Bioremediation: Features, Strategies and applications

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Abstract

In early times, we believed that we had an unlimited abundance of land and resources; today, however, the resources in the world show, in greater or lesser degree, our carelessness and negligence in using them. The problems associated with contaminated sites now assume increasing prominence in many countries. Contaminated lands generally result from past industrial activities when awareness of the health and environmental effects connected with the production, use, and disposal of hazardous substances were less well recognized than today. Environmental contamination is increasing day by day because of increase in population, industrialization and urbanization Bioremediation is the technology that uses microorganism metabolism to remove pollutants it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. This technology includes biostimulation (stimulating viable native microbial population), bioaugmentation (artificial introduction of viable population), bioaccumulation (live cells), biosorption (dead microbial biomass), phytoremediation (plants) and rhizoremediation (plant and microbe interaction).Rapid advances in the last few years has helped us in the understanding of process of bioremediation. The use of culture independent molecular techniques has definitely helped us to understand the microbial community dynamics, structure and assisted in providing the insight in to details of bioremediation which has surely facilitated to make the technology safer and reliable. This paper represents the special features, strategies, limitation and a variety of approaches of bioremediation.

Keywords: Bioremediation, Phtyoremediation .

Introduction

Bioremediation is the use of microorganism metabolism to remove pollutants. Bioremediation can occur on its own (natural attenuation or intrinsic bioremediation) or can be spurred on via the addition of fertilizers to increase the bioavailability within the medium (biostimulation). Recent advancements have also proven successful via the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Microorganisms used to perform the function of bioremediation are known as bioremediators[1]. It can be classified as in situ or ex situ .Bioremediation can be used at the site of contamination (in situ) or on contamination removed from the original site (ex situ). In the case of contaminated soil, sediments, and sludges, it can involve land tilling in order to make the nutrients and oxygen more available to the microorganisms.Some examples of bioremediation technologies are phytoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result. The conventional techniques used for remediation have been to dig up contaminated soil and remove it to a landfill, or to cap and contain the contaminated areas of a site. The methods have some drawbacks. The first method simply moves the contamination elsewhere and may create significant risks in the excavation, handling, and transport of hazardous material. Additionally, it is very difficult and increasingly expensive to find new landfill sites for the final disposal of the material. The cap and contain method is only an interim solution since the contamination remains on site, requiring monitoring and maintenance of the isolation barriers long into the future, with all the associated costs and potential liability. A better approach than these traditional methods is to completely destroy the pollutants if possible, or at least to transform them to innocuous substances. Some technologies that have been used are high-temperature incineration and various types of chemical decomposition (e.g., base-catalyzed dechlorination, UV oxidation). They can be very effective at reducing levels of a range of contaminants, but have several drawbacks, principally their technological complexity, the cost for small-scale application, and the lack of public acceptance, especially for incineration that may increase the exposure to contaminants for both the workers at the site and nearby residents.

Principle of Bioremediation:-

Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities [2] For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more acceptable than other technologies. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules [3]

Factors of Bioremediation:-

The control and optimization of bioremediation processes is a complex system of many factors. These factors include: the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environment factors (type of soil, temperature, pH , the presence of oxygen or other electron acceptors, and nutrients).

Table 1 Showing factors of bioremediation

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

Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. The main requirements are an energy source and a carbon source. of microbes and other biological systems, these can be used to degrade or remediate environmental hazards.

We can subdivide these microorganisms into the following groups:

Aerobic. In the presence of oxygen. Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.

Anaerobic. In the absence of oxygen. Anaerobic bacteria are not as frequently used as aerobic bacteria. 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.

Ligninolytic fungi. Fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs.

Methylotrophs. Aerobic bacteria that grow utilizing methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatics trichloroethylene and 1,2-dichloroethane.

Enironmental factors

Nutrients

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 The type of bioremediation depends on the concentration of soil contaminants Phosphorous and sulfur contribute with 70% of the remainders. The nutritional requirement of carbon to nitrogen ratio is 10:1, and carbon to phosphorous is 30:1.

Table 2 Showing environmental conditions [4]

Environmental Factor	Optimum conditions	Condition required for microbial Activity
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 ºC	15-45ºC
Contaminants	Hydrocarbon 5-10% of dry weight of soil	Not too toxic
Heavy metals	700ppm	Total content 2000ppm

Soil

1. High concentrations of contaminants (roughly 5% or more): The soil is agitated in a purifying water solution containing interface active agent, then separated from the oils. After that, bioremediation is started to efficiently clean the soil. At the experimental stage, bioremediation alone has been able to turn contaminated soil into soil suited for landscaping, and work is continuing to make this process even more efficient and effective.

