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Review Article

BIOREMEDIATION TECHNOLOGIES: CONTROLLING WASTE WATER AND SOIL POLLUTION

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ABSTRACT
Rapid industrialization and urbanization coupled with population explosion has resulted in generation of large quantities of wastewater and there is no horizontal expansion of land to increase the productivity we need to preserve our existing land and other natural resources too and develop such mechanism of bioremediation. Pollution is in tremendously increasing rate that it is a real challenge to treat the wastewater in a most cost effective and environmentally accepted way. Water is a key feature of public concern worldwide. Inappropriate use of water resources and poor management of waste water has an increasingly negative effect on economic growth on social welfare and on the world's eco-systems. Being a developing country the amount of waste water generation in India is on higher side as there is fast industrialization, urbanization and also population explosion. Bioremediation is defined as the process by which microorganisms are stimulated to rapidly degrade hazardous organic pollutants to environmentally safe levels in soils, sediments, substances, materials and ground water. Recently, biological remediation process have also been devised to either precipitate effectively immobilize inorganic pollutants such as heavy metals. Bioremediation technology uses micro-organisms to reduce, eliminate or transform contaminants present in soils, sediments or water. Bioremediation depends on the presence of specific microorganisms in the correct amounts and combination and in the appropriate environmental conditions. Microorganisms already living in contaminated environments are often well adapted to survive in the presence of existing contaminants and to the temperature, pH and oxidation/ reduction potential of the site. These indigenous microbes tend to utilize the nutrients and electron acceptors that are available, provided liquid water is present. Water also acts as a vehicle to transport both microorganism and dissolved substances including contaminants and their breakdown products. Bioremediation process involves biotransformation and biodegradation by transforming contaminants to non–hazardous or less hazardous chemicals. Often, the micro- organisms metabolize the chemicals to produce carbon dioxide or methane, water and biomass. Biotransformation is any alteration of the molecule or structure of a compound by micro-organisms. Methods dealing with remediation of metal polluted soils and waters are soil venting washing, chemical treatment, excavation and burial or simple isolation of contaminated sites. Such methods are only practical in small areas, as they are extremely costly. Therefore, alternative remediation methods, which are cost effective & environmentally friendly, need to be established. Phytoremediation an emerging technology that uses plants to remediate soil and water contaminated with various contaminants. This paper delineates the general processes, concepts and application of bioremediation and phytoremediation within the environment based on our knowledge in the field of science.
Keywords: *Bioremediation, Phytoremediation, Microbes, Hyper Accumulators, Bioremediation, Volatilization, Chelation, Sequestration, Heavy Metal Pollutants Bioaugmentation*

INTRODUCTION
Large amounts of water used for agricultural, municipal, and industrial purposes result in the generation of large volumes of waste water. Waste water treatment eliminates easily settled materials (primary treatment) and oxidizes the organic material present in waste water (secondary treatment). As the result, waste water is apparently clean; it is discharged into natural water bodies. However, this effluent contains large amounts of inorganic nitrogen and phosphorus which cause eutrophication of the water. Also, the presence of heavy metals and organic compounds can cause long-term problems (Gulhane and Pawar,

2014). Bioremediation is defined as the process by which microorganisms are stimulated to rapidly degrade hazardous organic pollutants to environmentally safe levels in soils, sediments, substances, materials and ground water. Recently, biological remediation process have also been devised to either precipitate effectively immobilize inorganic pollutants such as heavy metals. Stimulation of microorganisms is achieved by the addition of growth substances, nutrients, terminal electron acceptor/donors or some combination thereby resulting in an increase in organic pollutant degradation and bio-transformation. The energy and carbon are obtained through the metabolism of organic compounds by the microbes involved in bioremediation processes (Fulekar *et al.*, 2009).

Bioremediation technology uses micro-organisms to reduce, eliminate or transform contaminants present in soils, sediments or water. Bioremediation depends on the presence of specific microorganisms in the correct amounts and combination and in the appropriate environmental conditions. Microorganisms living already living in contaminated environments are often well adapted to survive in the presence of existing contaminants and to the temperature, pH and oxidation/ reduction potential of the site. These indigenous microbes tend to utilize the nutrients and electron acceptors that are available, provided liquid water is present (Pandey and Fulekar, 2012). Water also acts as a vehicle to transport both microorganism and dissolved substances including contaminants and their breakdown products. Bioremediation process involves biotransformation and biodegradation by transforming contaminants to non–hazardous or less hazardous chemicals. Often, the micro- organisms metabolize the chemicals to produce carbon dioxide or methane, water and biomass. Biotransformation is any alteration of the molecule or structure of a compound by micro-organisms. Biodegradation is the breaking down of organic or bioaccumulation and biotransformation of inorganic compounds into environmental friendly compounds. Biodegradation is the breaking down of organic or bioaccumulation and biotransformation of inorganic compounds into environmental friendly compounds (Sharma and Fulekar, 2009). Attempts at remediation contaminated sites have used conventional but often costly approaches, such as ‘pump and treat’, excavation and removal, soil vapour extraction, and other chemical treatments (McIntyre, 2003). These methods are time consuming, invasive, disruptive to natural habitats and usually result in a rearrangement of the problem (Shukla *et al.,* 2010). These methods, are estimated to be very costly for all contaminated sites in the country. Lately however, bioremediation has proven to be a safe, effective, low-cost and environmentally friendly alternative for sustainable remediation of environments contaminated by hazardous and recalcitrant pollutants (Shukla *et al.,* 2010; Singh, 2006; Eweiss *et al.,* 1998). Bioremediation uses biological processes and naturally occurring microbial catabolic activity to eliminate, attenuate or transform contaminants to less hazardous products such as carbon dioxide, water, inorganic salts, and microbial biomass (Alvarez and Illman, 2006; Testa and Jacobs, 2002; Kulkarni *et al.,* 2008). Bioremediation techniques can take place in situ and ex situ, and have been widely characterized (Shukla *et al.,* 2010; Irvine and Frost, 2003; Dott *et al.,* 1995; Vidali, 2001).

