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Microbiological Solution to Environmental Problems - A Review on Bioremediation

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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 use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms. Research has demonstrated that there are very few environments where microbes have not been able to survive, adapt, and indeed, thrive. Microbes are able to utilize a near infinite combination of electron donors and electron acceptors to drive their metabolism.

The purpose of this document is to provide fundamental information about factors that influence the rate and extent to which environmental contaminants are metabolized by microorganisms in the environment and technology involved including in situ (Bioventing, Biosparging, Biostimulation, Bioaugmentation) and ext site bioremediation (Landfarming, Soil Biopiles, Composting, Bioreactors) and also a short description about the phytoremediation and its types.

Keywords: *Bioremediation, Biostimulation, Bioaugmentation, Biosparging, etc.*

INTRODUCTION

Bioremediation is the use of living organisms, primarily microorganisms, to degrade environmental contaminants into less toxic forms. Microbes are able to utilize a near infinite combination of electron donors and electron acceptors to drive their metabolism. In addition to these redox (oxidation / reduction) reactions, they have also developed a myriad of other strategies enabling them to detoxify their environment. Bioremediation applies these principles to select a suitable combination of microbial community activity, electron donor / acceptor / contaminant concentrations and other physical and practical parameters to remediate / recover a targeted pollutant.

This process exploits such microorganisms and their enzymatic activities to effectively remove contaminants from contaminated sites. This process is a cost effective means of cleanup of hydrocarbon spills from contaminated sites as it involves simple procedures only and it is an environmentally friendly technology which optimizes microbial degradation activity via control of the pH, nutrient balance, aeration and mixing[11]. Also, bioremediation is a versatile alternative to physicochemical treatments[3, 5] and produces non-toxic end products such as CO2, water and methane from petroleum hydrocarbons (PHCs).

Biodegradable matter is generally organic material such as plant and animal matter and other substances originating from living organisms, or artificial materials that are similar enough to plant and animal matter to be put to use by microorganisms. Some microorganisms have a naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharma substances, pesticides, and metals.

Decomposition of biodegradable substances may include both biological and abiotic steps. Products that contain biodegradable matter and non-biodegradable matter are often marketed as biodegradable.

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.

FACTORS ASSOCIATED WITH 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

Environmental 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[33]

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.

CLASSIFICATION

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. This method is effective only when the subsurface soils are highly permeable, the soil horizon to be treated falls within a depth of 8-10 m and shallow groundwater is present at 10 m or less below ground surface. The depth of contamination plays an important role in determining whether or not an *in situ* bioremediation project should be employed. If the contamination is near the groundwater but the groundwater is not yet contaminated then it would be unwise to set up a hydrostatic system.

In situ bioremediation is a very site specific technology that involves establishing a hydrostatic gradient through the contaminated area by flooding it with water carrying nutrients and possibly organisms adapted to the contaminants. Water is continuously circulated through the site until it is determined to be clean.

The *in situ* treatment methods of contaminated soil include the following:

Bioventing - This process combines an increased oxygen supply with vapour extraction. A vacuum is applied at some depth in the contaminated soil which draws air down into the soil from holes drilled around the site and sweeps out any volatile organic compounds. The development and application of venting and bioventing for *in situ* removal of petroleum from soil have been shown to remediate approximately 800 kg of hydrocarbons by venting and approximately 572 kg by biodegradation[28].

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. In some instances air injections are replaced by pure oxygen to increase the degradation rates. However, in view of the high costs of this treatment in addition to the limitations in the amount of dissolved oxygen available for microorganisms, hydrogen peroxide (H_2O_2) was introduced as an alternative, and it was used on a number of sites to supply more oxygen. Each liter of commercially available H2O2 (30%) would produce more than 100 L of O2, and was more efficient in enhancing microbial activity during the bioremediation of contaminated soils and groundwaters[8, 18, 19].

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.

This process involves the introduction of preselected organisms to the site for the purpose of increasing the rate or extent, or both, of biodegradation of contaminants. It is usually done in conjunction with the development and monitoring of an ideal growth environment, in which the selected bacteria can live and work. The selected microorganisms must be carefully matched to the waste contamination present as well as the metabolites formed.

Effective seed organisms are characterized by their ability to degrade most petroleum components, genetic stability, and viability during storage, rapid growth following storage, a high degree of enzymatic activity and growth in the environment, ability to compete with indigenous microorganisms, nonpathogenicity and inability to produce toxic metabolites[4]

Mixed cultures have been most commonly used as inocula for seeding because of the relative ease with which microorganisms with different and complementary biodegradative capabilities can be isolated[2]. Different commercial cultures were reported to degrade petroleum hydrocarbons[9,10,20,22,23,29].

