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Review Article

DECONTAMINATION OF POLLUTED WATER EMPLOYING BIOREMEDIATION PROCESSES: A REVIEW

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Bioremediation is an ecologically sound and state-of-the-art technique that employs natural biological processes to completely eliminate toxic contaminants. Any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Bioremediation technologies can be generally classified as *in situ* or *ex situ*. In situ bioremediation involves treating the contaminated material at the site while ex situ involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation technologies are bioventing, land farming, bioreactor, compositing, bioaugmentation, rhizofiltration, and bio-stimulation. Microorganisms which perform the function of bioremediation is known as bioremediators. Not all contaminants, however, are easily treated by bioremediation using microorganisms. For example, heavy metals such as cadmium (Cd) and lead (Pb) are not easily absorbed or captured by organisms. The assimilation of metals such as mercury into the food chain may worsen matters. This manuscript gives an idea of what is bioremediation, principles of bioremediation, factors of bioremediation strategies, types, genetic engineering approaches, monitoring bioremediation and advantages or disadvantages of bioremediation.

Keywords: Groundwater, Hazardous substances, Landfill, Monitoring, Recalcitrant molecules, Methylotrophs, SDS, Bioventing, Biopiles, Bioreactors

INTRODUCTION

Enormous quantities of organic and inorganic compounds are released into the environment each year as a result of human activities. In some cases, these releases are deliberate and well regulated (e.g., industrial emissions) while in other cases they are accidental (e.g., chemical or oil spills). Many of these compounds are both toxic and persistent in terrestrial and aquatic environments. The contamination of soil, surface

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and groundwater is simply the result of the accumulation of these toxic compounds in excess of permissible levels. The quality of life on earth is linked inextricably to overall quality of the environment. In early times, we believed that we had an unlimited abundance of land and resources; today, (Rani *et al.*, 2010) 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. The problem is worldwide, and the estimated number of contaminated sites is significant. (Cairney, 1993). It is now well recognized that contaminated land is a potential threat to human health, and is continual discovery over recent years has led to international efforts to remedy many of these sites, either as response to the risk of adverse health on environmental effects caused by contamination or to enable the site to be redeveloped for use (Kensa, 2011).

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 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 in reducing levels of a range of contaminants, but have several drawbacks, principally their technologies complexity, the cost for small-scale application, and the lack of public acceptance, especially for incineration that may increase the exposure to contaminants that may increase the exposure to contaminants for both the workers at the site and nearby residents.

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 same 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. Because bioremediation seems to be a good alternative to conventional clean-up technologies research in this field, especially in the United States is rapidly increasing.

Bioremediation has been used at a number of sites worldwide, including Europe, with varying degrees of success. Techniques are improving as greater knowledge and experience are gained, and there is no dealing with certain types of site contamination. Unfortunately , the principles, techniques, advantages and disadvantages of bioremediation re not widely known or understood, especially among those who will have to deal directly with bioremediation proposals, such as site owners and regulators. Here, we intended to assist by providing a straightforward, bioremediation, the *pros* and *cons* of the technique, and the issues to be considered when dealing with a proposal for bioremediation.

PRINCIPLES OF BIOREMEDIATION

Environmental biotechnology is not a new field; composting and wastewater treatments are familiar examples of old environmental biotechnologies. However, recent studies in molecular biology and ecology offer opportunities for more efficient biological processes. Notable accomplishments of these studies include the clean-up of polluted water and land areas. 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 (Mueller, 1996). By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants (Rani *et al*., 2007) to degrade or detoxify substances hazardous to human health and/or environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated sites. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. 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 we have process known as bioaugmentation.

Foe bioaugmentation to be effective, microorganisms must enzymatically attack the pollutant and convert them to harmless products. A 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. Like other technologies, bioremediation has its limitations. Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, hence it is not easy to predict the rates of clean-up for a remediation; there are no rules to predict if a contaminant can be degraded. 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 result of transportation accidents. Since bioremediation is based on natural attenuation the public considers it more accepted than other technologies. Most remediation systems are run under aerobic conditions, but running a system under anaerobic conditions (Colberg, 1995) may permit microbial organisms to degrade otherwise recalcitrant molecules.

FACTORS FOR REMEDIATION

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).

MICROBIAL POPULATION FOR BIOREMEDIATION PROCESSES

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 excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or any waste stream. The main requirements are an energy source and a carbon source. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards. We can subdivide these microorganisms in Table 1.

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 amd polyaromatic 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.

