to mobilize and/or immobilize pollutants from different environmental conditions [80]. It includes a number of processes, including phytostabilization, rhizoremediation, phytoextraction, phytodegradation, and phytovolatilization [81]. Due to its effectiveness and cheaper cost compared to other remediation techniques for heavy metals and metalloids including chemical immobilization, excavating, and dumping, phytoremediation is becoming more and more popular. Numerous researches have looked into how phytoremediation might improve the effectiveness of bioremediation [82]. One example is the improvement of phytoremediation efficacy by the implication of plant growth-promoting rhizobacteria or PGPRs [83]. The potential for phytoremediation is increased by the discovery that biochar, a substance created by the pyrolysis of organic matter, dramatically reduces the bioavailability and leachability of cationic metals and metalloids in soils, improves soil structure, and promotes plant development [84]. Moreover, one of the most common type of secondary metabolite known as phlorotannins that belongs to the particular class of polyphenolic compound is present in brown algae and have the potential to improve the efficacy of phytoremediation with a sustainable approach [85]. In order to increase phytoextraction, plant growth, and toxicity tolerance, as well as phytoremediation efficacy, amendments, whether natural or synthetic, can also be added to soil [80]. It has also been investigated whether phytoremediation might be enhanced by bioaugmentation with endophytic microbes to lessen metal toxicity to plants [83]. Numerous methods and modifications have been investigated to increase phytoremediation's effectiveness since it has showed significant promise in improving the effectiveness of bioremediation [83] [85].

Several studies suggested the potentiality of plants in combating the removal of pollutants from the soil and environment. For instance, the ability of Brassica juncea or popularly called Indian mustard has the potentiality to absorb heavy metals from the soil, such as lead, selenium, zinc, mercury as well as copper and is well documented [86]. Fast-growing trees of the Salix genus, willows can endure a range of soil conditions. In addition to this, they have the ability to absorb and accumulate pollutants. Due to their rapid growth rate, absorb pollutants and ability to withstand a variety of soil conditions, they have been extensively used in phytoremediation operations [87]. Sunflower or Helianthus annuus are well known for their ability to extract heavy metals from contaminated soil, such as lead, arsenic and uranium [88]. The legume Medicago sativa, usually referred to as alfalfa, is useful for removing organic and heavy metal pollutants from soil [89]. The zinc-accumulating plant pennycress (Thlaspi caerulescens) has been frequently used in several phytoremediation related studies [90]. However, the plants that will be used for phytoremediation depend on the specific contaminants and the environmental conditions present on the particular location. Although, having countless advantages, there are some challenges that need to be addressed, to make the process more effective and popular. Despite the fact that it may be more economical than other cleanup methods, the treatment of plants under this process, occasionally exhibit high cost, for setting up and run the system.. Therefore, more comprehensive understanding is required from all stakeholders of society to implement this process without having any reservation or prejudice.

- **3. Role of Nanotechnology in Bioremediation:** Bioremediation strategies based on nanotechnology have showed potential. Nanomaterials can be utilized to speed up microbial decomposition or to get rid of impurities via catalytic or adsorption processes. Nano-Bioremediation is a promising approach that combines nanotechnology and bioremediation to enhance the efficiency of environmental cleanup either with the help of plants, microbes, algae [91]. In order to increase the effectiveness of bioremediation, nanoremediation may contribute as a promising method due to following advantages: (a) The surface area to volume ratio of nanoparticles is large, which might increase the contact between contaminants and the microorganisms utilized in bioremediation. (b) When utilized in bioremediation, they can act as electron acceptors or donors, which can boost the microbial activity and metabolism of the microorganisms. (c) Nanoparticles can facilitate the delivery of nutrients and other vital substances to bacteria, which can promote their activity and growth [92]. Microorganisms are more stable because nanoparticles can shield them from harmful environmental factors such high temperatures, acidic or basic environments, and salt. The effectiveness and specificity of bioremediation can be improved by functionalizing nanoparticles to target certain contaminants or contaminated locations. By immobilizing or decomposing contaminants, nanoparticles can lower their toxicity and lessen their negative effects on the environment and living things. Through increased microbial activity, improved nutrient transfer, increased stability of microorganisms, targeted distribution of microorganisms and decreased toxicity, nano-bioremediation methodology has the potential to increase the efficacy of bioremediation [91] [92]. Despite the fact that nanomaterials have many advantages, their potential hazards and effects on the environment should be carefully evaluated and handled by appropriate research, risk assessment, management and responsible use.
- **4. Role of Fungi in Bioremediation:** Mycoremediation is also a very popular methodology for bioremediation method that uses fungi to break down, detoxify and eliminate various environmental toxins [10]. Fungi are great candidates for boosting the effectiveness of bioremediation because they have special enzymatic abilities and can withstand harsh environmental conditions [17]. It uses fungi's inherent capacity to degrade a range of pollutants, including organic pollutants, heavy metals, pesticides, hydrocarbons, dyes and even some radioactive materials [13] [25] [26] . This environmentally benign technology has drawn attention as an alternative for conventional cleanup techniques, which frequently rely on expensive and occasionally harmful environmental activities [10] [11] [93].

