

EXPLORATION OF MODERN BIOREMEDIATION TECHNIQUES

Abstract

In the last few decades environmental pollution has emerged as one of the major challenges for humanity. Plants, bacteria, and fungi have the capacity to degrade and accumulate environmental pollution. Bioremediation is the process by which plants, bacteria, and fungi are employed to break the harmful chemical substances into smaller molecules, remove them from the soil or water, change, immobilize, and convert into a harmless form. The utilization, exploration and improvement in accumulation and degradation capacity of plants, bacteria and fungi can address the issues very efficiently for harvesting the harmful chemicals from the polluted site. We have discussed recent developments utilization of plants, bacteria, and fungi in their natural form for bioremediation which is a very cost-effective technique as well as other emerging tools such as use of engineered plants, engineered microbes, modern genetic engineering tools in bioremediation, and other *in-situ* and *ex-situ* techniques.

Keywords: Bioremediation, accumulation, degradation.

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I. INTRODUCTION

The production of chemicals has supported humans at various stages such as controlling disease, food security, and protection from harsh environments. Simultaneously these chemicals or their by-products have negative impacts (US-EPA. 2017). The scientific research and exploration have registered across 350000 chemicals and mixtures. Over 50000 chemicals are confidential and 70000 are not properly described (Wang *et al.*, 2020). In the last few decades, the problem of chemical contaminants has adversely affected human health (Munzel *et al.*, 2023, Rodriguez Eugenio, 2021). The contaminants have also affected the other living organisms such as nematodes beetles, pollinators, etc. The various contaminants such as chemical, radioactive, and persistent organic pollutants have caused health as well as environmental damage. (Brusseu, and Artiola, 2019, Ashraf., 2017). Pesticides, sugar, textile, paper, and pulp industries have produced various contaminants which are causing serious damage to human health and various ecological interactions of the ecosystem (Sargent *et al.*, 2023). Chemical pollutants have the potential to become one of the largest environmental threats to humanity.

Table 1: Pollutants, their Sources, and Harmful Effects on Human Health

Sr. No.	Pollutants	Source	Disease/Harmful Effects	References
1.	Cadmium	Soil	Endothelial dysfunction and Atherosclerosis	Munzel <i>et al.</i> , 2023
2.	Lead	Soil	Multiple Non-communicable Diseases (NCDs) and Cardiovascular diseases	Nediani <i>et al.</i> , 2019
3.	Gasoline and Benzene	Soil	Leukaemia	Lin <i>et al.</i> , 2022
4.	Mercury	Soil	Kidney and Liver damage	Jyothi and Farook, 2020
5.	Arsenic	Water	Melanosis and Keratosis	Safiuddin <i>et al.</i> , 2011
6.	Chromium	Water	Gastric and Respiratory Cancer	Suh <i>et al.</i> , 2019
7.	Trihalomet hane	Water	Brain and Bladder Cancer	Zumel Marne <i>et al.</i> , 2021
8.	Chlorine	Water	Bladder and Rectal Cancer	Helte <i>et al.</i> , 2023
9.	Nitrogen	Water and Soil	Oesophageal Cancer	Yang <i>et al.</i> , 2023

10.	Copper	Water and Soil	Methemoglobinemia, Hepatic necrosis	Babuji <i>et al.</i> , 2023
11.	Manganese	Soil	Neurological disorder and sexual dysfunction	Rodriguez Eugenio, 2021
12.	Polycyclic Aromatics Hydrocarbons (PAH)	Soil	Cataract, hemolysis	Zungum and Imam, 2021
13.	Phthalates	Soil	Liver cancer and Testicular Atrophy	Zhao <i>et al.</i> , 2023
14..	Radionuclides	Soil	Lung cancer	Timin <i>et al.</i> , 2022
15.	Dichlorodiphenyltrichloroethane (DDT)	Soil	Neurotoxic, Carcinogenic, Immunotoxic and Reproductive effects	Van den Berg, 2011
16.	Selenium	Soil	Brittle hair and nails, Gastrointestinal problems, Kidney and Liver cancer, Heart diseases and Nerve damage	Steffan <i>et al.</i> , 2018
17.	Zinc	Soil	Tachycardia, Hyperglycemia, Gastroduodenal corrosive injury	Plum <i>et al.</i> , 2010;