Biostimulation - This process involves the stimulation of indigenous microorganisms to degrade the contaminant. The microbial degradation of many pollutants in aquatic and soil environments is limited primarily by the availability of nutrients, such as nitrogen, phosphorus, and oxygen availability. The addition of nitrogen- and phosphorus-containing substrates has been shown to stimulate the indigenous microbial populations.

Zucchi *et al*.[35] while studying the hydrocarbon-degrading bacterial community in laboratory soil columns during a 72-day biostimulation treatment with a mineral nutrient and surfactant solution of an aged contamination of crude oil-polluted soil, found a 39.5% decrease of the total hydrocarbon content. The concentrations of available nitrogen and phosphorus in seawater have been reported to be severely limiting to microbial hydrocarbon degradation[1, 17]. The problem of nutrient limitations has been overcome by applying fertilizers[2,12,15,16,22,32] which, range from soluble and slow release agricultural fertilizers of varying formulations to specialized oleophilic nitrogen-and phosphorus-containing fertilizers for use in treating oil spills.

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[7].

This process allows for better control of the system by enabling the engineering firm to dictate the depth of soil well as the exposed surface area. As a consequence of the depth and exposed surface area of the soil being determined, one is able to better control the temperature, nutrient concentration, moisture content and oxygen availability.

Composting - Solid-phase treatment carried out after extraction. A process that treats soils in above-ground treatment areas equipped with collection systems to prevent any contaminant from escaping the treatment. This involves mixing contaminated materials with compost containing bioremediation organisms. The mixture incubates under aerobic and warm conditions. The resultant compost can be used as a soil augmentation or be placed in a sanitary landfill.

Moisture, heat, nutrients, or oxygen are controlled to enhance biodegradation for the application of this treatment and are relatively simple to operate and maintain, require a large amount of space, and cleanups require more time to complete than with slurry-phase processes. This treatment increases temperature to 65 ºC, as a result of microbial activity.

Landfarming - The use of farming tilling and soil amendment techniques to encourage the growth of bioremediation organisms in a contaminated area. It has been used successfully to remove large petroleum spills in soil. Contaminated soils are excavated and spread on a pad with a built-in system to collect any contaminated liquids that seep out of contaminant soaked soil. The soils are periodically turned over to mix air into the waste. Moisture and nutrients are controlled to enhance bioremediation.

Rate of degradation depends on: 1- Microbial population, 2-Type of contamination, 3- Level of contamination and 4- Soil type.

Soil Biophiles - Contaminated soil is piled in heaps within a lined area (to prevent leaching) several meters high over an air distribution system. Aeration is provided by pulling air through the heap with a vacuum pump. Moisture and nutrient levels are maintained at levels that maximize bioremediation.

The soil heaps can be placed in enclosures. Volatile contaminants are easily controlled since they are usually part of the air stream being pulled through the pile.

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.

Biofilters - Biofilter is a reactor containing inert support packing with a high surface area which supports an active microbial film. This film can be maintained by continuous supply of nutrients & high humidity. It has been used to remove volatile organic compounds (VOC's) from air. It helps in removal of organic gases by passing air through compost or soil containing microorganisms capable of degrading the gases.

DEVELOPMENTS OF PHYTOREMEDIATION

Phytoremediation is the use of green plants and their associated microorganisms, soil amendments, and agronomic practices to remove, contain or render harmless environment contaminants. Terrestrial, aquatic and wetland plants and algae can be used for the phytoremediation process under specific cases and conditions of hydrocarbon contamination[25, 27].

The first plant based remediation system was installed over 300 years ago in Germany for the treatment of municipal sewage, since then this use of plants has become rather common. But plants are also used to decontaminate soils polluted by organic wastes. Plants roots absorb organic in nearly direct proportion to their relative lipophilicity. Remediation may occur due to (1) accumulation of the organics in plant tissues, (2) translocation of the organics to leaf and volatilization from the leaf surfaces or (3) the organics may be metabolized in plant tissues or in the rhizosphere itself (by enzymes secreted by the plants)[6].

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. The health effects of arsenic include liver, lung, kidney and bladder cancers. 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[14].