The process of mycoremediation typically involves several steps [94]. Firstly, the ability of a particular fungus species to target and digest the specific pollutants present in the polluted site is taken into consideration while selecting it. Because different fungi have different enzymatic capacities, they can break down various kinds of contaminants. Secondly, the chosen fungi are introduced into the contaminated environment, either by adding fungal mycelium (the fungi's thread-like structures) or by directly applying fungal spores. The mycelium of the fungus grows and spreads throughout the polluted area. And finally, fungi produce extracellular enzymes that convert complicated organic chemicals into less hazardous or more readily metabolizable forms [93] [94]. One of the main mechanisms of mycoremediation, this procedure is referred to as enzymatic degradation. Some fungi have the capacity to bio- or myco-accumulate contaminants within their cells. This enables the fungi to concentrate the pollutants, making their eventual separation or removal more practical. Mycorrhizal fungus occasionally coexists harmoniously with plants. When paired with mycoremediation, these linkages, often referred to as mycorrhizae, increase the ability of plants to absorb toxins from the soil, increasing the effectiveness of phytoremediation [95].

Mycoremediation is especially useful for treating persistent or resistant contaminants that are difficult to remove using traditional techniques. Fungi are useful instruments in the restoration of polluted landscapes because they can survive in a variety of settings and endure extreme conditions, such as high pollution levels or harsh pH levels [96]. The selection of the proper fungal species, ambient circumstances, pollutant concentrations and the ecosystem's general health are all important considerations for mycoremediation [93] [96]. Mycoremediation techniques require ongoing study and implementation in order to reach their full potential as a sustainable and environmentally acceptable bioremediation strategy. Studies revealed that it has a lot of potential for improving bioremediation effectiveness, particularly when it comes to dealing with stubborn and emerging contaminants plus difficult environmental circumstances [97]. Therefore, it can provide a strong and sustainable method to recovering contaminated sites and maintaining environmental health when used in conjunction with other bioremediation approaches, such as phytoremediation. Mycoremediation can be applied in realistic situations more effectively with continued research and development in this area, which will open up new opportunities for research.

5. Role of Algae in Bioremediation: The need to provide sustainable solutions that can provide additional benefits in addition to environmental remediation has been sparked by the rapidly evolving bioremediation prospects. Due to its captivating sustainable characteristics, which include reducing odor and toxicity, co-remediating a wide range of prevalent and new inorganic and organic contaminants from gaseous and aquatic environments and generating biomass for a variety of useful product refinement, algal remediation is attracting the uttermost attention in present scenario [98]. Popularly known as phycoremediation, this technique is becoming a promising in the coming future for restoring the polluted system with added advantages and high potential. Algae are photosynthetic microorganisms that can quickly multiply and flourish in a variety of aquatic conditions and due to these properties scientists genetically alter algae to exhibit particular features that enhance their capacity to remove contaminants [99]. It can increase the expression of transporters and receptors on the surface of algal cells, allowing them to more effectively absorb a wider variety of contaminants [100]. In order to improve the algae's ability to concentrate toxins from the environment, genes linked to the accumulation of particular contaminants, such as heavy metals, can also be added. Through genetic engineering, specialized enzymes that can break down contaminants can be produced and secreted by algae. Algae can be given the ability to directly decompose complex organic contaminants like hydrocarbons, pesticides, plastics by inserting genes that code for the enzymes necessary for their breakdown, hence boosting the effectiveness of bioremediation as a whole [101]. Algae strains that are more resistant to certain environmental stressors, such as high levels of pollutants, harsh temperatures, or salt, can be created with the aid of genetic engineering [98] [99].