The word Bioremediation is derived from ‘Bio’ has Greek roots which means ‘Life’ and the Latin word ‘remedium’ which means to correct or remedy. Bioremediation means the remedy from environmental problems with the help of living organisms (Nithya *et al.*, 2021). The biotics convert the harmful and toxic components into non-toxic components by degradation. In the era of large amounts of pollution with heavy metals, pesticides, industrial wastes, greenhouse gases, hydrocarbons, nuclear wastes and toxic chemicals, this field emerges to focus on sustainable development (Azubuike *et al.*, 2016). Naturally many plants and microbes help to convert or reduce the toxic components by converting them into non-toxic forms, but as the amount of toxicant increases with anthropogenic activities, the responsibility comes on the science, or specifically, bioengineering and other emerging fields to engineer those microbes and plants so they can withstand with the high amount of pollutants and to help us to eradicate it (Bibi *et al.*, 2019). Bioremediation is a technique that basically focuses on wastewater treatment and soil pollution. Various types of bioremediation treatments to those hazardous chemicals result in the formation of methane, carbon dioxide

and water, which is useful in one or the other way (Hussain *et al.*, 2022). Till date, few bacteria, fungi and plants are recorded for the process of bioremediation and still it is the area of research to add more information about the organisms which can help the nations to solve the problems of rising pollutants (Singh *et al.*, 2021). The term phytoremediation is used for the plants as they can uptake certain heavy metals to reduce them in soil, still the crop or food plants cannot be used for the purpose, generally, ornamentals and certain weeds are useful so it cannot harm the living beings to a certain extent (Ansari, 2016). But during research, it should also be kept in mind that those weeds and ornamentals which are used for the remediation should not be used by the pollinators or other insects or if they are using it as food, it should be tested that pollutants are not passing to that trophic levels (Sardrood *et al.*, 2012). The landfill capping is the alternative technique which is used in which the contaminated soil is covered by other layers of soil which is not a permanent solution (Kumar *et al.*, 2021). Bioremediation is somewhat a slow process as the biotics convert contaminants into some useful molecules and the process takes time, but recent advances in technologies are trying to improve the process and this can be the permanent solution for the increasing amount of hazardous components increasing in the environment (Kensa, 2011). Countries like the United States, Europe, etc. started using and also improving the techniques of bioremediation (Verma *et al.*, 2021). The main aim of this article is to bring major issues related to the environment and its solution by recently advanced techniques of bioremediation in light. This will focus on the emerging techniques which can help in removing the toxicants permanently from nature with easy applications, cheapest costs and highly acceptable.

II. *IN SITU* TECHNIQUES OF BIOREMEDIATION

In situ techniques involve the remediation of soil on the site of contamination with the help of alpha, beta and gamma bacteria, fungi and plants (Bokade *et al.*, 2023). As compared to *ex situ* bioremediation, *in situ* bioremediation is cost effective and cheaper as transportation is not required for the treatment. Aerobic microbes are introduced in the soil and it helps to degrade the contaminants from the soil (Simarro *et al.*, 2013).

III. PHYTOREMEDIATION

Phytoremediation is a very popular technique where plants are used as accumulators where plants are using energy to remove contaminants from the soil. They are used as pumps for removing contaminants from soil and water. Various processes can be adopted such as Phytotransformation (McCutcheon, and Schnoor, 2003, Gao *et al.*, 2000, Caçador, and Duarte, 2015), Phytostabilization (Shackira, and Puthur, 2019, Galal *et al.*, 2017), Phytovolatilization (Sakakibara *et al.*, 2010), Phytoextraction (Bhargava *et al.*, 2012), Rhizodegradation (Li *et al.*, 2016) and Rhizofiltration (Dushenkov *et al.*, 2012, Verma *et al.*, 2006, Yadav *et al.*, 2011, Bakshe and Jugade., 2023).