2. Low concentrations of contaminants : Soils that have low concentrations of contaminants can be treated using bioremediation alone. It takes about 6 months to a year to purify soil containing two percent heavy oils, but at a concentration of 0.8 percent, the job can be done in only about one to two months. This environmentally-friendly method makes it possible to recycle and reuse soil without much effort.

Bioremediation strategies:-

***In-Situ* Bioremediation:**

In situ bioremediation is the application of biological treatment to the cleanup of hazardous chemicals present in the subsurface. The optimization and control of microbial transformations of organic contaminants require the integration of many scientific and engineering disciplines.

Biosparging.

Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system.

Bioventing

Bioventing is a promising new technology that stimulates the natural *in-situ* biodegradation of any aerobically-degradable compounds in NAPL within the soil by providing oxygen to existing soil microorganisms. In contrast to soil-vapor extraction (SVE), bioventing uses low air-flow rates to provide only enough oxygen to sustain microbial activity. Oxygen is most commonly supplied through direct air injection into residual contamination in soil by means of wells. Adsorbed fuel residuals are biodegraded, and volatile compounds also are biodegraded as vapors move slowly through biologically active soil.[5]

Bioaugmentation

Bioaugmentation is the introduction of a group of natural microbial strains or a genetically engineered variant to treat contaminated soil or water. It is commonly used in municipal wastewater treatment to restart activated sludge bioreactors. Most cultures available contain a research based consortium of Microbial cultures, containing all necessary microorganisms At sites where soil and groundwater are contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene**,** bioaugmentation is used to ensure that the *in situ* microorganisms can completely degrade these contaminants to ethylene and chloride, which are non-toxic[6] Monitoring of this system is difficult

Biopiling

Biopile treatment is a full-scale technology in which excavated soils are mixed with soil amendments, placed on a treatment area, and bioremediated using forced aeration. The contaminants are reduced to carbon dioxide and water. The basic biopile system includes a treatment bed, an aeration system, an irrigation/nutrient system and a leach ate collection system. Moisture, heat, nutrients, oxygen, and pH are controlled to enhance biodegradation. The irrigation/nutrient system is buried under the soil to pass air and nutrients either by vacuum or positive pressure. Soil piles can be up to 20 feet high and may be covered with plastic to control runoff, evaporation and volatilization, and to promote solar heating. If volatile organic compounds (VOCs) in the soil volatilize into the air stream, the air leaving the soil may be treated to remove or destroy the VOCs before they are discharged into the atmosphere. Treatment time is typically 3 to 6 months [7]

***Ex-Situ* Bioremediation**

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° to 65° C. The increased temperatures result from heat produced by microorganisms during the degradation of the organic material in the waste. Windrow composting has been demonstrated using the following basic steps. First, contaminated soils are excavated and screened to remove large rocks and debris [8,9]

Bioreactors

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. In general, the rate and extent of biodegradation are greater in a bioreactor system than *in situ* or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages. The contaminated soil requires pre treatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washingor physical extraction (e.g., vacuum extraction) before being placed in a bioreactor.

Table 3. Developmental methods applied in bioremediation [10,11]

Technique	Examples	Benefits	Applications	References
In Situ	Biosparging Bioventing Bioaugmentation	Most efficient Non Invasive Relative passive Naturally attenuated process, treat soil and water	Biodegradative abilities of indigenous microorganisms Presence of metals and inorganic compounds Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants	[12,6,13]
Ex situ	Land farming (Solid-phase treatment system) Composting (Anaerobic,converts solid organic wastes into humus-like material) Biopiles	Cost efficient ,Simple, Inexpensive ,self-heating Low cost Rapid reaction rate, Inexpensive, self heating Can be done on site	Surface application, aerobic process, application of organic materials to natural soils followed by irrigation and tilling To make plants healthier good alternative to land filling or incinerating practical and convenient. Surface application, agricultural to municipal waste	[8,9]
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetic Optimized environmental parameters Enhances mass transfer Effective use of inoculants and surfactant	Bioaugmentat Toxicity of amendments Toxic concentrations of contaminants	[14]
Precipitation or Flocculation	Non-directed physico-chemical complex -ation reaction between dissolved contamin - ants and charged cellular components (dead Biomass)	Cost-effective	Removal of heavy Metals	[15]
Microfiltration	Microfiltration membranes are used at a constant pressure	Remove dissolved solids rapidly	Waste water treatment; recovery and reuse of more than 90% of original waste water	-
Electrodialysis	Uses cation and anion exchange membrane pairs	Withstand high temperature and can be reused	Removal of dissolved solids efficiently	-