Microbes are nature’s ultimate garbage disposal, consuming the dead, decomposing and inert material that litters Earth’s surface. This concept is called bioremediation; it involves degradation of toxic pollutants to non-toxic products. Scientists are designing or deploying microbes to purge sites of contaminants such as oil, radioactive waste, gasoline and mercury etc. Oil-eating super bugs are used to the cleanup large oil spills. Widely used approach to bioremediation involves stimulating a group of organisms in order to shift the microbial ecology toward the desired process known as ‘Biostimulation’ and the other widely used approach is termed ‘Bioaugmentation’ where organisms selected for high degradation abilities are used to inoculate the contaminated site. These two approaches are not mutually exclusive, they can be used simultaneously. Instead of adding organisms to the environment, often the best way to get rid of chemical pollutants is to help the bacteria already on the job. Bioremediation using both indigenous and genetically modified microbes is a cost-effective way to clean up contamination. By rebalancing the system with the proper microbial processes, the time period required for chemical breakdown can be reduced. Energy production is the chief metabolic activity of microorganisms. Most organic substances can serve as the source of energy to diverse group of microorganisms and hence, they are transformed or degraded in the polluted environment. Ammonia from urea and uric acid waste produced by large chicken farms has a tremendous impact on water and soil quality. The concentrations of certain types of fungus increased threefold in chicken waste when it was treated by commercially available additives to acidify the waste in hopes of controlling the ammonia going into the environment. There are reports saying that uric acid degrading fungi could significantly decrease the amounts of ammonia in waste within two weeks. Bacterial genera, such as Pseudomonas, degrade pesticides in polluted bodies of water (Patwardhan *et al.*, 2014).

The studies have found that increased concentrations of Pseudomonas, degraded pesticides much more efficiently as compared to water samples with lesser amounts of the microbe. Explosives are synthesized globally mainly for military munitions. Nitrate esters, such as GTN and PETN, nitroaromatics like TNP and TNT and nitramines with RDX, HMX and CL20, are the main class of explosives used. Their use has resulted in severe contamination of environment and strategies are now being developed to clean these substances in an economical and eco-friendly manner. The incredible versatility inherited in microbes has rendered these explosives as a part of the biogeochemical cycle. Several microbes catalyze mineralization and/or nonspecific transformation of explosive waste either by aerobic or anaerobic processes (Ramakrishnan, 2013). Anaerobic microbial mineralization of recalcitrant organic pollutants is of great environmental significance and involves intriguing novel biochemical reactions. In particular, hydrocarbons and halogenated compounds have long been doubted to be degradable in the absence of oxygen, but the isolation of unknown anaerobic hydrocarbon-degrading and reductively dehalogenating bacteria during the last decades provided ultimate proof for these processes in nature (Dusenbery, 1996). Petroleum oil contains aromatic compounds that are toxic for most life forms. Episodic and chronic pollution of the environment by oil causes major ecological perturbations. Marine environments are especially vulnerable since oil spills of coastal regions and the open sea are poorly containable and mitigation is difficult. In addition to pollution through human activities, about 250 million liters of petroleum enter the marine environment every year from natural seepages. Despite its toxicity, a considerable fraction of petroleum oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCB) In addition to hydrocarbons, crude oil often contains various heterocyclic compounds, such as pyridine, which appear to be degraded by similar, though separate mechanisms than hydrocarbons. Bioremediation of hydrocarbons, from oil spills or storm water run-off, depends upon the initial contaminant, level of contamination, temperature, the type of soil, the availability of oxygen in the area and the ability of the microbes to reach the contaminant (Patwardhan *et al.*, 2014).

Heavy metals (metals with an atomic number > 20) and other inorganic and organic pollutants are present in the soil and water as natural components or as a result of human activities. Mining metal, smelting, energy and fuel production, burning of fossils fuels, use of fertilizers and pesticides in agriculture, dumping of industrial wastes and chemical spills incorporate hazardous pollutants into the environment (Salt *et al.*, 1998). These pollutants accumulate in the water and soil and eventually translocate in plant tissue (Brown, 1995) or contaminate water sources and pose a threat to human health. Once absorbed by plants, some of these contaminants are either broken down (organic contaminants) into non hazardous materials or concentrate (inorganic contaminants) within the plant body. Consumption of such polluted plants by herbivorous animals introduces contaminants like heavy metal ions into the food chain. Soil contaminants such as Copper (Cu), Zinc (Zn), Nickel (Ni), Aluminum (Al), Cadmium (Cd), Lead (Pb), and other heavy metals have been Proven to cause hazardous effects in animals and humans (Adriano, 1992; KIimisch, 1993; Salt *et al.*, 1998).