Several plants have been reported for phytoremediation of aquatic ecosystem such as *Eichhornia crassipes*, *Pistia stratiotes* L, *Wolffia*, *Salvinia auriculata*, *Ceratophyllum demersum*, *Potamogeton Crispus*, *Vallisneria spiralis*, *Phragmites australis*, (Ali *et al.*, 2020). *Cyperus rotundus*, *Parthenium hysterophorus* have been used for remediation of soil (Boruah *et al.*, 2020). The transgenic plants have been produced by overexpression of the genes for enhancing the extraction capacities of the plants. The extraction potential for plants has been enhanced by overexpression of genes such as tobacco, rapeseed for Cd tolerance

(Misra and Gedamu 1989). The genes which are in biosynthetic pathways of metabolism of chemical compounds can be exploited from various other living beings which includes microorganism, fungus, plants, and animals. The sequences of codons are then incorporated into desired plants. Transgenic plants which can express mammalian P450s and the other enzymes are the efficient candidates for tolerance and phytoremediation of the chemicals of herbicides. Plants can also be engineered for the better absorption and detoxification of contamination. (Kawahigashi, 2009).

Cell suspension culture has already been utilized for the production of secondary metabolites (Yue *et al.*, 2016). Kagalkar *et al.*, 2011 have reported cell culture of *Blumea malcolmii* Hook can play a significant role in the remediation of textile industries. It can decrease the parameters such as biological oxygen demand and chemical oxygen demand of effluent within 48 hours. Malachite Green is one the dyes used in the textile industry. Study reports 93.41 percent decolorization. Cells of *Blumea malcolmii* Hook have tolerated and degraded higher concentrations of dyes.

Table 2: The Phytoremediation Plants and the Hazardous Elements which they Accumulate

Sr. No.	Hazardous Element	Accumulating Plants	References
1.	Arsenic	<i>Holcus lanatus</i> <i>Pteris vittata</i>	Peer <i>et al.</i> , 2006 Wan <i>et al.</i> , 2018
2.	Cadmium	<i>Helianthus annuus</i>	Ali <i>et al.</i> , 2018 Junior <i>et al.</i> , 2015
3.	Lead	<i>Tithonia rotundifolia</i> <i>Mangifera indica</i> <i>Brassica oleracea</i> <i>Helianthus annuus</i> <i>Ocimum sanctum</i>	Collin <i>et al.</i> , 2022
4.	Mercury	<i>Jatropha curcas</i>	Marrugo-Madrid <i>et al.</i> , 2021
5.	Chromium	<i>Helianthus annuus</i> <i>Pennisetum sp.</i> <i>Portulaca oleraceae</i>	Bahadur <i>et al.</i> , 2017 Jia <i>et al.</i> , 2022 Kale <i>et al.</i> , 2015
6.	Trihalomethane	<i>Medicago falcata</i>	Panchenko <i>et al.</i> , 2017
7.	Copper	<i>Corchorus sp.</i>	Saleem <i>et al.</i> , 2020
8.	Manganese	<i>Polygonum pubescens</i> <i>Jatropha curcas</i> <i>Vetiveria Zizanioides</i>	Yu <i>et al.</i> , 2020 Nero, 2021

9.	Polycyclic Aromatics Hydrocarbons (PAH)	Phoenix sp. Juncus subsecundus	Xiao <i>et al.</i> , 2015 Zhang <i>et al.</i> , 2012
10.	Phthalates	<i>Helianthus annuus</i>	Mustafa <i>et al.</i> , 2021
11.	Radionuclides	<i>Amaranthus retroflexus</i> <i>Vetiveria Zizanioides</i>	Yan <i>et al.</i> , 2021
12.	Dichlorodiphenyltric hloroethane (DDT)	<i>Ricinus communis</i>	Rissato <i>et al.</i> , 2015
13.	Selenium	<i>Brassica sp.</i>	Dhillon and Banuelos, 2017
14.	Zinc	<i>Brassica napus</i>	Belouchrani, 2016
15.	Nickel	<i>Brassica napus</i> <i>Helianthus annuus</i>	Boros-Lajszner, 2021 Majeed <i>et al.</i> , 2023

