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Review Article

TO DECONTAMINATE WASTEWATER EMPLOYING BIOREMEDIATION TECHNOLOGIES

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ABSTRACT

Bioremediation is an ecologically sound and state-of-the-art technique that employs natural biological processes employing microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. To completely eliminate toxic contaminants occurring in sludges, and ground water contaminated with petroleum hydrocarbons, solvents, pesticides, wood preservatives, non-halogenated SVOCs, and BTEX and other organic chemicals, especially effective for remediating low level residual contamination in conjunction with source removal. Compared with other technologies, such as thermal desorption and incineration (which require excavation and heating), thermally enhanced recovery (which requires heating), chemical treatment (which may require relatively expensive chemical reagents), and *in situ* soil flushing (which may require further management of the flushing water), bioremediation may enjoy a cost advantage. Not all contaminants, however, are easily treated by bioremediation using microorganisms. For example, heavy metals such as Cd & Pb aren't easily absorbed or captured by organisms. The assimilation of metals such as Hg into the food chain may worsen matters. While bioremediation (nor any other remediation technology) can't degrade inorganic contaminants, can be used to change the valence state of inorganics and cause adsorption, immobilization onto soil particulates, precipitation, uptake, accumulation, and concentration of inorganics in micro or macroorganisms. These techniques, while still largely experimental, show considerable promise of stabilizing or removing inorganics from soil. This manuscript delineates the general processes of bioremediation within the soil environment, factors of bioremediation strategies, genetic engineering approaches, monitoring bioremediation, and further, the *pros* & *cons* of the technique, limitations and potential of both *ex situ* and *in situ* bioremediation as viable alternatives to conventional remediation are explained and addressed.

Keywords: Groundwater; Hazardous substances; Radionuclide; Phytoremediation; Recalcitrant molecules; Methylotrophs; SDS; Bioreactors; BTEX; VOC; PAH; SVOC; Heavy metals; Genetic engineering

1. 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 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 [1], 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. 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 [2].

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 US is rapidly increasing.

Three primary ingredients for bioremediation are: 1) presence of a contaminant, 2) an electron acceptor, and 3) presence of microorganisms that are capable of degrading the specific contaminant. Generally, a contaminant is more easily and quickly degraded if it is a naturally occurring compound in the environment, or chemically similar to a naturally occurring compound, because microorganisms capable of its biodegradation are more likely to have evolved. Petroleum hydrocarbons are naturally occurring chemicals; therefore, microorganisms which are capable of attenuating or degrading hydrocarbons exist in the environment. Development of biodegradation technologies of synthetic chemicals such DDT is dependent on outcomes of research that searches for natural or genetically improved strains of microorganisms to degrade such contaminants into less toxic forms. Microorganisms have limits of tolerance for particular environmental conditions, as well as optimal conditions for pinnacle performance. Factors that affect success and rate of microbial biodegradation are nutrient availability, moisture content, pH, and temperature of the soil matrix. Inorganic nutrients including, but not limited to, N & P are necessary for microbial activity and cell growth. It has been shown that treating petroleum-contaminated soil with nitrogen can increase cell growth rate, decrease the microbial lag phase, help to maintain microbial populations at high activity levels, and increase the rate of hydrocarbon degradation [4].

All soil microorganisms require moisture for cell growth and function. Availability of water affects diffusion of water and soluble nutrients into and out of microorganism cells. However, excess moisture, such as in saturated soil, is undesirable because it reduces the amount of available oxygen for aerobic respiration. Anaerobic respiration, which produces less energy for microorganisms (than aerobic respiration) and slows the rate of biodegradation, becomes the predominant process. Soil pH is important because most microbial species can survive only within a certain pH range. Furthermore, soil pH can affect availability of nutrients. Biodegradation of petroleum hydrocarbons is optimal at a pH 7 (neutral); the acceptable range is pH 6 – 8. Temperature influences rate of biodegradation by controlling rate of enzymatic reactions within microorganisms. Generally, speed of enzymatic reactions in the cell ~ doubles for each 10°C rise in temperature. There is an upper limit to the temperature that microorganisms can withstand. Most bacteria found in soil, including many bacteria that degrade petroleum hydrocarbons, are mesophiles which have an optimum temperature ranging from 25°C to 45°C. Thermophilic bacteria (those which survive and thrive at relatively high temperatures) which are normally found in hot springs and compost heaps exist indigenously in cool soil environments and can be activated to degrade hydrocarbons with an increase in temperature to 60°C. This finding suggested an intrinsic potential for natural attenuation in cool soils through thermally enhanced bioremediation techniques [5].