Phytoremediation is defined as the use of plants to remove pollutants from the environment or to render them harmless. Phytoremediation is often referred as Bio-remediation, Botanical- Bioremediation and Green remediation. Phytoremediation is the plant based technology used to remove the pollutants from the environment. Some plant species have the unique ability to tolerate, take up, and hyper accumulate heavy metals from the soil through their roots and concentrate them in the stem, roots, and leaves (Brown, 1995; Zaman, 2003). These plant parts can be harvested, compacted and incinerated to minimize the volume of waste which needs to be treated or disposed off properly at hazardous waste sites. Such incinerated product containing high concentrations of metals may be used as bio-ore. For example, incinerated product of alpine pennycress (*Thlaspi caerulescens*), grown on zinc contaminated soil, may yield 30 to 40 percent zinc which is as high as high- grade ore (Becker, 2000). Such a use of vegetation to remove, accumulate, degrade or immobilize harmful environmental pollutants from soil or water is called phytoremediation. The plants that can accumulate such contaminants in high concentrations are the hyper accumulators. Hyper accumulation can be define as a plant's ability to accumulate metals at levels 100-fold greater in the shoot tissue as compared to the common non accumulator plants. A typical hyper accumulator plant should accumulate at least 10 ppm Hg; 100 ppm Cd; 1000 ppm Co, Cr, Cu and Pb; 10000 ppm Zn and Ni (Baker *et al.*, 2000). Hyper accumulator plant species must be able to tolerate soil metal toxicity and carry out normal physiological processes. According to Rufus Chaney of the United States Department of Agriculture, the best hyper accumulators should be tall, high yielding, fast growing, easy to harvest, deep rooted, and hold onto their leaves so they can be harvested along with the plant (Becker, 2000). Although, such an ideal hyper accumulator is yet to be found in the nature, with the advancement of genetic engineering technology, creation of such transgenic plants may not be so far from reality. Although, phytoremediation technology is in its infancy, with advances in biological, chemical, and engineering technologies, this emerging green technology has the potential to serve as a cost effective, sustained, nonintrusive and ecologically sound method to remediate contaminated soils and water. It promises to be a safe alternative to conventional cleanup techniques. Phytoremediation occurs through complex interactions between plants, soil and soil microbes. Soil Pollution by heavy metals and other organic or inorganic pollutants is a major environmental problem as it poses a threat to human health. The conventional methods to cleanup such contaminated sites are not always practical as they are costly, environmentally intrusive and cannot be used for larger contaminated sites. Phytoremediation an emerging technology uses plants to remediate soil and water contaminated with various contaminants. Studies indicate that it is a novel strategy that is cost effective, nonintrusive to the environment and can be applied to remediate large contaminated sites (Baker and McGrath, 2000).

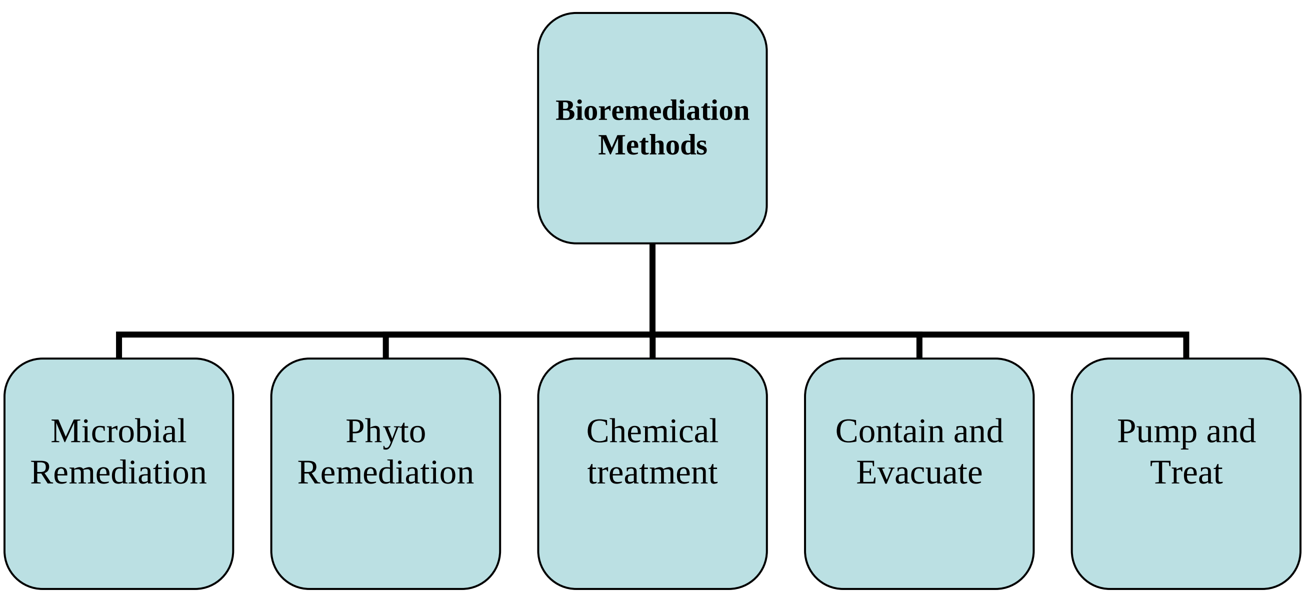


Figure No. 1- Showing preferred Bioremediation methods

Bioremediation Process

The process of bioremediation enhances the rate of the natural microbial degradation of contaminants by supplementing there microorganisms with nutrients, carbon sources or electron donors. This can be done by using indigenous micro-organisms or by adding an enriched culture of micro-organisms that have specific characteristics that allow them to degrade the desired contaminant at a quicker rate. Ideally, bioremediation results in the complete mineralization of contaminants to H_2O and CO_2 without the build-up of intermediates (Sharma and Fulekar, 2009). Bioremediation processes can be broadly categorized into two groups: ex situ and in situ. Ex situ bioremediation technologies include bioreactors, bio-filters, land farming and some composting methods. In situ bioremediation technologies include bioventing, bio-sparing, bio-stimulation liquid delivery system and some composting methods. In situ treatments tend to be more attractive to vendors and responsible parties because they require less equipment, generally have a lower cost and generate fewer disturbances to the environment. However, the difficulties associated with implementing in situ processes have limited their application in the field. Bioremediation using white rot fungi to inoculate contaminated media is a promising technology that is currently being researched. This technology can be used in an ex situ or in situ manner. Generally, this fungus is used to inoculate a composting process, but it does have other bioremediation applications (Jorgensen, 2007).