Bioremediation by microbes is a very easy, cost-effective, sustainable, eco-friendly, and fast process as compared to plants (Alori, 2015). Research has found how microbes and metals interact and what kind of biochemical reactions are formed. Recently, the application of those microbes which are associated with plants. These microbes not only remediated the soil but also enhanced the growth of plants and were helpful for plant remediation (Saba *et al.*, 2019). The soil Polycyclic Aromatics Hydrocarbons (PAH) was degrading by microbial-associated phytoremediation, and the process of degradation was enhanced by the number of bacteria, their activity, and ergosterol content available to microbes (Garcia-Sanchez *et al.*, 2018). Microbial phytoremediation is a potent technique for the degradation of Total Petroleum Hydrocarbon (TPH), the petroleum-degrading bacteria and their enzymatic activity plays an important role in the remediation of oil-contaminated soil (Wang *et al.*, 2022).

The molecular mechanism of microbes for degrading soil pollutants discovered like the *Trichoderma virens* fungi remediated by glutathione transferase which is helpful for PAHs. Some endophytic bacterial species such as *Pseudomonas sp.* and *Pantoea sp.* reduce the toxic effect of petroleum pollutants. Two major genes of *Pseudomonas* such as CYP153 and alkB increase the stress tolerance power and other genes nh, pan, phn helpful in microbial-associated phytoremediation process for PHAs and TPH (Rai *et al.*, 2020). Cyanobacteria and other green algae work as biodegradation agents in farms against the toxic chemicals of fertilizers and pesticides (Basit *et al.*, 2021). Phosphate solubilizing microbes (PSM) impact toxins more efficiently as compared to other conventional methods. PSM forms the microbial consortium with other bacteria and enhances the production of more chemicals which shows their effect on the remediation process such as phytostabilization and phytoextraction (Gupta and Kumar, 2017). Arbuscular mycorrhizal fungi (AMF) secrete the glomalin protein which forms the complex with metal and protects the plant from its adverse effects and overcomes the organic pollution from the soil (Aransiola *et al.*, 2019).

IV. ENGINEERED MICROORGANISM FOR BIOREMEDIATION

Microbes play an important role in biodegradation. It has the capability to reduce the toxicity of pollutants. Many different metabolic pathways and genetic approaches are discovered for an enzymatic reaction, but some xenobiotics are still not degraded by conventional metabolic pathways because of the lack of known metabolic pathways. It has no such information for the degradation of that type of contamination (Peper and Reineke, 2000). Recombinant DNA technology and metabolic engineering are used to understand different metabolic pathways. Various biotechnological processes are used for xenobiotic remediation (Sanghvi *et al.*, 2020). Genetically modified microbes have modified codons for different pollutants. The first genetically modified microbes were developed by the US EPA in 1996. These bacteria produce more protein that enhances the metabolic pathways (Sharma *et al.*, 2021). These cellular transporters enhance the absorption of As⁺³ and Hg. In *E. coli* bacteria glycerol facilitators (Homotetramer) increase the bioaccumulation by the uptake of Hg (Singh *et al.*, 2010). *Pseudomonas* bacteria use the MerT/P, MerC, Merp, and MerF as importers for the absorption of Hg (Sone *et al.*, 2013). During the metal stress in the plant, the phytochelatin synthetase gene is expressed and makes the PC synthetase. PC synthetase is a metal-binding cysteine-rich peptide that plays a crucial role in metal accumulation. This gene is isolated from the arabidopsis and introduced in *E. coli* for higher accumulation of heavy metals (Sauge-Merle *et al.*, 2003). The mutualism between root hair and bacteria plays a vital role in rhizoremediation because the bacteria synthesise Toluene Ortho Monooxygenase (TOM) that is responsible for the degradation of trichloroethane and the plant provides the habitat and food for the growth of bacteria (Wood, 2008).