Special Features of Bioremediation

- It is a natural process , it takes a little time, as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded,the biodegradative population declines. The residues for the treatment are usually harmless productsort
- Bioremediation also requires a very less effort and can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.
- Bioremediation is also a cost effective process as it lost less than the other conventional methods that are used for clean-up of hazardous waste.
- It also helps in complete destruction of the pollutants , many of the hazardous compounds can be transformed to harmless products ,this feature also eliminates the chance of future liability associated with treatment and disposal of contaminated material.
- It does not use any dangerous chemicals. The nutrients added to make microbes grow are fertilizers commonly used on lawns and gardens. Because bioremediation changes the harmful chemicals into water and harmless gases, the harmful chemicals are completely destroyed..

Limitations of Bioremediation :-

• Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.

• There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound. Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.

• It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.

• Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids, and gases.

• Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration.

• Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation.
There is no accepted definition of “clean”, evaluating performance of bioremediation is difficult

Table 4 Some contaminants potentially suitable for bioremediation. [6]

Class of Contaminants	Examples	Aerobic	Anaerobic	Potential Sources
Chlorinated solvents	Trichloroethylene	-	+	Drycleaners
Polychlorinated biphenyls	4-Chlorobiphenyl	-	+	Electrical manufacturing
“BTEX”	Benzene Toluene Ethylbenzene Xylene	+	+	Oil production and storage Gas work sites Airports Paint manufacture
Polyaromatic hydrocarbons	Naphthlene,Anthracene,Pyrene	+	-	Oil production and storage.Engine works
Pesticides	Atrazine, 2 4 D , Parathon	+	+	Agriculture. Pestcides manufacture

Developments of Phytoremediation:-

Microbes are not the only species that can be enhanced by genetic modification for bioremediatory purposes. Plants have also been studied and used. Bioremediation by plants is called *phytoremediation*. Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water. This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites. The U.S. Environmental Protection Agency (EPA) seeks to protect human health and the environment from risks associated with hazardous waste sites, while encouraging development of innovative technologies such as phytoremediation to more efficiently clean up these sites. Arsenic is one target of phytoremediation[16]. The health effects of arsenic include liver, lung, kidney and bladder cancers. One plant, *Arabidopsis thaliana* has been genetically modified to over express two bacterial genes, *arsC* and g-*ECS*1. The gene *arsC* codes for arsenate reductase, which allows the plant to modify arsenate into aresenite, and g-*ECS* codes for gglutamylcysteine synthase, which makes a thiol that can detoxify aresenite by forming arsenic-protein thiates that are then stored in vacuoles1. Essentially this genetically modified plant can take up arsenate, detoxify it and store it. Phytoremediation can also be used to destroy high-energy compounds such as TNT, GTN, RDX, TETRYL and HMX9. Tobacco plants have been genetically modified to express bacterial pentaerythritol tetanite (PETN) reductase allowing these plants to take up high-energy compounds and reduce them to non-explosive substances[17]. Another genetically modified plant possesses a bacterial mercuric reductase gene allowing it to take up mercury(Hg) out of the soil and store it safely. A recent publication of some workers describes the development of transgenic poplars (*Populus*) over expressing a mammalian cytochrome P450, a family of enzymes commonly involved in the metabolism of toxic compounds. The engineered plants showed enhanced performance about the metabolism of trichloroethylene and the removal of a range of other toxic volatile organic pollutants, including vinyl chloride, carbon tetrachloride, chloroform and benzene. Some workers suggested that transgenic plants might be able to contribute to the wider and safer application of phytoremediation[18] Herbicides are economically important, but the non-point pollution that they cause may disrupt the surrounding environment. Phytoremediation of herbicides has been well studied using conventional plants.