Metabolic Process

The control and optimization of bioremediation processes is a complex system of many factors. The metabolism of organic contaminants can be broadly disseminated by the ability of the organisms to gain energy for cell growth from the process. These include:

Primary Substrates: If the metabolism of a compound provides energy for cell maintenance and division, the contaminant is referred to as a primary substrate.

Secondary Substrates: In some cases, a compound is metabolized and provides the cell with energy but does not support growth. Contaminants of this type are referred to as secondary substrates.

Co-metabolisms: If a compound is transformed with benefit of the cell (no energy or carbon provided for use by the organism) while the cell is obtaining energy from another transformable compound, the biotransformation is referred to as co- metabolic.

Energy Biotransformation: Finally, an additional classification has been recently identified in which some contaminants are capable of serving as the terminal electron acceptor in the respiratory chain of certain anaerobic (without oxygen) bacteria. In this case, energy is not obtained from the contaminant itself, but its transformation is a component of metabolic processes that provide energy to the cell for growth.

Growth Requirement

Microorganisms can be isolated from almost any environmental conditions. The potential of microorganisms for bioremediation process depends on the existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population and the environmental factors. As essential element of bioremediation process is the ability to sustain enhanced levels of metabolic activity for extended period of time. The studies on assessment of conditions of contaminated sites demonstrate the following factors essential for bioremediation (Pandey and Fulekar, 2012).

Microbial Bioremediation

Micro-organisms are now known to be the principal agents, which can clean and modify the complex lipophilic organic molecules, once considered recalcitrant, to simple water soluble products. They first attack these organic chemicals by the enzymatic apparatus acquired during the course of enrichment, when they are exposed to these specific or structurally related compounds. Presence of these contaminants in the environment either induces or depresses the enzymatic function of microorganisms. This capability largely depends upon the selective microbial community as well as on the structural and functional groups of toxic compounds. These water soluble intermediates are usually attacked by primary or secondary groups of organisms to form inorganic end products, resulting in complete biodegradation. Bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacterial and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The micro-organisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminated compounds are transferred by living organisms through reactions that take place as a part of their metabolic processes (Ambade and Raut, 2014). Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation, the process is called as "Bio-augmentation". The microorganisms with the genetic capacity to transform compounds of interest must be present in contaminant metabolism to occur in a bioremediation process. In certain cases, the addition of organisms acclimated to specific contaminants, or bio-augmentation, may decrease the duration of lag phases. The ability to effectively bio-augment bioremediation system is a function of the process used (Pandey and Fulekar, 2012).

Table 1: Requirements for Microbial Growth in Bioremediation Process

Requirements	Description
Carbon source	Carbon is the most basic element of living forms and is needed in greater quantities than other elements. Carbon contained in many organic contaminants may serve as a carbon source for cell growth. If the organism involved is an autotroph CO2 or HCO3 in solution is required. In some cases, contaminant levels may be too low to supply adequate levels of cell carbon, or the contaminant is metabolized via co-metabolism. In these cases the addition of carbon sources may be required. Nutrients
Nutrients	The growth and activity of the microorganisms must be estimated by adequate maintenance and supply of nutrients. Bio-stimulation usually involves the addition of nutrients and oxygen to help indigenous microorganisms. These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. Nutrients: Nitrogen (ammonic, nitrate, or organic nitrogen) and phosphorous (ortho-phosphate or organic phosphorous) are generally the limiting nutrients. In certain anaerobic systems, the availability of trace metals (e.g. iron, nickel, cobalt, molybdenum and zinc) can be of concern.
Energy source	In the case of primary metabolism, the organic contaminant supplies energy required for growth. This is not the case when the contaminant is metabolized via secondary metabolism or co-metabolism or as a terminal electron acceptor. If the contaminant does not serve as a source of energy, the addition of a primary substrate(s) is required.
Electron acceptor	All respiring bacteria require a terminal electron acceptor. In some cases, the organic contaminant may serve in this capacity. Dissolves oxygen is a common electron acceptor in aerobic bioremediation processes. Under anaerobic conditions, NO3-, SO43-, Fe3+, and CO2 may serve as terminal electron acceptors. Certain co-metabolic transformations are carried out by fermentative and other anaerobic organisms, in which terminal electron acceptors are not required.
Temperature	Rates of growth and metabolic activity are strongly influenced by temperature. Surface soils are particularly prone to wide fluctuations in temperature. Generally, mesophilic conditions are best suited for most applications (with composting being a notable exception).
pH	A pH is another important factor that influences bioremediation process. If the soil is acidic, it is possible to raise pH by adding lime. A pH ranging between 6.5 and 7.5 is generally considered optimal. The pH of most ground water (8.0–8.5) is not considered inhibitory.

Absence of toxic metals Many contaminated sites contain a mixture of chemicals, organic and inorganic, which may be inhibitory or toxic to microorganisms. Heavy metals and phemelic compounds are of particular concerns.

Soil moisture Moisture content affects the microbial growth and activity. The water-holding capacity recommended for bioremediation process may range from 25% – 28%.

Adequate contact between micro-organisms and substrates For contaminants to be available for microbial uptake it must be present in aqueous phase. Thus contaminants that exist as non-aqueous phase liquids or are sequestered within a solid phase may not be readily metabolized. For degradation it is necessary that bacteria and the contaminants be in contact. This is not easily achieved, as neither the microbes nor contaminants are uniformly spread in the soil. Some bacteria are mobile and exhibit a chemotactic response, sensing the contaminant and moving towards it. Other microbes such as fungi grow in a filamentous, form towards the contaminant. It is possible to enhance the mobilization of the contaminant utilizing some surfactants such as sodium dodecyl sulphate (SDS).

