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Review Article

ECO-FRIENDLY APPROACH FOR ENVIRONMENT POLLUTION: A REVIEW ON BIOREMEDIATION

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A B S T R A C T

Rapid industrial development has led to the recognition and increasing understanding of the interrelationship between pollution, public health, and the environment. Industrial development results in the generation of industrial effluents, and if untreated results in water, sediment and soil pollution. The indiscriminate release of heavy metals into the environment is a major concern worldwide, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the environment. Many of them are toxic even at very low concentrations; arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc etc. are not only cytotoxic but also carcinogenic and mutagenic in nature. Bioremediation is an effective process over the chemical methods also it is eco-friendly and low-cost as compared with other methods. The relief of organic and inorganic pollutants in the environment cause many problems to the soil, aquatic systems and consequently affects the human health. Therefore, it is necessary to find out unambiguous strains of microorganism which are proficient of biodegradation or detoxification of such pollutants from the environment. In the bioremediation process, bacteria alone, consortia, or combination of bacteria, fungi, and plants can be used for effective biodegradation. Microbes could be isolated from almost all types of environmental conditions and also have a wide range of adaptability. To survive under a stressed condition, bacteria had evolved several types of mechanisms to tolerate the uptake of contaminants. In general, the immobilization and mobilization are the two main techniques used for the bioremediation of contaminants mainly heavy metals by microbes.

INTRODUCTION

The quality of life on Earth is linked inextricably to the overall quality of the environment. Rapid industrial development has led to the recognition and increasing understanding of the interrelationship between pollution, public health, and environment. Industrial development results in the generation of industrial effluents, and if untreated results in water, sediment and soil pollution (1). Heavy metals (elements with an atomic density greater than 6 g/cm3) are one of the most persistent pollutants in the environment. The indiscriminate release of heavy metals into the soil and waters is a major health concern worldwide, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the environment. Many of them are toxic even at very low concentrations; arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc etc. are not only cytotoxic but also carcinogenic and mutagenic in nature (2).

Heavy metals occur naturally in the environment from pedogenetic processes and also through anthropogenic sources (5). The most significant natural sources are weathering of minerals, erosion and volcanic activity, while the anthropogenic sources depend upon human activities such as mining, smelting, electroplating, use of pesticides and phosphate fertilizer discharge, as well bio-solids (e.g., livestock manures, composts, and municipal sewage sludge), atmospheric deposition, etc. (4,6-8). The disturbance of nature's slowly occurring geochemical cycle of metals by man results in accumulation of one or more of heavy metals in the soil and waters, and above defined levels, this is enough to cause risk to human health, plants, animals and aquatic biota (10). The heavy metals essentially become contaminants in the soil and water environment because of their excess generation by natural and man-made activities, transfer from mines to other locations where higher exposure to humans occurs, discharge of high concentration of metal waste through industries, and greater bioavailability(9).

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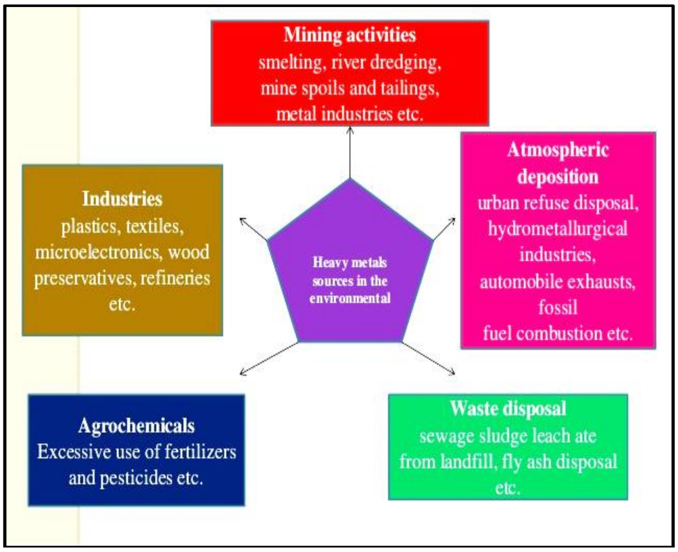