The metallothioneins expressing genetically engineered bacteria enhance the heavy metals accumulation (Fasani *et al.*, 2018). Water, soil and sediments contamination with mercury can be cleared effectively with the help of genetically engineered *E. coli* strain JM109 (Priyadarshane *et al.*, 2022). Polyphosphate kinase and metallothioneins expressing transgenic bacteria are also effective for the removal of mercury (Sharma, 2021). Lindane and trichloroethylene are the highly toxic compounds for the human and genetically engineered bacteria are capable of removing it from the environment (Rafeeq *et al.*, 2023). Genetically engineered *E. coli* SE5000 strain is the best option to accumulate the nickel from the environment (Azad *et al.*, 2014). Arsenite S-adenosylmethionine methyltransferase (*arsM*) gene from *Rhodospseudomonas palustris* engineered into *E. coli*. The *arsM* gene has capability of converting toxic methylated inorganic arsenic into its less toxic volatile Trimethylarsine (TMA) and proven as an effective way to remove arsenic from the contaminated soil. In genetically modified bacteria, the overexpression of *nixA* encoded membrane transport protein, Metallothionein (MT) protein and Glutathione S-transferase fusion protein (GST-MT) have the ability to accumulate large amounts of nickel (Kumar *et al.*, 2013). Genetically engineered, *Pseudomonas sp.* LB400 and *E. coli* JM109 strains are effective on Polychlorinated Biphenyl (PCB) (Sintaha, 2013), *Pseudomonas pseudoalcaligenes* KF707-D2 and *E. coli* FM5/pKY287 can bioremediate Trichloroethylene (TCE) and toluene (Zhang *et al.*, 2017) while *Pseudomonas sp.* B13 strain is effective on mono/dichlorobenzoate (Menn *et al.*, 1999).

Table 3: Bioremediation Microbes and the Element which they Degrade

Sr. No.	Hazardous Element	Microbes	References
1.	Arsenic	<i>Bacillus sp.</i> <i>Pseudomonas aeruginosa</i>	Akhtar <i>et al.</i> , 2013 Sher and Rehman, 2019
2.	Cadmium	<i>Caulobacter crescentus</i> <i>Escheria coli</i> <i>Moraxella sp.</i> <i>Ralstonia eutropha</i> <i>Mesorhizobium huakuii</i> <i>Pseudomonas fluorescens</i> <i>Pseudomonas putida</i> <i>Bacillus subtilis</i>	Azad <i>et al.</i> , 2014
3.	Lead	<i>Mucor circinelloides</i> <i>Alcaligenes eutrophus</i>	Sun <i>et al.</i> , 2017 Hou <i>et al.</i> , 2020 Sevak <i>et al.</i> , 2021
4.	Mercury	<i>Alcaligenes faecalis</i> <i>Bacillus pumilus</i> <i>Pseudomonas aeruginosa</i> <i>Brevibacterium iodinum</i>	Sarao and Kaur, 2021
5.	Chromium	<i>Nitrosomonas</i>	Naz <i>et al.</i> , 2021 Guo <i>et al.</i> , 2021
6.	Trihalomethane	<i>Escheria coli</i> <i>Pseudomonas sp.</i>	Zamule <i>et al.</i> , 2021
7.	Copper	<i>Pseudomonas stutzeri</i> <i>Escheria coli</i>	Palanivel, 2020 Nurlaila <i>et al.</i> , 2021
8.	Manganese	<i>Providencia sp.</i>	Wu <i>et al.</i> , 2022
9.	Polycyclic Aromatics Hydrocarbons (PAH)	<i>Pseudomonas</i> <i>Acromobacter</i> <i>Acinetobacter</i> <i>Flavobacterium</i>	Abatenh <i>et al.</i> , 2017
10.	Phthalates	<i>Gordonia sp.</i> <i>Singulisphaera sp.</i> <i>Sphingobacterium sp.</i> <i>Brevundimonas sp.</i> <i>Dyella sp.</i>	Kong <i>et al.</i> , 2019 Song <i>et al.</i> , 2019