Time Time is an important factor in the start-up of bioremediation systems. Even the above mentioned considerations are met, lag phases are often observed prior to the onset of activity. In some cases, the dramatic bacterial population shifts that are required for bioremediation will lengthen periods of slow activity.

Environmental requirements The optimum environmental conditions that requires for bioremediation of contaminant sites are presented in table given below.

Parameters	**Conditions Required for Microbial Activity**	**Optimum Value for Organic Pollutant Degradation**
Soil moisture	25 - 28% of water holding capacity	30 – 90%
Soil pH	5.5 – 8.8	6.5 to 8.0
Oxygen content	Aerobic, minimum air filled pore space of 10%	10 – 40%
Nutrient content	N and P for microbial growth	C:N:P=100:10:1
Temperature (°C)	15 – 45	20 – 30
Contaminants	Not too toxic	Hydrocarbon 5 – 10% of dry weight of soil
Heavy metals	Total content – 2000 ppm	700 ppm
Type of soil	Low clay or silt content	700

(source, Vidali, 2001)

Microbes are everywhere in the biosphere, and their presence invariably affects the environment where they are growing in. Several of the processes which microorganisms perform are of critical importance for the cycling of nutrients, degradation of various compounds, and the global climate. Microbes make up the major portion of the biomass present on the earth. Therefore, the nutrients they eat and the products they form greatly influence the environment. Microbes release nutrients from dead organisms, making them available to the rest of the ecosystem. Some microbes play a role in the production of energy (Atlas and Bartha, 1981).

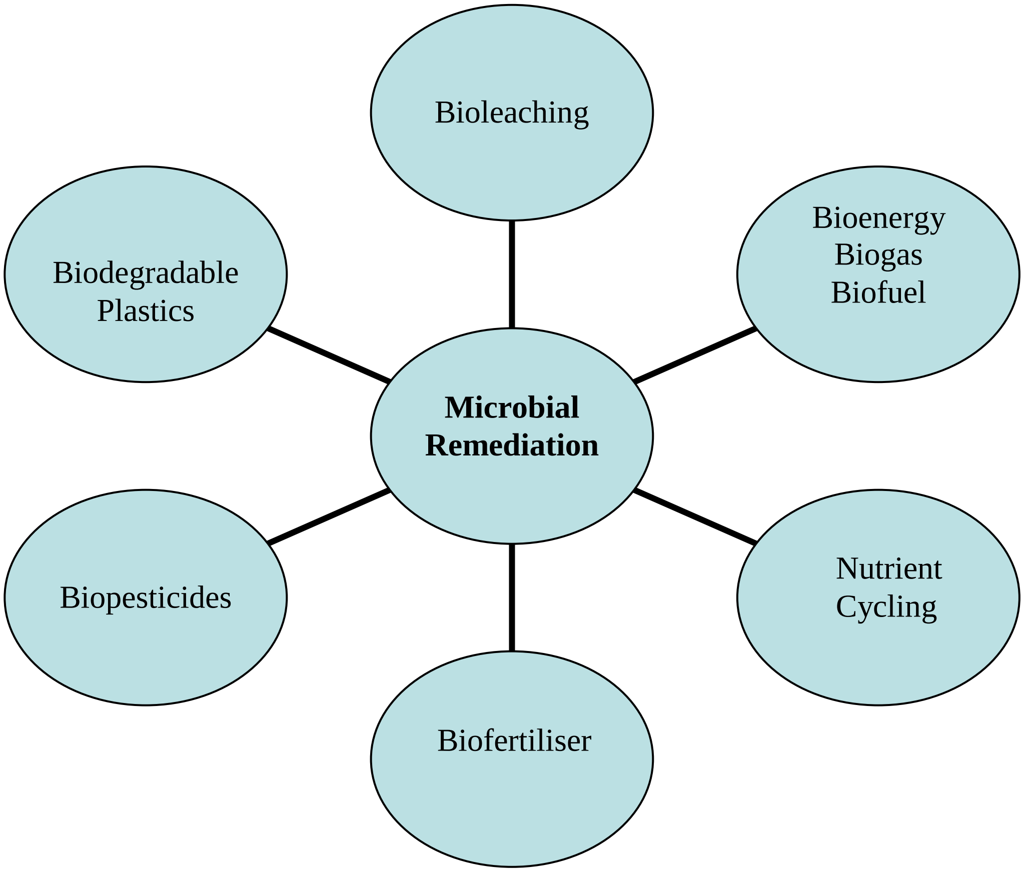


Figure 2: Power of Microbes to Remedy the Environmental Problems

Phytoremediation Process

The term phytoremediation originated from the words phyto meaning 'plant' and remediation meaning 'to correct evil.' The use of phytoremediation has been recognized for over 300 years (Anderson *et al.*, 1993). Phytoremediation of environmental contamination is an emerging and fascinating area of research. Hyper accumulator plants have the potential to remove various contaminants from soils and water. Phytoremediation describes the bioremediation through the use of plants that solve the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere. Phytoremediation lower down pollutant concentrations in contaminated soils, water, or air, with plants able to contain, degrade, or eliminate metals, pesticides, solvents, explosives, crude oil and its derivatives, and various other contaminants from the media that contain them. According to Baker and McGrath (2000) an ideal phytoremediators is a plant that grows fast, creates large biomass, and is equipped with an extensive root system.