Figure 1

Sources of heavy metals in the environment (53)

Bioremediation

There are numerous methods currently employed to remove these metals from the environment. Some of these methods are chemical precipitation and sludge separation, chemical oxidation or reduction, ion exchange, reverse osmosis, membrane separation, electrochemical treatment, and evaporation. These techniques are very expensive and not environmentally acceptable, hence new biotechnological methods for remediation of these toxic metals are being considered recently (11). The term bioremediation has been introduced to describe the process of using biological agents such as bacteria, fungi, and/or green plants to remove contaminants from the environment. It is defined as the elimination, attenuation or transformation of polluting or contaminating substances by the application of biological processes. Developing of biological-based treatment system considered as economically cheaper and more environment-friendly (12). In the bioremediation process, bacteria alone, consortia, or combination of bacteria, fungi, and plants can be used for effective biodegradation (13, 14).

Table 1 Summary of bioremediation strategies (15)

Technology	Example	Benefit	Limitation
In-situ	Bioventing Bioaugmentation	Relatively passive	Extended treatment time
Ex-situ	Landfarming composting	Extended space and time	Environmental parameters
Bioreactors	Slurry reactors Aqueous reactors	High cost	Toxicity of contaminants

Factors allied with Bioremediation

The control and optimization of bioremediation processes is a complex system of many factors.

Table 2 Factors associated with Bioremediation (16)

Factors	Condition Required
Microorganisms	Aerobic or Anaerobic
Natural Biological processes of microorganism	Catabolism and Anabolism
Environmental Factors	Temperature, pH ,Oxygen content, Electron acceptor/donor
Nutrients	Carbon ,Nitrogen ,Oxygen etc.
Soil Moisture	25-28% of water holding capacity
Type of soil	Low clay or silt content

These factors include the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population and the environment factors (type of soil, temperature, pH, presence of oxygen or other electron acceptors, and nutrients) (15, 16).

Desire of Environmental Factors and Nutrients by Microorganisms

Although the microorganisms are present in the contaminated environment, they cannot necessarily be there in the numbers required for bioremediation of the site. Their growth and activity must bestimulated. Bio-stimulation usually involves the addition of nutrients and oxygen to help indigenous microorganisms (16).These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. All of them will need nitrogen, phosphorous, and carbon. Carbon is the most basic element of living forms and is needed in greater quantities than other elements. In addition to hydrogen, oxygen, and nitrogen it constitutes about 95% of the weight of cells (15).

Table 3 Environmental Circumstances for Microbial Activities (16)

Environmental Factor	Optimum conditions	Condition required for microbial Activities
Available soil moisture	25-85% water holding capacity	25-28% of water holding capacity
Oxygen	>0.2 mg/L DO, >10% air-filled pore space for aerobic degradation	Aerobic, minimum air-filled pore space of 10%
Redox potential	Eh > 50 mill volts	-
Nutrients	C:N:P= 120:10:1 molar ratio	N and P for microbial growth
pH	6.5-8.0	5.5 to 8.5
Temperature	20-30 °C	15-45°C
Contaminants	Hydrocarbon 5-10% of dry weight of soil	Not too toxic
Heavy metals	700ppm	Total content 2000ppm

Bioremediation of Heavy Metals

The microbes can't degrade heavy metals directly but they can change the valence state of metals which may convert them into immobile or less toxic forms. Collins and Stotzky (17) stated that several metals are essential for biological systems and present in a range of certain concentration. The metals always have been associated with metalloproteins and enzymes as co-factors. Its low concentrations lead to decrease in metabolic activity while high concentrations could act in a deleterious way by blocking essential functional groups, displacing other metal ions, or modifying the active conformation of biological molecules. Besides, they are toxic to both higher organisms and microorganisms. The progressive accumulation of metals may inhibit the degradation of organic pollutants or humic substances in the environment. This problem can be solved by an increase of the heavy metal resistance of the bioremediating system (18,19). Heavy metals are present in soils and aqueous streams as both natural components or as a result of human activity (i.e., metal-rich mine tailings, metal smelting, electroplating, gas exhaust, energy and fuel production, downwash from power lines, intensive agriculture, sludge dumping (18).