11.	Radionuclides	<i>Mycobacterium</i> <i>Rhodococcus</i> <i>Sphingomonas</i> <i>Flavobacterium</i> <i>Bacillus</i> <i>Alcaligenes</i> <i>Pseudomonas</i>	Thakare <i>et al.</i> , 2021 Francis and Nancharaiah, 2015
12.	Dichlorodiphenyltric hloroethane (DDT)	<i>Sedum alfredii</i> <i>Pseudomonas sp.</i>	Zhu <i>et al.</i> , 2012 Wang <i>et al.</i> , 2017
13.	Zinc	<i>Tricholoma lobynis</i> <i>Rhodobacter sphaeroides</i>	Ji <i>et al.</i> , 2012 Peng <i>et al.</i> , 2018
14.	Nickel	<i>Caulobacter sp.</i> <i>Bacillus cereus</i> <i>Bacillus thuringiensis</i>	Naveed <i>et al.</i> , 2020 Zhu <i>et al.</i> , 2016 Chen <i>et al.</i> , 2019

- Bioventing:** Bioventing is a process in which the organic pollutants of water are degraded with the help of microorganisms. The archaea and algae increase this process, provide the appropriate airflow, and maintain O₂ levels and nutrients (Yadav *et al.*, 2021). This process increases the microbial population and enhances the biological activity for the removal of oil pollutants and hydrocarbons from the soil. It stimulates hydrocarbon degradation and is the first technique applied on a large scale (United States EPA, 2020; Zouboulis *et al.*, 2020). This technique is commercially useful for the treatment of polluted soil, and the rate of oxygen flow plays a crucial role in the biodegradation of organic components (Doudu *et al.*, 2022).
- Bioattenuation:** The process of natural attenuation enhances microbial activity by adding nutrients and microbes. These techniques reduce the toxicity of contamination by the help of aerobic and anaerobic biodegradation, volatilization, and transformation of contamination (Vasquez-Murrieta, 2016). The chlorinated organic compound is one of the major pollutants of water that is secreted by industries, Perchloroethane (PCE) enhances the dechlorination of vinyl chloride to ethene. This process is facilitated by the microbial consortium (Distefano, 1999). Acid mine drainage (AMD) acts as a pollutant of soil and water, which decreases agricultural practices. This type of pollutant is removed by the bio-attenuation process (Anekwe and Isa, 2022). Paint industries and urban areas efflux the water waste which is harmful to land water. Bioattenuation by fungi and bacteria such as *Saccharomyces*, *Penicillium*, *Aspergillus*, *Rhodotorula*, *Bacillus*, *Staphylococcus*, and *E.coli*. Made slightly alkaline soil as compared to polluted soil which is acidic in nature. The alkaline pH of soil shows a reduction in heavy metal components and toxicity of soil pollutants (Chukwuma *et al.*, 2022).
- Biosparging:** Biosparging is the process of administration of air and nutrition into polluted sites. The air starts the aerobic activity and supports the bacteria growth. The bacterias in soil degrades the contamination. Kao *et al.*, 2008 reveal that Biosparging increases Dissolved oxygen, NO₃⁻, SO₄²⁻ and it decreases sulphide, and methane as well as it enhances heterotrophs and reduced anaerobes, and methanogen. About 75 % of

contamination has been removed from jet fuel-contaminated soil (Machackova *et al.*, 2012).

V. *EX SITU* TECHNIQUES OF BIOREMEDIATION

The process of transporting polluted soil from its site to the site of treatment is considered as *ex-situ* bioremediation. The *ex-situ* techniques are generally applicable to those soils where the pollution level is high and in deeper layers (Maitra, 2018). The contaminants are in very high amounts and thus have the risk of leaching to lower layers and the aerobic microbes are unable on-site to degrade the contaminants due to the absence of oxygen in deeper layers of soil. But during transporting soil, care should be taken so that contaminants are not introduced to other places during transportation (Paul *et al.*, 2021).