Table 1: Some Contaminants Potentially Suitable for Bioremediation

Class of Contaminants	Specific Examples	Aerobic	Anaerobic	More Potential Sources
Chlorinated solvents	Trychloroethylene Perchloroethylene		+	Drycleaners
Polychlorinated biphenyls	4-Chlorobiphenyl4, 4-Dichlorobiphenyl		+	Electrical manufacturing Power station
Chlorinated phenol 'BTEX'	Pentachlorophenol Benzene, Toluene, Ethylbenzene, Xylene	+	++	Timber treatment Landfills, Oil production and storage gas work sites, Airport Paint manufacture, Port facilities, Railway yards, Chemical manufacture
Polyarmatic hydrocarbons (PAHs)	Naphthalene, Anthracene Bezopyrene	+		Oil production and storage Gas work sites, Coke plant Engine works landfill
Pesticides	Atrazine Carbaryl Carbofuran Diazine Glycophosphate Parathion Propham 2,4-D	+	+	Agriculture Timber treatment plants, Pesticide manufactureRecreational areas Landfills

Liginolytic Fungi: Fungi such as white rot fungus *Phanaerochaete chrysoporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental toxicants. 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 anaerobic 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. 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 toward it. Other microbes such as fungi grow in a filamentous form toward the contaminant. It is possible to enhance the mobilization of the contaminant utilizing some surfactants such as sodium dodesyl sulphate (SDS)*.

ENVIRONMENTAL FACTORS

Nutrients: Although the microorganisms are present in contaminated soil, they cannot necessarily be there in the numbers required for bioremediation of the site. Their growth and activity must be stimulated. Biostimulation 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 necessary enzymes to break down the contaminants. All of them will need nitrogen, phosphorus and carbon (Table 2). Carbon is the most basic element of living forms and is needed in greater quantities than other elements. In addition to hydrogen, oxygen and nitrogen it constitutes about 95% of the weight of cells. Phosphorus and sulphur contribute with 70% of the remainders. The nutritional requirement of carbon to nitrogen ratio is 10:1 and carbon to phosphorus 30:1.

ENVIRONMENTAL REQUIREMENTS

Optimum environmental conditions for the degradation of contaminants are reported in Table 3.

Table 2: Composition of a Microbial Cell

Element	Percentage	Element	Percentage
Carbon (C)	15	Sodium (Na)	1
Nitrogen (N)	14	Calcium (Ca)	0.5
Oxygen (O)	20	Magnesium (Mg)	0.5
Hydrogen (H)	8	Chloride (Cl)	0,5
Phosphorus (P)	3	Iron (Fe)	0.2
Sulphur (S)	1	All others	0.3
Potassium (K)	1		

Microbial growth and activity are readily affected by pH, temperature and moisture. Although microorganisms have been also isolated in extreme condition, most of them grow optimally over a narrow range, so that it is important to achieve optimal conditions. If the soil has too much acid it is possible to rinse the pH by adding lime. Temperature affects bio-chemical reactions rates, and the rates of many of them double for each 10ºC rise in temperature. Above a certain temperature however, the cells die. Plastic covering can be used to enhance solar warming in the late spring, summer and autumn. Available water is essential for all the living organisms and irrigation is needed to achieve the optimum moisture level. Soil structure controls the effective delivery of air, water, and nutrients. To improve soil structure, materials such as gypsum or organic matter can be applied. Low soil permeability can impede movement of water, nutrients, and oxygen; hence, soil with low permeability may not be appropriate for *in situ* clean-up techniques.

Basic Types of Bioremediation Techniques

Biostimulation provides nutrients and suitable physiological conditions for the growth of the indigenous microbial populations. This promotes increased metabolic activity, which then degrades the contaminants. Bioaugmentation means introduction of specific blends of laboratory-cultivated microorganisms into a contaminated environment or into a bioreactor to initiate the bioremediation process. The process of developing bioremediation techniques may involve the following steps:

a) Isolating and characterizing naturally-occurring microorganisms with bioremediation potential

b) Laboratory cultivation to develop viable populations

c) Studying the catabolic activity of these microorganisms in contaminated material through bench scale experiments

d) 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 micro-organisms and nutrients and the optimization of environmental factors at the contaminated site itself (2) Ex-situ restoration of contaminated material in specifically designated areas by land-forming and composting methods.

Table 3: Environmental Conditions Affected Degradation

Parameters	Condition Required for Microbial Activity	Optimum Value for an Oil Degradation
Soil moisture	25-28% of water holding capacity	30-90%
Soil pH	5.5-8.8	6.5-8.0
Oxygen content	Aerobic. Minimum air-filled pore space of 10%	10-40%
Nutrient content	N & P for microbial growth	C:N:P = 100:10:1
Temperature ^{0}C	15-45	20-30
Contaminants	Not too toxic	Hydrocarbon 5-10% of dry weight of soil
Heavy metals	Total content 2000ppm	700ppm
Type of soil	Low clay or silt content	

BIOREMEDIATION STRATEGIES

Different techniques are employed depending on the degree of saturation and aeration of an area. In situ techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. Ex situ techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation (soil) or pumping (water). Bio-augmentation techniques involve the addition of microorganisms with the ability to degrade pollutants. It frequently involves the addition of microorganisms indigenous or exogenous to contaminated sites. Two factors limit the use of added microbial cultures in a land treatment unit1) non-indigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and 2) most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degrades if the land treatment unit is well managed.

