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Bioremediation: Bioremediation defined as a biological process of the decontamination is the use of living microorganisms (bacteria, fungi, actinomycetes, cyanobacteria etc) to degrade environmental pollutants or to prevent pollution. Bioremediation is a cleaning up contaminated environments by exploiting the diverse metabolic abilities of microorganisms to convert contaminants to harmless products by mineralization, generation of carbon (IV) oxide and water, or by conversion into microbial biomass.

Bioremediation consists of using living organisms (usually and to a lesser extent, plants) to reduce or eliminate toxic pollutants. These organisms may be naturally occurring or laboratory cultivated. These organisms either eat up the contaminants or assimilate within them all harmful compounds from the surrounding area, thereby, rendering the region virtually contaminant-free. Generally, the substances that are eaten up are organic compounds, while those, which are assimilated within the organism, are heavy metals. Bioremediation harnesses this natural process by promoting the growth and/or rapid multiplication of these organisms that can effectively degrade specific contaminants and convert them to nontoxic by-products. Importantly, bioremediation can also be used in conjunction with a wide range of traditional physical and chemical technologies to enhance their efficacy. Comments Dr Banwari Lal, the TERI researcher working on this project, "Bioremediation is an ecologically sound and state-of-the-art technique that employs natural biological processes to completely eliminate toxic contaminants".

Types of Bioremediation: On the basis of place where wastes are removed, there are principally two ways of bioremediation:

1. ***In Situ Bioremediation:*** Most often, in situ bioremediation is applied to eliminate the pollutants in contaminated soils and groundwater. It is a superior method for the cleaning of contaminated environments because it saves transportation costs and uses harmless microorganisms to eliminate the chemical contaminations. These microorganisms are better to be of positive chemotactic affinity toward contaminants. This feature increases the probability of the bioremediation in close points where bioremediants have not distributed. Also, the method is preferred as it causes the least disruption of the contaminated area. This would be of much relevance either where the least investment and pollution are favored (for example in factories) or in areas contaminated with dangerous contaminants (for example in areas contaminated with chemical or radioactive materials). Another advantage of in situ bioremediation is the feasibility of synchronous treatment of soil and groundwater. However, in situ bioremediation posses some disadvantages: the method is more time-consuming compared to other remedial methods, and it leads to a changed seasonal variation in the microbial activity because of the direct exposure to the variations in uncontrollable environmental factors, and the use of additives may lead to additional problems. The yield of bioremediation is determined by the kind of waste materials, namely if wastes could provide the required nutrients and energy, then microorganisms would be able to bioremediate. However, in the absence of favorable wastes, the loss of bioactivity may be compensated through stimulation of native microorganisms. Another choice of less preference is to apply genetically engineered microorganisms.

Two types of *in situ* bioremediation are distinguished based on the origin of the microorganisms applied as bioremediants:

i) **Intrinsic bioremediation**: This type of in situ bioremediation is carried out without direct microbial amendment and through intermediation in ecological conditions of the contaminated region and the fortification of the natural populations and the metabolic activities of indigenous or naturally existing microfauna by improving nutritional and ventilation conditions.

ii) **Engineered in situ bioremediation**: This type of bioremediation is performed through the introduction of certain microorgansims to a contamination site. As the conditions of contamination sites are most often unfavorable for the establishment and bioactivity of the exogenously amended microorganisms, therefore here like intrinsic bioremediation, the environment is modified in a way so that improved physico-chemical conditions are provided. Oxygen, electron acceptors, and nutrients (for example nitrogen and phosphorus) are required to enhance microbial growth.

2. Ex Situ Bioremediation: The process of bioremediation here takes place somewhere out from contamination site, and therefore requires transportation of contaminated soil or pumping of groundwater to the site of bioremediation. This technique has more disadvantages than advantages. Depending on the state of the contaminant in the step of bioremediation, ex situ bioremediation is classified as:

a) Solid phase system (including land treatment and soil piles): The system is used in order to bioremediate organic wastes and problematic domestic and industrial wastes, sewage sludge, and municipal solid wastes. Solid-phase soil bioremediation includes three processes including land-farming, soil biopiling, and composting.
b) Slurry phase systems (including solid–liquid suspensions in bioreactors): Slurry phase bioremediation is a relatively more rapid process compared to the other treatment processes.

Contaminated soil is mixed with water and other additives in a large tank called a bioreactor and intermingled to bring the indigenous microorganisms in close contact with soil contaminants. Nutrients and oxygen are amended, and the conditions in the bioreactor are so adjusted that an optimal environment for microbial bioremediation is provided. After completion of the process, the water is removed, and the solid wastes are disposed off or processed more to decontaminate remaining pollutants.