Since the natural phytoremediators often lack these qualities, scientists are focusing on developing bioengineered plants to fit the requirements of different cleanup conditions (Zhao and McGrath, 2009). With the advancement of transgenic biotechnology, it is expected that plants can be genetically modified to tolerate various stressed environments better and bioaccumulate greater quantities of pollutants. In addition to transgenic modification of plants, investigators are also focusing on the possibility of increasing pollutants bioavailability in the soil to enhance plant uptake of the pollutants (McGrath and Zhao, 2003; Krammer, 2005).

Phytoremediation may be applied wherever the soil or static water environment has become polluted or is suffering ongoing chronic pollution. It may involve uptake and concentration of substances from the environment into the plant biomass (phytoextraction), rhizofiltration, chemical modification by plants or microbes and by enhancing soil microbial activity (Zaman, 2003).

Phytoextraction or Phytoaccumulation

The process of up taking of contaminants through plant roots and accumulating them in the above ground plant tissues is Phytoextraction. Plants suitable for this process must be able to tolerate the high level of contaminants in the soil, and with a higher biomass, these plants may be able to accumulate larger volumes of the pollutants. It is a technique of using pollutant accumulating plants to remove metals or organic components from the soil and water by concentrating them in the harvestable parts. Hyper accumulator plants can be used to extract pollutants from the soil and water and harvested plant's material can be utilized for non-edible purposes (Khunte, 2014).

Rhizofiltration

The process of uptake of inorganic contaminants dissolved in water and accumulating them in plant roots is Rhizofilteration. The efficient plants for rhizofilteration should have rapidly growing roots with high root biomass. Rhizofilteration method involves the use of plant roots to absorb waste streams containing mainly metals. This process utilizes the advantage of plants with dense and deeper root system in wet lands and marshy areas. Rhizofilteration can partially treat industrial discharge, agricultural runoff or acid mine drainage. It can be used for lead, copper, nickel, zinc and chromium, which are primarily retained within the roots. Plants like Sunflower, Indian mustard, Tobacco, Rye, Spinach, and Corn have been used for their ability to remove lead from effluents with sunflower having greatest ability. Similarly, the aquatic plants like *Eichornia crassipes*, *Lemna minor*, etc. will be best suited for this technology (Khunte, 2014).

Phytotransformation or Phytodegradation

The process of uptake of organic contaminants such as pesticides, herbicides, hydrocarbons, etc. which are broken down or metabolized within the plant tissue is Phytotransformation. The use of plants capable of removing organic pollutants by phytodegradation is an important option for phytoremediation. In this process, plant enzymes act on organic pollutants and mineralize them either completely into inorganic compounds, such as CO_2, water and C_{l2} or partially into stable intermediates that arrested in the plants (Khunte, 2014).

Rhizodegradation

The release chemicals by plant root systems that enhance biodegradation of organic contaminants with the help of soil microorganisms is Rhizodegradation (Khunte, 2014).

Phytovolatilization

The process of transporting organic contaminants to the leaves that transpire, evaporate, or volatize the contaminants into the atmosphere is Phytovolatilization (Khunte, 2014).

Phytostabilization

The use of plants to eliminate the bioavailability of contaminants in the environment is phytostabilization. Barren lands are more susceptible to erosion and leaching. This spreads the pollutants in the environment. Revegetation of such soils with tolerant plant species will help stabilize such lands. Trees are ideal for phytostabilization as they are perennial and their extensive root systems can reach greater areas as compared to smaller plants (Khunte, 2014).

Techniques of Bioremediation

The process of developing bioremediation techniques may involve the following steps (Storm, 1985).

- Isolating and characterizing naturally occurring microorganisms with bioremediation potential.
- Laboratory cultivation to develop viable population.
- Studying the catabolic activity of these microorganisms in contaminated material through bench scale experiments
- Monitoring and measuring the progress of bioremediation through chemical analysis and toxicity testing in chemically contaminated media. Field applications of bioremediation techniques using either /

both steps (1) In-situ stimulation of microbial activity by the addition of microorganisms and nutrients and optimization of environmental factors at the contaminated site itself (2) Ex-situ restoration of contaminated material in specially designated areas by land filling and composting methods.