In Situ Bioremediation: These techniques are generally the most desirable options due to lower cost and fewer disturbances since they provide the treatment in place avoiding exaction and transport of contaminants. In situ treatment is limited by the depth of the soil that can be effectively treated in some cases. The most important land treatments are:

Bioventing is the most common in situ treatment and involves supplying nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface. In situ biodegradation involves supplying oxygen and nutrients by circulation aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. It can be used for soil and groundwater. Generally, this technique includes conditions such as the infiltration of water-containing nutrients and oxygen or other electron acceptors for groundwater treatment.

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 installing small-diameter air injection points allows considerable flexibility in the design and construction of the system.

***Ex Situ* Bioremediation:** This technique involves the excavation or removal of contaminated soil from ground. Land farming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative micro-organisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10-35 cm in soil. Since land farming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attention as a disposal alternative.

Composting is a technique that involves combining contaminated soil with nonhazardous organic amendments such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristics of composting.

Biopiles are a hybrid of land farming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of land farming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic microorganisms.

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 mat 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 microorganism) capable of degrading target contaminants. In general, the rate and extent of biodegradable 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 predicable. Despite the advantages of reactors systems, there are some disadvantages. The contaminated soils require pre treatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction (e.g., vacuum extraction) before being placed in a bioreactor.

Genetic Engineering Approaches: The use of genetic engineering to create organisms specifically designed for bioremediation has great potential. The bacterium *Deinococcus radiodurans* (the most radioresistant organism known) has been modified to consume and digest toluene and ionic mercury from highly radioactive nuclear waste. Most commonly, the process is misunderstood. The microbes are ever-present in any given context generally referred to as "normal microbial flora". During bioremediation (biodegradation) processes, fertilizers/nutrients supplementation is introduced to the environments, in efforts to maximize growth and production potential. Common misbelieve is that microbes are transported and dispersed into an unadulterated environment.

Mycoremediation: Mycoremediation is a form of bioremediation in which fungi are used to decontaminate the area. The term mycoremediation was coined by Paul Stamets and refers specifically to the use of fungal mycelia in bioremediation. One of the primary roles of fungi in the ecosystem is decomposition, which is performed by mycelium. The mycelium secretes extra cellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fibre. These are organic compounds composed of long chain of carbon and hydrogen, structurally similar to many organic pollutants.

Monitoring Bioremediation: The process of bioremediation can be monitored indirectly by measuring the Oxidation Reduction Potential or redox in soil and groundwater, together with pH, temperature, oxygen content, electron receptor/ donor concentrations and concentration of breakdown products (e.g., CO_2) (Table 4).

Table 4: Reactions and Redox Potentials of Some Processes

Process	Reactions	Redox Potentials (Eh in mV)
Aerobic	$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$	600 ~400
Anaerobic		
Denitrification	$2NO_3^- + 10e^- + 12 H^+ \rightarrow N_2 + 6H_2O$	500 ~ 200
Manganese IV reduction	$MnO_2 + 2e^- + 4H^+ \rightarrow Mn^{2+} + 2H_2O$	400 ~ 200
Iron III reduction	$Fe(OH)_3 + 3e^- + H^+ \rightarrow Fe^{2+} + 3H_2O$	300 ~ 100
Sulphate reduction	$SO_4^- + 8e^- + 10H^+ \rightarrow H_2S + 4H_2O$	0 ~ 150
Fermentation	$2CH_2O \rightarrow CO_2 + CH_4$	–150 ~ -220

ADVANTAGES OF BIOREMEDIATION

Bioremediation is natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminants 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 products and include carbon dioxide, water and cell biomass. Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material. Instead of transferring contaminants from one environmental medium to another, for example from land to water or air, the complete destruction of target pollutants is possible.

- Bioremediation can often be carried out on site, often without causing a major disruption or 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 can prove less expensive than other technologies that are used for clean-up of hazardous waste.

DISADVATAGES OF BIOREMEDIATION

Bioremediation is limited to those compounds that are biodegradable, not all compounds are susceptible to rapid and complete degradation (Table 5).

- 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 successes 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

Table 5: Advantages and Disadvantages of Bioremediation

Technology	Examples	Benefits	Limitations	Factors to Consider
In situ	Bioremediation Biosparging Bioventing Bioaugmentation	Most efficient Non-invasive Relatively passive Natural attenuation Process treats soil and water	Environmental constraints Extended treatment time difficulties	Biodegradative abilities of indigenous microorganism Presence of metals and other inorganics Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants
Ex situ	Land farming Composting Biopiles	Cost efficient Low cost, can be done on site	Space requirements Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation	As above
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation Kinetic optimized environmental parameters Enhances mass transfer effective use of inoculants and surfactants	Soil requires excavation Relatively high cost capital Relatively high operating cost Toxicity of amendments Toxic concentrations of contaminants	

contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids and gases.

- Bioremediation often takes longer time 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, and there are no acceptable endpoints for bioremediation treatments.

CONCLUSION

Bioremediation is far less expensive than other technologies that are often used to clean up hazardous waste. There are a number of cost or efficiency advantages to bioremediation which can be employed in areas that are inaccessible without excavation.

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