TYPES OF PHYTOREMEDIATION

There are five types of phytoremediation techniques which are:

1. Phytoextraction

It is the process used by the plants to accumulate contaminants into the roots and above ground shoots or leaves. This technique saves tremendous remediation cost by accumulating low levels of contaminants from a widespread area. The 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 cleanup process. As this process is controlled by plant, so it takes more time than any traditional soil cleanup process. Some of the plants responsible are *Viola baoshanensis, Sedum alfredii, Rumex crispus*[21,34].

2. Phytotransformation

It 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 non carcinogenic form[24].

3. Phytodegradation

It 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[26].

4. Phytostabilization

It refers to the 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. Phytostabilization is especially applicable for metal contaminants at waste sites where the best alternative is often to hold contaminants in place. Metals do not ultimately degrade, so capturing them *in situ* is the best alternative at sites with low contamination levels (below risk thresholds) or vast contaminated areas where a large-scale removal action or other *in situ* remediation is not feasible[30].

5. Rhizofiltration

Rhizofiltration is a type of phytoremediation, which refers to the approach of using hydroponically cultivated plant roots to remediate contaminated water through absorption, concentration, and precipitation of pollutants. It also filters through water and dirt. This process is very similar to phytoextraction in that it removes contaminants by trapping them into harvestable plant biomass. Both phytoextraction and rhizofiltration follow the same basic path to remediation. 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. 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[13,31].

LIMITATION OF RHIZOFILTRATION

- The pH of the influent solution may have to be continually adjusted to obtain optimum metals uptake.
- The chemical speciation and interaction of all species in the influent have to be understood for proper application.
- A well-engineered system is required to control influent concentration and flow rate.
- Plants (especially terrestrial plants) may have to be grown in a greenhouse or nursery and then placed in the rhizofiltration system.
- Periodic harvesting and plant disposal are required.
- Metal immobilization and uptake results from laboratory and greenhouse studies might not be achievable in the field

ADVANTAGES OF BIOREMEDIATION TECHNIQUES

The use of intrinsic or engineered bioremediation processes offers several potential advantages that are attractive to site owners, regulatory agencies, and the public. These include:

- Lower cost than conventional technologies.
- Contaminants usually converted to innocuous products.
- Contaminants are destroyed, not simply transferred to different environmental media.
- Nonintrusive, potentially allowing for continued site use.
- Relative ease of implementation.

DISADVANTAGES OF BIOREMEDIATION TECHNIQUES

There are potential disadvantages to bioremediation as well, these include:

- May be difficult to control.
- Amendments introduced into the environment to enhance bioremediation may cause other.
- Contamination problems.
- May not reduce concentration of contaminants to required levels.
- Requires more time.
- May require more extensive monitoring.
- Lack of (hydraulic) control.
- Dynamic process, difficult to predict future effectiveness

DISCUSSION AND CONCLUSION

Increased population, industrialization and urbanization are responsible for environmental contamination. Environmental decontamination is an enigma. However, advances in science and technology enabled us to apply the potential of biological diversity for pollution abatement which is termed as Bioremediation. This is emerging as an effective innovative technology for treatment of a wide variety of contaminants. Bioremediation approach is currently applied to contain contaminants in soil, groundwater, surface water, and sediments including air. These technologies have become attractive alternatives to conventional cleanup technologies due to relatively low capital costs and their inherently aesthetic nature.

Microbial diversity and its diverse metabolic activities are of critical importance to the sustainability of life on our planet, including recycling elements on which primary productivity depends, producing and consuming gases important for maintaining our climate, and destroying the wastes of human civilization. Besides that, microbes often play key roles in conservation of higher organisms and in restoration of degraded ecosystems. Hence, microbial diversity goes hand in hand with goals for maintenance of higher organism diversity and ecosystem management. The scope of environmental bioremediation extends to: Inorganics viz., Arsenic, Mercury, Chromium, Fluoride, Cyanide, abandoned mines, fl y ash disposed sites, engineered phytotreatment technologies, biological permeable barriers; and Organics viz., petroleum hydrocarbons, pesticides and explosives. Quite a variety of plants, natural, transgenic, and/ or associated with rhizosphere micro-organisms are extraordinarily active in these biological interventions and in cleaning up pollutants by removing or immobilizing them.

As new bioremediation techniques are brought from the lab into commercial practice, the importance of sound methods for evaluating bioremediation will increase. Therefore, the application of bioremediation technology in the restoration of ecosystem and soil management is used less compared to Europe and USA. Hence, we need extensive research programs to increase the capabilities of bioremediation to deep, extensive, subsurface contamination due to chlorinated hydrocarbons and complex mixed wastes, including soils and groundwater

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