- 1. Bioaugmentation and Biostimulation:** Biostimulation is the process in which the environment is modified to stimulate bacteria which are capable of bioremediation. Chaudhary *et al.*, 2021 have studied the effects of combinations of bioaugmentation and various biostimulation treatments on the remediation and bacterial diversity of diesel-contaminated soil. The bacterial consortium has degraded 81.9% diesel degradation 60 days in liquid media, Consortium bioaugmentation with nutrients, zero-valent iron nanoparticles, nZVI have shown 99 percent of hydrocarbon (TPH) degradation. The study also concludes biostimulation alone is not adequate. *Dehalococcus* and *Desulfuromonas* sp. Containing PCE was used for the dechlorination of water with the help of bioaugmentation and biostimulation (Lendvay *et al.*, 2003).
- 2. Windrows:** In windrow, ex-situ technique, the piled up contaminated soil is turned periodically and water is added to it for aeration and to speed up the process of remediation. Biotransformation, assimilation and mineralization, enhances the process by providing a suitable environment to the aerobic microbes for degradation activities (Patel *et al.*, 2022). As compared to biopile, windrow is more effective for the removal of hydrocarbons from the soil (Azubuikie *et al.*, 2016; Sharma 2020). *Enterobacteria* and *Pseudomonas* are most effective bacteria for the removal of polycyclic aromatic hydrocarbons from the coal tar contaminated soils (Lors *et al.*, 2010).
- 3. Biopile:** The technique is the best suitable option for the low molecular weight volatiles and for the colder environments. Nutrients, aeration, leachate collection, irrigation and treatment bed system are the important steps for biopiling. Maintains pH and other required factors for the microorganisms to enhance the remediation (Tyagi and Kumar, 2021). The saw dust, straw and wood chips are added to the soil and the warm air is blown at regular intervals to maintain optimum temperature and aeration in the soil. Alpha, beta and gamma proteobacteria are useful for biopiling and about 93% of total hydrocarbons are removed from the diesel contaminated soil, within one year (Bala *et al.*, 2022).
- 4. Landfarming:** The technique is also known as land treatment is applicable where the contaminants in soil are not able to degrade anaerobically and in deeper layers, oxygen is not present. The tilling of soil is done periodically to provide oxygen and nutrients to degrade the contaminants with the help of aerobic microbes. Polyethylene geomembrane and layers of sand are also used below the contaminated soil on the site of ex-situ

bioremediation to prevent leaching of hazardous substances (Rawe *et al.*, 2006). The moisture content, nutrients and pH is maintained during processing, and the United States Environmental Protection Agency and Federal Remediation Technology Roundtable Agency considered it as the technique which prevents leaching to the other ground levels (Rubinos *et al.*, 2007). The combination of earthworm enzyme extract, biosurfactants, nutrients, bulking and sorption agents removes about 53% of petroleum hydrocarbons in 16 weeks whereas the combination of biosurfactants, nutrients and biochar eradicates about 23% total petroleum hydrocarbons (Brown *et al.*, 2017).

- 5. Limitations of Bioremediation:** Bioremediation is only applicable to the contaminants which are biodegradable and also a time-consuming process (Kensa, 2011). Most of the humans are unaware about the harmless and harmful microbes and thus they avoid the microbic treatment as they consider microbes will be more harmful than the chemicals with which the soil is contaminated (Fernandez Pinas, 2014). The optimum temperature, aeration, nutrients, pH and other factors required for the removal of toxic substances should be maintained (Abatenh *et al.*, 2017). Pilot or bench studies to full field application is difficult (Harekrushna and Kumar, 2012).

VI. CONCLUSION

Remediation has received importance in various fields such as textile, dye, pharmaceuticals, rubber, plastics, food, fisheries, wood, soil, water waste management. Other waste such as heavy metals, hydrocarbons have got more attention in the last few decades. The degradation pathways need to be understood for the better remediation implementation. Biotechnological tools can be an important tool for the complete picture of remediation. The engineered plants can be utilised for remediation of specific pollutants. The genomics, metabolomics, and proteomics have played significant solutions for bioremediation and improvements in remediation. Still there is more additional efforts required for the development of more cost effective, efficient and accurate bioremediation ways.

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