Techniques of Phytoremediation

Flow chart represents the techniques being involved in Phytoremediation

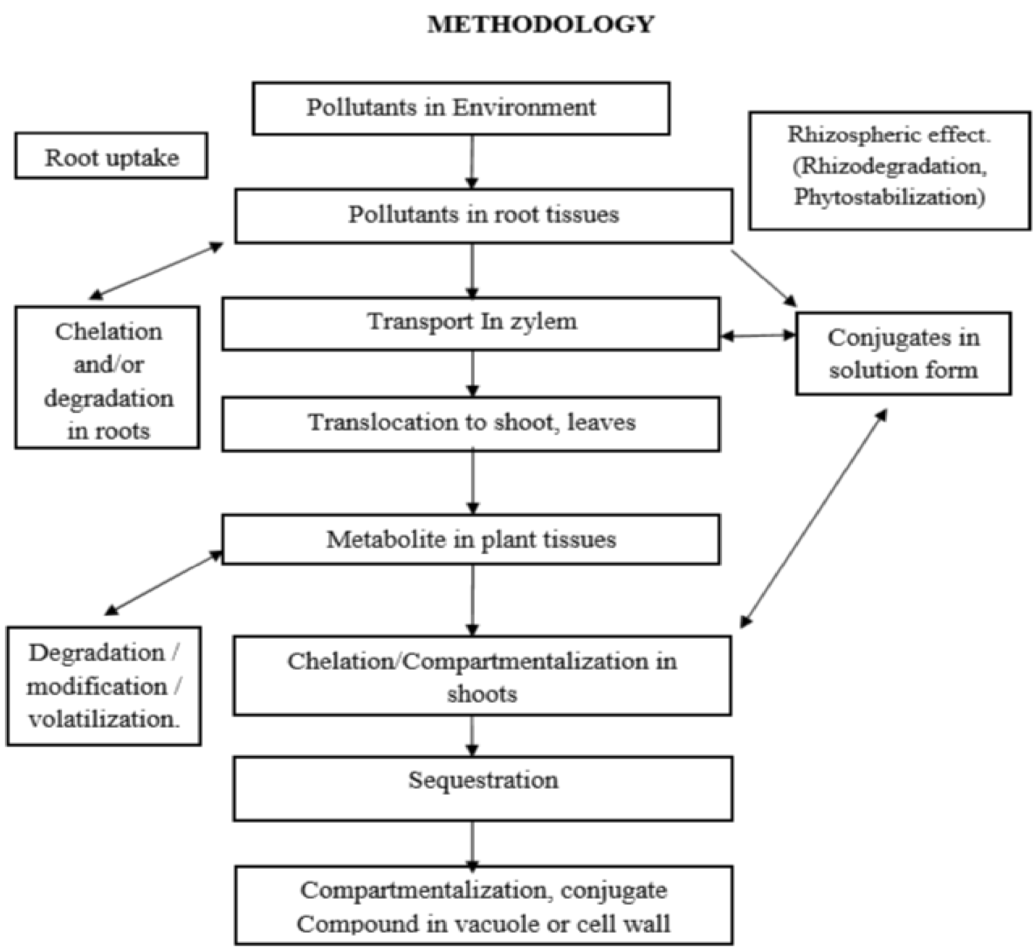
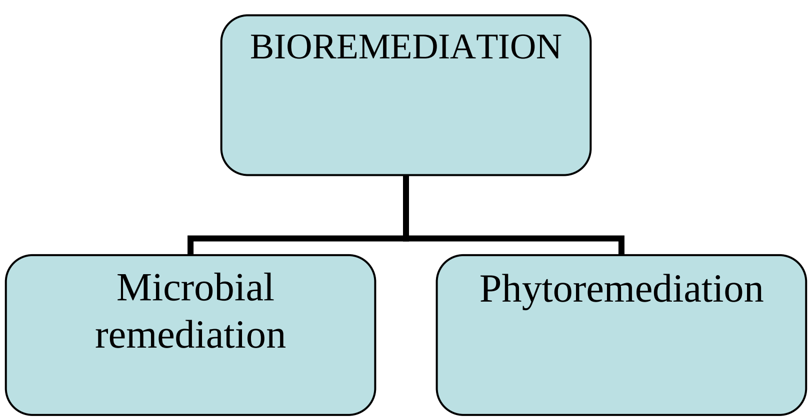


Figure 3: Microorganisms and Plants Playing Efficient Role in Bioremediation Process



Review Article

Natural communities of microorganisms in various habitats have an amazing physiological versatility, they are able to metabolize and often mineralize an enormous number of organic molecules. Certain communities of bacteria and fungi metabolize a multitude molecules that can be degraded is not known but thousands are known to be destroyed as a result of microbial activity in one environment or another.
Microorganisms that carry out biodegradation in many different environments are identified as active members of microbial consortiums. These microorganisms include: *Acinethobacter, Actinobacter, Acaligenes, Arthrobacter, Bacillins, Berijerinckia, Flavobacterium, Methylosinus, Mycrobacterium, Mycococcus, Nitrosomonas, Nocardia, Penicillium, Phanerochaete, Pseudomonas, Rhizoctomia, Serratio, Trametes* and *Xanthofacter*.
The list and description of plants with different capacities to accumulate metals has been given in the following table 2.

Table 2: List of Plants Suitable for Phytoremediation

Scientific Name	Common Name	Origin and Characteristics	Element and Degree of Accumulation
Azolla filicoides	Water fern	Africa, floating	Cu, Ni, Pb, and Mn
Bacopa monnieri	--	India, emergent species	Hg, Cu, Cr, Pb and Cd
Eichhornia crassipes	Water hyacinth	Troublesome weed	Cd, Zn, Hg, Pb and Cu
Hydrilla aquatic	Hydrilla	Southern Asia	Cd, Cr, Hg and Pb
Salvinia molesta	Water fern	India	Cr, Ni, Pb and Zn
Brassica juncea	Indian Mustard	Cultivated	Pb, Zn, Ni, Cu, Cr, Cd and Ur,
Helianthus annus	Sunflower	Cultivated	Pb, Ur ,Sr, Cs, Cr, Cd, Cu, Mn, Ni, and Zn
Thaspi caerulescens	Apline pennycress	Europe	Zn, Cd, Co, Cu, Ni, Pb and Cr
Lemna minor	Duckweed	Native to North America	Pb, Cd, Cu and Zn

(Source Khunte,2014)

Table 3: Applications of Plants in Phytoremediation (Source Khunte, 2014)

Plant Type	Applications
Thlaspi, Alyssum and Brassica species	Phytoextraction and Phytomining
Grasses and food crops	Stabilization and Rhizodegradation
Atriplex and Salicornia species	Salt extraction and volume reduction of oilfield brines
Poplar , Willow and Cottonwood	Contaminant biotransformation, barrier for hydraulic contaminant and vegetative cover for landfills
Duckweed (lemna minor) and Pennywood (Hydrocotyle species)	Rhizofiltration and Phytotransformation
Reeds (Phyagmites species) and Cattails (Typha species)	Constructed wet lands

Advantages of Bioremediation

- Bioremediation is useful for the complete destruction of a wide variety of contaminants Bioremediation is a highly cost effective method as compared to the effective method as compared to the conventional remediation techniques.
- It is nonintrusive to the environment, thus, friendly to the ecosystem.
- Plant roots stabilize the soil, reduce soil erosion, and prevent off site on.