Contaminants can adsorb to soil particles, rendering some contaminants unavailable to microorganisms for biodegradation. Thus, in some circumstances, bioavailability of contaminants depends not only on the nature of the contaminant but also on soil type. Hydrophobic contaminants, like petroleum hydrocarbons, have low solubility in water and tend to adsorb strongly in soil with high organic matter content. In such cases, surfactants are utilized as part of the bioremediation process to increase solubility and mobility of these contaminants. Additional research findings of the existence of thermophilic bacteria in cool soil also suggest that high temperatures enhance the rate of biodegradation by increasing the bioavailability of contaminants. It is suggested that contaminants adsorbed to soil particles are mobilized and their solubility increased by high temperatures. If the challenges of bioremediation, particularly of *in situ* techniques, can be overcome, bioremediation has potential to provide a low cost, non-intrusive, natural method to render toxic substances in soil less harmful or harmless over time.. On a broader scope, much research has been and continues to be developed enhance understanding of the essence of microbial behavior as microbes interact with various toxic contaminants. Additional research continues to evaluate conditions for successful introduction of exogenic and genetically engineered microbes into a contaminated environment, and how to translate success in the laboratory to success in the field [6].

2. APPROACH OF BIOREMEDIATION

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. According to the EPA, bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances". 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). Bioremediation may occur on its own (natural attenuation or intrinsic bioremediation) or may only effectively occur through the addition of fertilizers, oxygen, etc., that help encourage the growth of the pollution-eating microbes within the medium (biostimulation). Depleted soil nitrogen status may encourage biodegradation of some nitrogenous organic chemicals, and soil materials with a high capacity to adsorb pollutants may slow down biodegradation owing to limited bioavailability of the chemicals to microbes [6-8].

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. However, not all contaminants are easily treated by bioremediation using microorganisms. For example, heavy metals such as Cd & Pb are not readily absorbed or captured by microorganisms. A recent experiment, however, suggests that fish bones have some success absorbing lead from contaminated soil. Bone char has been shown to bioremediate small amounts of Cd, Cu & Zn. The assimilation of metals such as mercury into the food chain may worsen matters. Phytoremediation[#] is useful in these circumstances because natural plants or transgenic plants are able to bioaccumulate these toxins in their above-ground parts, which are then harvested for removal. The heavy metals in the harvested biomass may be further concentrated by incineration or even recycled for industrial use[9]. Some damaged artifacts at museums contain microbes which could be specified as bio remediating agents. The elimination of a wide range of pollutants and wastes from the environment requires increasing our understanding of the relative importance of different pathways and regulatory networks to carbon flux in particular environments and for particular compounds, and they will certainly accelerate the development of bioremediation technologies and biotransformation processes [10].

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 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 [11]. For 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 [12].

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 may permit microbial organisms to degrade otherwise recalcitrant molecules [13].

3. INIMITABILITY 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) [14].

4. 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 [15-17]. We can subdivide these microorganisms in Table 1.

Aerobic

Number of bacteria viz. *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus,* and *Mycobacterium are e*xamples of aerobic bacteria recognized for their degradative abilities. 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

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-Chlorobiphenyl 4,4-Dichlorobiphenyl		+	Electrical manufacturing Power station
Chlorinated phenol	Pentachlorophenol		+	Timber treatment Landfills, Oil production and storage gas work sites, Airport Paint manufacture, Port facilities, Railway yards, Chemical manufacture
'BTEX'*	Benzene, Toluene, Ethylbenzene, Xylene	+	+	
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 manufacture Recreational 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)□.

5. ENVIRONMENTAL REQUIREMENTS

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 10°C 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 [18]. 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. Although the microorganisms are present in contaminated soil, they can't 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 C, N & P (Table 2). C is the most basic element of living forms and is needed in greater quantities than other elements. In addition to H, O & N, it constitutes about 95% of the weight of cells. P & S contribute with 70% of the remainders [19-21]. The nutritional requirement of C to N is 10:1 and C to P is 30:1.