This improved tolerance and response of different micro algal strains towards bioaccumulation ability for heavy metals make sure that these altered algae can work well in difficult situations where other ambient microorganisms could fail [102]. Genetic alterations can be used to make "biomarker" algae, which respond to the presence of specific contaminants by changing their color, fluorescence, or producing particular chemicals [103]. With real-time input on the efficiency of the bioremediation process, this feature makes it simpler to monitor pollution levels. Algae-based bioremediation projects can be made more feasible and economically beneficial by using genetic engineering to create strains of algae that are better suited for large-scale production [100]. Moreover, it also expands carbon footprint via carbon-capturing that propose an enhanced opportunity than any other non-algal process for several high carbon dioxide emitting industries [104]. Despite these potential benefits, it's essential to consider the ecological and ethical implications of genetically modified organisms in the environment. Careful risk assessments and regulatory oversight are necessary to ensure the responsible use of genetically engineered algae for bioremediation purposes. Continued research and development in this field can unlock the full potential of genetically modified algae as powerful tools in the ongoing efforts to remediate and restore polluted ecosystems. These new bioremediation technologies have the potential to transform the industry and deliver more efficient and long-lasting solutions for environmental cleanup. New methods and uses for bioremediation are being explored by ongoing research and development in this field.

VI. APPLICATION OF BIOREMEDIATION WITH REFERENCE TO CASE STUDIES

 In this chapter, from the above mentioned topics, it has been revealed that bioremediation makes use of the environment's hidden decontamination workforce in the nature's way of repairing itself. The effectiveness and decontaminating capacity of biological agents, such as algae, bacteria, and fungi, depends on a number of variables, including oxygen, nutrients, moisture, pH, and temperature. By guaranteeing several aspects including cost, the concentration of the pollutant, and the makeup of the degrading site, the practice of bioremediation can be made successful in many parts of the world. These elements eventually guarantee the viability of the proposed ex situ or in situ bioremediation approach. Ex-situ treatments can treat a far greater number of contaminants than in situ methods, but they are more expensive because of excavation and transportation costs. Increasing the cost effectiveness of the specific site remediation is a national priority. Hence, the selection and use of more cost-effective strategies need improved access to data on the performance and cost of technologies used in the field with proper surveillance. In this regard, some prominent and successful case studies of bioremediation using different techniques and types of bioremediation are enlisted in Table 3.

Table 3: List of the Successful Case Studies Using Bioremediation Techniques

VII.CONCLUSION AND FUTURE PROSPECTS

 There are a number of important lessons and best practices that have been learned from numerous successful bioremediation programs in varied environmental situations. These lessons learned can assist direct future bioremediation initiatives and guarantee more successful and long-lasting results. Each bioremediation project should take into account the particular characteristics of the contaminated site, such as the kind and quantity of contaminants, the properties of the soil or water, the climate, and the existence of native microorganisms. To be as effective as possible, the bioremediation strategy must be tailored to the unique site conditions. A complete comprehension of the polluted site's microbial ecology is essential. The right bioremediation technique can be chosen by identifying the natural microbial communities and their ability to breakdown contaminants. This information also helps prevent unforeseen repercussions, such as encouraging the spread of harmful microbes. It is crucial to continuously examine the bioremediation process in order to determine its progress and make the required corrections. Regular evaluation of pollutant concentrations, microbial populations, and ecosystem health overall gives helpful feedback to improve the bioremediation strategy over time. When several approaches are used together, bioremediation frequently performs best. Combining various techniques, such as phytoremediation with mycoremediation or bioaugmentation with bio-stimulation, can increase overall effectiveness and expand the range of toxins targeted. When using genetically modified organisms in bioremediation initiatives, safety concerns should come first, and ethical principles should be followed.

 To prevent unforeseen repercussions and potential environmental impact, rigorous risk assessments are essential. The effectiveness of bioremediation programs depends on involving stakeholders and the local community. The project is more likely to be sustained in the long run if the community is involved from the planning stage on. This creates understanding, support, and cooperation. Often a slow process, bioremediation can take years to completely clean up an area. To make sure that contaminants don't resurface or spread to other places, long-term monitoring and upkeep of the treated locations are essential. A culture of collective learning is fostered when researchers, practitioners, and policymakers exchange knowledge, data, and experiences. Collaboration between professionals and stakeholders can result in bioremediation techniques that are more effective and efficient. Bioremediation solutions should be flexible and adaptable because environmental conditions and problems can vary over time. Success depends on having the flexibility to modify the plan in response to fresh information or unanticipated circumstances. Prioritizing environmentally sound and sustainable bioremediation methods reduces the impact of the cleanup procedure on the environment. Environmental professionals can raise the success rate of remediation initiatives, aid in the restoration and preservation of damaged habitats, and learn from these lessons and best practices for use in future bioremediation projects. Innovation and developments in bioremediation technologies will be fueled by ongoing research and knowledge gained from prior experiences, making them even more efficient and practical solutions to environmental problems.

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