Protagonist of Bacteria in Bioremediation Process

Remediation of ecological niches such as soil, sediments, and water amended with heavy metals can be achieved through biologically encoded changes in the oxidation state.(21) Bioremediation is the microbe-mediated process for clearance or immobilization of the contaminants, including all possible toxins like hydrocarbons, agrochemicals, and other organic toxicants. But for inorganic toxic compounds such as heavy metals, microbes are unable to simplify them into harmless compounds, and they should be used according to their specialization for the type of contaminants (20). White et al. (22) reported that bacterial remediation is the process of using metal-reducing bacteria to break down the contaminants. To survive under a metal stressed condition, bacteria have evolved several types of mechanisms to tolerate the uptake of heavy metal ions. These mechanisms of removal of heavy metals include the efflux of metal ions outside the cell, accumulation and forms complex of the metal ions inside the cell and later reduce the toxic metal ions to a non-toxic state. Some of the effective bacterial species against metal chromium were P.aeruginosa, Bacillus sp., Streptomyces sp., P. fluorescens (23). The study of the genetics of such metal accumulator microorganisms can help us to transfer the traits in the microbes that are missing through the development of microarrays, which result in differentially expressed microbe genes. On another side, several microorganisms are known to require varying amounts of heavy metals as essential micronutrients for growth and development. For example, Fe^{3+} is essentially required by all bacteria while Fe^{2+} is important for anaerobic bacteria [24]. However, the adsorption capacity depends on microbial total biomass and geochemistry of the system. Some oxyanions of metals do not interact with microbes, and their bioremediation is based on their catalyzed redox conversion to insoluble forms. These reduction or oxidation reactions take place due to enzymatic activity and biomass concentration of microbes (25).

Mechanism of Bioremediation by Microorganisms

Microbes could be isolated from almost all types of environmental conditions and also have a wide range of adaptability. It can survive from zero to extremely high and desert conditions. In water, it can survive in presence and absence of oxygen and also in presence of hazardous compounds (26). To survive under a stressed condition, bacteria have evolved several types of mechanisms to tolerate the uptake of contaminants. In general, the immobilization and mobilization are the two main techniques used for the bioremediation of contaminants mainly heavy metals by microbes (23, 26).

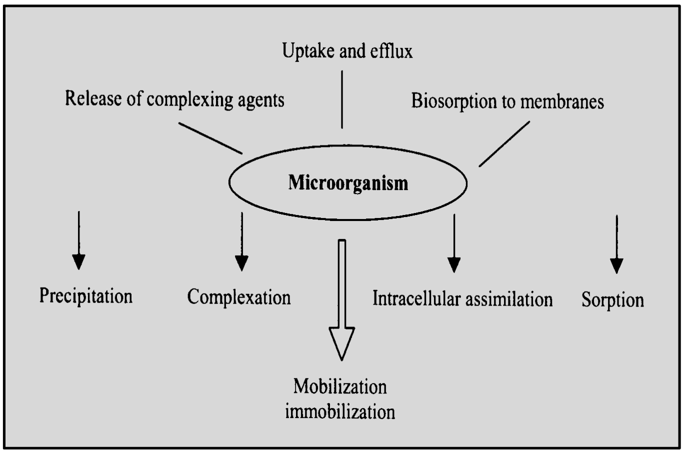

Figure 2 Metal processing mechanisms of microorganisms (52)

Immobilization

Immobilization is a technique used to reduce the mobility of contaminants by altering the physical or chemical characteristics of the contaminant. This remediation approach can utilize microorganisms to immobilize metal contaminants. It is usually accomplished by physically restricting contact between the contaminant or by chemically altering the contaminant (28). Chemical reagents and bacterial reagents assist with the immobilization of metal contaminants. Most sites contaminated with metals use the solidification and stabilization approach to immobilize metals. Solidification treatment involves mixing or injecting chemical agents to the contaminated environment. The prominent mechanism by which metals are immobilized is by precipitation of hydroxides. The chemical composition of the contaminated site, the amount of water present, and the temperatures are all factors important to the successful use of the solidification/ stabilization mechanism (25,28). The stabilization and solidification technique is achieved by mixing the contaminated material with appropriate amounts of stabilizer material and water. The mixture forms a solidified matrix with the waste. The stabilization and solidification techniques can occur both in-situ or ex-situ. In-situ is preferred for volatile or semi-volatile organics. The in-situ process is useful for treating surface or shallow contamination (25).