Bioremediation Techniques: There are several bioremediation techniques, some of them have been listed as follows

A) **Biostimulation:** Biostimulation provides nutrients and suitable physiological conditions for the growth of the indigenous microbial populations. This promotes increased metabolic activity, which then degrades the contaminants.
B) **Bioaugmentation:** Bioaugmentation means introduction of specific blends of laboratory-cultivated microorganisms into a contaminated environment or into a bioreactor to initiate the bioremediation process. The process of developing bioremediation techniques may involve the following steps: 1) Isolating and characterizing naturally-occurring microorganisms with bioremediation potential, 2) Laboratory cultivation to develop viable populations. 3) Studying the catabolic activity of these microorganisms in contaminated material through bench- scale experiments
C) **Biofilters:** The use of microbial stripping columns used to treat air emissions.
D) **Bioreactors:** The use of biological processes in a contained area or reactor for biological treatment of relatively small amounts of waste. This method is used to treat slurries or liquids. Slurry reactors or aqueous reactors are used for ex situ treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors

involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil-bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants

E) **Bioventing:** The process of drawing oxygen through the contaminated medium to stimulate microbial growth and activity. Bioventing is the most common in situ treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface (Vidali 2001). In many soils effective oxygen diffusion for desirable rates of bioremediation extend to a range of only a few centimeters to about 30 cm into the soil, although depths of 60 cm and greater have been effectively treated in some cases

F) **Composting:** An aerobic and thermophillic process that mixes contaminated soil with a bulking agent. Composting may be performed using static piles, aerated piles, or continuously fed reactors. Composting is a technique that involves combining contaminated soil with nonhazardous organic amendments such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristic of composting (Vidali 2001). Composting is a process by which organic wastes are degraded by microorganisms, typically at elevated temperatures. Typical compost temperatures are in the range of 55–65 C. The increased temperatures result from heat produced by microorganisms during the degradation of the organic material in the waste.

Phytoremediation: Phytoremediation basically refers to the use of plants and associated soil microbes to reduce the concentrations or toxic effects of contaminants in the environment. Phytoremediation is widely accepted as a cost-effective environmental restoration technology. Phytoremediation is an alternative to engineering procedures that are usually more destructive to the soil. Phytoremediation of contaminated sites should ideally not exceed one decade to reach acceptable levels of contaminants in the environment. Phytoremediation is, however, limited to the root-zone of plants. Also, this technology has limited application where the concentrations of contaminants are toxic to plants. Phytoremediation technologies are available for various environments and types of contaminants. These involve different processes such as in situ stabilization or degradation and removal (i.e., volatilization or extraction) of contaminants

Phytostabilization: Phytostabilization aims to retain contaminants in the soil and prevent further dispersal. Contaminants can be stabilized in the roots or within the rhizosphere (Figure 3B). Revegetation of mine tailings is a common practice to prevent further dispersal of contaminants (Figure 4). Mine tailings have been stabilized using commercially available varieties of metal tolerant grasses such as Agrostis tenuis cv. Goginan and cv. Parys and Festuca rubra cv. Merlin. Metal tolerance of plants is generally increased by symbiotic, root-colonizing, arbuscular mycorrhizal fungi (AMF), through metal sequestration in the AMF hyphae. In addition, excretion of the glycoprotein glomulin by AMF hyphae can complex metals in the soil. Populations of AMF that are adapted to metal contaminated soils have great potential in phytostabilization. Soil microbes can decrease toxic effects of contaminants in the soil. For example, exudates (peptides) from the bacterium Pseudomonas putida can decrease Cd toxicity in plants. Plants can also convert contaminants into less toxic forms, or decrease their bioavailability.

Phytodegradation: Phytodegradation involves the degradation of organic contaminants directly, through the release of enzymes from roots, or through metabolic activities within plant tissues (Figure 3B). In phytodegradation organic contaminants are taken up by roots and metabolized in plant tissues to less toxic substances. Phytodegradation of hydrophobic organic contaminants have been particularly successful. Poplar trees (Populus spp.) have been used successfully in phytodegradation of toxic and recalcitrant organic compounds. Rhizodegradation involves attenuation of organic contaminants into less toxic substances within the rhizosphere through biodegradation of soil microbes.

Phytovolatilization: Phytovolatilization involves the uptake of contaminants by plant roots and its conversion to a gaseous state, and release into the atmosphere. This process is driven by the evapotranspiration of plants (Figure 3B). Plants that have high evapotranspiration rate are sought after in phytovolatilization. Organic contaminants, especially volatile organic compounds (VOCs) are passively volatilized by plants. For example, hybrid poplar trees have been used to volatilize trichloroethylene (TCE) by converting it to chlorinated acetates and CO2. Metals such as Se can be volatilized by plants through conversion into dimethylselenide [Se(CH3)2]. Genetic engineering has been used to allow plants to volatilize specific contaminants. For example, the ability of the tuliptree (Liriodendron tulipifera) to volatilize methyl-Hg from the soil into the atmosphere (as Hg0) was improved by inserting genes of modified E. coli that encode the enzyme mercuric ion reductase (merA).

Phytoextraction: Phytoextraction uses the ability of plants to accumulate contaminants in the aboveground, harvestable biomass (Figure 3B). This process involves repeated harvesting of the biomass in order to lower the concentration of contaminants in the soil. Phytoextraction is either a continuous process (using metal hyperaccumulating plants, or fast growing plants), or an induced process (using chemicals to increase the bioavailability of metals in the soil). Continuous phytoextraction is based on the ability of certain plants to gradually accumulate contaminants (mainly metals) into their biomass. Certain plants can hyperaccumulate metals without any toxic effects. These plants are adapted to naturally occurring, metalliferous soils.