- As roots absorb water and nutrients from the soil and water, downward movement of chemicals by percolation is prevented and groundwater contamination is reduced.
- Plants may increase the amount of organic carbon in the soil, limiting contaminant movement and help soil to stabilize pollutants.
- Bioremediation can be used on large scale where the other technologies fail. It provides the permanent solution and requires low capital investment. Hence, it is cost effective alternative to physical remediation system.

Bioremediation technology is environmentally safe and aesthetically acceptable as well as least harmful method.

Figure 4: ***Azolla Filicoides*** **(Encyclopedia, 1783)**

Figure 5: ***Phyagmites sp.*** **(Deng *et al.*, 2004)**

Figure 6: ***Eichhornia Crassipes*** **(Nigam, 2002)**

Figure 7: ***Lemna Minor*** **(White and Wise, 1998)**

Figure 8: ***Salvinia Molesta*** **(Choudhary *et al.*, 1998)**

Figure 9: ***Thaspi Caerulescens*** **(Comis, 2000)**

Figure 4 to 9: Photographs of Plants Playing Role in Phytoremediation

Disadvantages of Bioremediation

- Bioremediation is limited to those compounds that are biodegradable; all compounds are not susceptible to rapid and complete degradation.
- Products of biodegradation may be more persistent and toxic than the parent compound.
- This is a slower process compared to conventional soil and water remediation processes.
- Complete removal of metal pollutants from the contaminated site is not possible.
- In Bioremediation, there are possibilities of accumulation of metals in the food chain.
- True costs and benefits of bioremediation technology to the ecosystem are yet to be determined.

Recent Approaches

Scientists are currently looking into certain genetically engineered microorganisms to increase their ability to metabolize specific chemicals such as hydrocarbons and pesticides. The possibilities of using genetic engineering for improvement of bioremediation process had an early boost in the late 1980's. Recombinant DNA techniques have been studied intensively to improve the degradation of hazardous waste under laboratory condition. The genetically engineered microorganisms have higher degradative capacity and have been demonstrated successfully for the degradation of various pollutants under defined conditions. Genetic modification technology has resulted often in a wide variety of current and potential applications for use in the process of bioremediation. Bioremediation explores gene diversity and metabolic versatility of microorganisms (Boricha and Fulekar, 2009). The genetic architecture of these organisms makes them valuable in biodegradation, biotransformation, biosorption and bioaccumulation. The necessary blue print of gene encoding for biodegradative enzymes is present in chromosomal and extra- chromosomal DNA of such microbes. Recombinant DNA techniques facilitate to evolve the ability of an organism to metabolize a xenobiotic by detection of such degradative genes and transforming them into appropriate host via suitable vector under the tight control of appropriate promoters. It depends on susceptibility to alteration and exchange of genetic information. The recombinant DNA technology explores PCR, anti-sense RNA technique, site directed mutagenesis, electroporation and particle bombardment techniques. The biotechnology armed with recombinant DNA technology is now fine tuning the bioremediation technology by improving pollutant–degrading microbes through strain improvement and genetic modification of specific regulatory and metabolic genes that are crucial in developing effective, safe and economical techniques for bioremediation (Pandey and Fulekar, 2012).

Conclusion

Although, remediation of contaminated areas is a common international concern, the context, awareness and approach taken to address the problem have been found to be country specific. This agrees with the findings of Rivett *et al.*, (2002) which indicated that the factors driving the use of bioremediation include economics/cost considerations, relative perceptions or ignorance of the extent of contamination, country-specific policies and bio-safety legislation, and stronger focus on an environmental medium such as soil at the expense of others. This has been observed that a marked contrast is important according to process-based remediation versus physical methods like land filling, and the lack of centralized information on remediation activity.

Sustainable energy development focuses on fulfilling the needs of today but also making the resources available for tomorrow. The building of a sustainable society will require reduction of dependency on fossil fuels and lowering of the amount of pollution that is generated. For energy production, microbes offer efficient and sustainable ways to convert biomass into liquid fuels and electricity which are currently derived from fossil fuels. They are also important tools for generation of alternatives such as biogas which can reduce our dependency on fossil fuels. Microbes are the best hope in producing renewable energy in large quantities without damaging the environment or competing with our food supply. Microbes surround plant roots; inhabit stems, roots, and leaves and microbial activities also provide plants with nitrogen, phosphorus and other nutrients, they protect them from diseases, recycle nutrients, and improve soil structure. They can carry out bioremediation and thus, help in decontamination of environment and help in production of clean energy and resources from waste. They are helpful in extraction of minerals from their ores (bioleaching). Microbes can thus help address society's energy, environmental, and food challenges. Thus, microbes are versatile enough to address various environmental problems with a neutral carbon footprint and make the earth a better place to live (Olson *et al.*, 2003).

An ideal phytoremediators are the plant that grows fast, creates large biomass, and is equipped with an extensive root system. Since the natural phytoremediators often lack these qualities. Scientists are focusing on developing bioengineered plants to fit the requirements of different cleanup conditions With the advancement of transgenic biotechnology, it is expected that plants can be genetically modified to tolerate various stressed environments better and bioaccumulate greater quantities of pollutants. In addition to transgenic modification of plants, investigators are also focusing on the possibility of increasing pollutants bioavailability in the soil to enhance plant uptake of the pollutants (Zaman, 2003).

Microbial remediation and Phytoremediation has many limitations, and it is not a remedy for all hazardous waste problems.

Moreover, this technology has to be fully established before it can be commercially available. Nevertheless, research findings clearly indicate that this is a feasible and novel technology that holds greater promises for the future.

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