Table 5 Overview of phytoremediation applications

The major advantages of phytoremediation are as follows

i) The cost of the phytoremediation is lower than that of traditional processes both *in-situ* and *ex- situ*.

ii) The plants can be easily monitored.

iii) The possibility of the recovery and re-use of valuable products.

iv) It uses naturally occurring organisms and preserves the natural state of the environment.

v) The low cost of phytoremediation (up to 1000 times cheaper than excavation and reburial) is the main advantage of phytoremediation.

Table:5 five types of phytoremediation techniques

Process	Function	Pollutant	Medium	Plants	References
Phytoextraction	Remove metals pollutants that accumulate in plants. Remove organics from soil by concentrating them in plant parts	Cd, Pb, Zn, As, Petroleum, Hydrocarbons and Radionuclides	Soil & Groundwater	*Viola baoshanensis, Sedum alfredii, Rumex crispus*	[19,20]
Phytotranformation	Plant uptake and degradation of organic Compounds	xenobiotic substances	Soil	*Cannas*	[21]
Phytodegradation	Plants and associated microorganisms degrade organic pollutants	DDT, Expolsives, waste and Nitrates	Groundwater	*Elodea Canadensis, Pueraria*	[22,23]
Rhizofiltration	Roots absorb and Zn, Pb, Cd, As Groundwater adsorb pollutants, mainly metals, from water and aqueous waste streams	Zn, Pb, Cd, As	Groundwater	*Brassica juncea,*	[24,25]
Phytostabilization (Immobilization)	Use of plants to reduce the bioavailability of pollutants in the environment	Cu, Cd, Cr, Ni, Pb, Zn	Soil	*Anthyllis vulneraria, Festuca arvernensis*	[26]

Phytoextraction

This process has been tried more often for extracting heavy metals than for organics, We can find five types of phytoremediation techniques, classified based on the contaminant fate:
phytoextraction, phytotransformation, phytostabilization, phytodegradation, rhizofiltration, even if a combination of these can be found in natureThe main advantage of phytoextraction is environmental friendly. The traditional methods those are used for cleaning up the heavy metal contaminated soil are responsible for disruption of soil structure and reduce soil productivity, whereas phytoextraction can clean up the soil without causing any kind of harm to the soil quality. Another benefit of phytoextraction is less expensive than any other clean up process. As this process is controlled by plant, so it takes more time than any traditional soil cleanup process.
Phytoextraction or *phytoaccumulation* is the process used by the plants to accumulate contaminants into the roots and aboveground shoots or leaves. This technique saves tremendous remediation cost by accumulating low levels of contaminants from a widespread area. Unlike the degradation mechanisms, this process produces a mass of plants and contaminants (usually metals) that can be transported for disposal or recycling.
Phytotransformation or *phytodegradation* refers to the uptake of organic contaminants from soil, sediments, or water and, subsequently, their transformation to more stable, less toxic, or less mobile form. Metal chromium can be reduced from hexavalent to trivalent chromium, which is a less mobile and noncarcinogenic form.
Phytostabilization is a technique in which plants reduce the mobility and migration of contaminated soil. Leachable constituents are adsorbed and bound into the plant structure so that they form a stable mass of plant from which the contaminants will not reenter the environment.
Phytodegradation or *rhizodegradation* is the breakdown of contaminants through the activity existing in the rhizosphere. This activity is due to the presence of proteins and enzymes produced by the plants or by soil organisms such as bacteria, yeast, and fungi. Rhizodegradation is a symbiotic relationship that has evolved between plants and microbes. Plants provide nutrients necessary for the microbes to thrive, while microbes provide a healthier soil environment.

Rhizofiltration

Rhizofiltration is similar in concept to Phytoextraction but is concerned with the remediation of contaminated groundwater rather than the remediation of polluted soils. The contaminants are either adsorbed onto the root surface or are absorbed by the plant roots. Plants used for rhizofiltration are not planted directly in situ but are acclimated to the pollutant first. Plants are hydroponically grown in clean water rather than soil, until a large root system has developed. Once a large root system is in place, the water supply is substituted for a polluted water supply to acclimatize the plant. After the plants become acclimatized they are planted in the polluted area where the roots uptake the polluted water and the contaminants along with it. As the roots become saturated, they are harvested and disposed of safely. Repeated treatments of the site can reduce pollution to suitable levels as was exemplified in sunflowers were grown in radioactively contaminated pools.

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