Mobilization

Microorganisms can mobilize metals through autotrophic and heterotrophic leaching, chelation by microbial metabolites and siderophores, methylation, and redox transformations. Heterotrophic leaching is when microorganisms can acidify their environment by proton efflux thus leading to the acidification resulting in the release of free metal cations (27). Autotrophic leaching is when acidophilic bacteria retrieve CO^2 and obtain energy from the oxidation of the ferrous iron or reduced sulfate compounds, which causes solubilization of metals. Siderophores are specific iron chelating legends and are able to bind to other metals, such as magnesium, manganese, chromium, gallium, and radionuclide, such as plutonium. Methylation involves methyl groups that are enzymatically transferred to a metal, forming a number of different metalloids. Redox transformations can allow microorganisms to mobilize metals, metalloids, and organo-metallic compounds by reduction and oxidation processes. There are various metal-mobilization techniques that can also occur in nature (29).

Role of Bacteria in refurbishing Soil Condition

Detoxification and rehabilitation of contaminated soil with the use of microbes have emerged as the safest, easy and effective technology. Native soil microorganisms have been explored and harnessed for their ability to remove or detoxify toxic products released due to human activities in the environment viz. mining of ores, oil and gas extraction, pesticides, pigments, plastic, organic solvents, fuel and industrial processes (30). The biochemical route for the redistribution of the organic pollutant in the soil starts from various physical, chemical and biological processes resulting in adsorption by soil particles and root tissues, volatilization, transport through water and air, microbial degradation and leaching (27). Microorganisms have a great deal of undiscovered and unexplored potential for remediation of soil pollutants and increasing the production of agricultural crops with low input.

For endurance under metal-stressed environment, plant growth promoting rhizobacteria have evolved several mechanisms by which they can immobilize, mobilize or transform metals rendering them inactive to tolerate the uptake of heavy metal ions. These mechanisms include (a) exclusion-the metal ions are kept away from the target sites; (b)extrusion-the metals are pushed out of the cell through chromosomal/plasmid-mediated events; (c) accommodation metals form complex with the metal binding proteins or other cell components; (d) bio-transformation-toxic metal is reduced to less toxic forms, and (e) methylation and demethylation. Thus, in general, the immobilization and mobilization are the two main techniques used for the bioremediation of metals by bacteria (28, 30).