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		

6. 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. Optimum environmental conditions for the degradation of contaminants are reported in Table 3. The process of developing bioremediation techniques may involve the following steps [22]:

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 microorganisms 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 ºC	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	

7. 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). Bioaugmentation 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) nonindigenous 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 is 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 [23-29].

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 [30].

Ex situ bioremediation: This technique involves the excavation or removal of contaminated soil from ground. Landfarming 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 microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10-35 cm in soil. Since landfarming has the potential ti 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 landfarming and composting [31-35].

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 landfarming 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 [36-38].

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. *Micoremediation* is a form of bioremediation in which fungi are used to decontaminate the area. 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 [39-41].

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

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

Table 5: Pros & Cons 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 & other inorganics Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants
Ex situ	Landfarming 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

8. 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.

9. 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 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.

10. CONCLUSION

The emerging of recent studies in molecular biology and ecology offers opportunities for more efficient biological processes to detoxify contaminants. Notable accomplishments of these studies include the clean-up of polluted water and land areas. Bioremediation is far less expensive than other technologies that are often used to clean up hazardous waste. Bioremediation technology exploits various naturally occurring mitigation processes: natural attenuation, biostimulation, and bioaugmentation. Bioremediation which occurs without human intervention other than monitoring is often called natural attenuation. This natural attenuation relies on natural conditions and behavior of soil microorganisms that are indigenous to soil. Biostimulation also utilizes indigenous microbial populations to remediate contaminated soils. Biostimulation consists of adding nutrients and other substances to soil to catalyze natural attenuation processes. Bioaugmentation involves introduction of exogenic microorganisms (sourced from outside the soil environment) capable of detoxifying a particular contaminant, sometimes employing genetically altered microorganisms. There are a number of cost or efficiency advantages to bioremediation which can be employed in areas that are inaccessible without excavation. 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 is to be explored with advanced innovations.

***BTEX** is the term used for benzene, toluene, ethylbenzene, & xylene- **(VMAOCs)** volatile, monocyclic aromatic compounds present in coal tar, petroleum products, and various organic chemical product formulations. These are the most soluble of the major gasoline compounds and, therefore, are common indicators of gasoline contamination.

#Phytoremediation consists of mitigating 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. Phytoremediation may be applied wherever the soil or static water environment has become polluted or is suffering ongoing chronic pollution. Examples where phytoremediation has been used successfully include the restoration of abandoned metal mine workings, reducing the impact of contaminants in soils, water, or air. Contaminants such as metals, pesticides, solvents, explosives, and crude oil and its derivatives, have been mitigated in phytoremediation projects worldwide. Many plants such as mustard plants, alpine pennycress, hemp, and pigweed have proven to be successful at hyperaccumulating contaminants at toxic waste sites. Over the past 20 years, this technology has become increasingly popular and has been employed at sites with soils contaminated with lead, uranium, and arsenic. While it has the advantage that environmental concerns may be treated in situ; one major disadvantage of phytoremediation is that it requires a long-term commitment, as the process is dependent on a plant's ability to grow and thrive in an environment that is not ideal for normal plant growth. Phytoremediation may be applied wherever the soil or static water environment has become polluted or is suffering ongoing chronic pollution. Examples where phytoremediation has been used successfully include the restoration of abandoned metal-mine workings, reducing the impact of sites where polychlorinated biphenyls have been dumped during manufacture and mitigation of ongoing coal mine discharges. Phytoremediation refers to the natural ability of certain plants called hyperaccumulators to bioaccumulate, degrade, or render harmless contaminants in soils, water, or air. Breeding programs and genetic engineering are powerful methods for enhancing natural phytoremediation capabilities, or for introducing new capabilities into plants. Genes for phytoremediation may originate from a micro-organism or may be transferred from one plant to another variety better adapted to the environmental conditions at the cleanup site. For example, genes encoding a nitroreductase from a bacterium were inserted into tobacco and showed faster removal of TNT and enhanced resistance to the toxic effects of TNT. Researchers have also discovered a mechanism in plants that allows them to grow even when the pollution concentration in the soil is lethal for non-treated plants. Some natural, biodegradable compounds, such as exogenous polyamines, allow the plants to tolerate concentrations of pollutants 500 times higher than untreated plants, and to absorb more pollutants.

11. REFERENCES

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