Wide-reaching Scenario of Microbial Bioremediation on Various Contaminants

Research is underway at a number of facilities using exogenous, specialized microbes or genetically engineered microbes to optimize bioremediation (31). In any bioremediation process, the introduced microorganisms use the contaminants as nutrients or energy sources (32). Bioremediation activity through microbes is stimulated by supplementing nutrients (Nitrogen and phosphorus), electron acceptor (oxygen), and substrates (methane, Phenol, and toluene) or by introducing microorganisms with desired catalytic capabilities (33,34). Some common microorganism used in the process of remediation are Acromobacter, Alcaligenes, Arthrobacter, Bacillus, Acinetobacter, Corneybacterium, Flavobacterium, Micrococcus, Mycobacterium, Nocardia, Pseudomonas, Vibrio, Rhodococcus and Sphingomonas species (35, 36, 37). The main species involved in effective wastewater treatment include lactic acid *bacteria-Lactobacillus Plantarum, L. casei and Streptococcus lacti and Photosynthetic bacteria-Rhodopseudomonaspalustrus, Rhodobacter spheroid*, etc (38). Arthrobacter species was first isolated from natural environment which has the capability to reduce nitrogen by heterotrophic nitrification process [39]. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform (40). Endosulfan, a pesticide residual, is extremely toxic to fish and aquatic invertebrates. It can bind to soil particles and persist for a relatively long period, with a half-life of 60-800 days. Bacteria such as *Pseudomonas sp.* and Arthrobacter sp. can degrade up to 57-90% of α-endosulfan and 74-94% β – Endosulfan in a period of 7-days (41). The first patent for a biological remediation agent was registered in 1974 is a strain of *Pseudomonas putida* that was able to degrade petroleum. Several studies reveal, 42 different pollutants including black liquor from a kraft pulp and paper mill effluent, tannery effluent, steel industrial effluent etc., can be biodegraded by using *Pseudomonas species. Pseudomonas syringae* also showed the formation of a bond which plays an important role in the accumulation of cadmium, calcium, magnesium, zinc, copper, and mercury (42). In the presence of oxygen, the aerobic bacteria such as *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus, and Mycobacterium* are capable of degrading pollutants. These microbes degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy (43). A pure culture of Exiguobacteriumaurantiacum has the capability of phenol degradation and PAHs in batch culture when provided with pure compounds as a source of carbon and energy (44). Other studies show that *Pseudomonas sp*. acts as fuel eating bacteria which can degrade the hydrocarbons (45). *Pseudomonas sp*. and Brevibacillus species were nitrate-reducing bacterial strains isolated from petroleum contaminated soil (46). The most common bacteria that carry out ammonia oxidation is Nitrosomonas species, while nitrite oxidation is carried out by *Nitro spina, Nitrococcus, and Nitrocystis* (47). Biological treatment by autotrophic nitrogen removal is preferred for concentrated wastewater streams with high ammonia concentrations in the range of 100 -5000 mg N/L (48). The oxidized nitrogenous compounds (Nitrite to nitrate) are reduced to gaseous nitrogen by heterotrophic microorganisms that use nitrite or nitrate instead of oxygen as an electron acceptor and organic matter as carbon and energy source. Denitrification is common among the gram-negative bacteria such as *Pseudomonas, Alcaligenes, Paracoccus, and Thiobacillus*. Denitrification is the slow process particularly for industrial wastewaters that contain high concentrations of nitrate. The decrease of ammonia nitrogen concentration from 10 mg/l to below 0.3 mg/l was obtained within 3 days after inoculation of microbial inoculums with aeration in polluted water with the use of nitrifiers (49). *Bacillus methylotrophic* L7, the first reported gram-positive bacterial strain to denitrify nitrite to N_2 and denitrifying nitrite and nitrate to N_2O under aerobic condition. The strain L7 exhibited ability with maximum NH4-N removal rate of 51.58 mg/l/d and maximum NO_2-N removal rate of 5.81 mg/l/d, besides, more than 90 mg/d ammonia removal efficiency was obtained even in the extremely high ammonia load (>1000 mg/L) (50). The maximum ammonia removal of 95% was observed in activated sludge of wastewater was achieved using heterotrophic bacterium *Alcaligenes* species in a period of four days (51).

Conclusion and Imminent Prospects

In this review, we have haggard age stalt the potential of microbes for bioremediation of different environmental pollutants. Bioremediation is an effective process over the chemical methods since bioremediation is eco-friendly and low-cost as compared with other methods. The relief of organic and inorganic pollutants in the environment cause many problems to the soil, aquatic systems and consequently affects the human health. Therefore, it is necessary to find out unambiguous strains of microorganism which are proficient of biodegradation or detoxification of such pollutants from the environment. The isolated bacterial species from the polluted sites as certained tobe more effective in bioremediation and this depend on the adaptation of the organism to grow for long time on the pollutants. The application of microorganisms in bioremediation still encounter with some difficulties since some organic pollutants are difficult to be degraded by all bacteria. The genetic engineering can overcome this problem and expand the bioremediation of many organic, inorganic pollutants and increase microorganism tolerance to these pollutants. The environmental condition of are necessary to be understood for each strain in order to increase the absorption, accumulation and or biodegradation of the pollutants. It is also necessary for understanding the mechanism of biodegradation of microorganisms for the different pollutants (oil spills, organic hydrocarbons, dyes, heavy metals, phenols or polycyclic aromatic compounds etc.) which cause serious problems to the environment and arerecorded as carcinogenic compounds for safe and clean environment. Therefore, based on the present review, it may be concluded that degradation of environmental pollutants through various bacterial strains can be considered as a significant approach for bioremediation.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

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