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Bioremediation techniques and strategies on removal of polluted environment

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Abstract
By reason of human activities are more greater content than natural process, many organic chemical substances such as pesticides, halogenated and nitroaromatic compounds, petroleum hydrocarbons, phthalate esters, solvents pollute the aquatic and soil environments. Bioremediation is known as one of the cleaner, safer, environmental friendly technology and cost effective for cleaning up sites that are contaminated by various pollutants. The bioremediation process uses different agents such as bacteria, fungi, yeast, algae and plants for treating environment included heavy metal, organic and inorganic compound. The objective of this paper is to enlarge a better understanding of the strategies and techniques for the bioremediation. Besides the paper addresses the main features of the following issue and techniques: comparing with conventional remediation techniques, advantageous, disadvantageous and limitations of bioremediations, principles of bioremediation, bioremediation system and process (*In situ* and *Ex situ* remediation techqniques), phytoremediation, electrobioremediation, bioremediation organisms, using biomolecular engineering techniques for bioremediation.

Keywords : Bioremediation; polluted soil; electrobioremediation; phytoremediation; phycoremediation.

1.Introduction

Our planet 'Earth' has unique natural resources like soil, air, water, plant, animals and forests. The result of advent civilization and industrialization, overusing and misusing various natural resources has led to decrease efficiency and consumption of them. There are various reasons for depletion of wildlife and natural habitat like the use of chemical fertilizers, herbicides and pesticides in agriculture, industrial and anthropogenic activities. Due to the increase in urbanization, industrilization and population, enviromental contamination has been increased for recent century. The application of biotreatment is increasing rapidly to investigate the treatment of contaminated areas.

The conventional techniques used for remediation is digged up contaminated soil and remove it to a different area or covers the contamined sites. These methods may be risks in the excavation, handling, and transport hazardous material. Besides, methods such as incineration, excavation, landfilling and storage are very expensive, difficult to find new landfill sites for the final disposal of material, and inefficient [1, 2]. To overcome these drawbacks, "Bioremediation" is defined as the process by way of various microorganisms to degrade the environmental contaminants into less toxic forms, is a significant option. Compared with conventional treatment, the main advantages of bioremediation: it uses relatively low-cost, high efficiency, no additional nutrient requirement, site distruption is minimal it can often be done on site and has greater public acceptance. However, Bioremediation has also its limitations such as limiting their availability to microorganisms, some chemicals (heavy metals, some chlorinated compounds, radionuclides) are not amenable to biodegredation and low water solubility of the contaminants. Research in Bioremediation field rapidly increasing because of it seems to be a good alternative to conventional methods. Bioremediation studies on the increase in a number of sites worlwide especially United States and Europe. Using techniques, knowledge and experience are gained are improving, therefore bioremediation applications have great important to remove particular types of contamination recently. Unfortunately, the techniques, principles, advantages and disadvantages of bioremediation are not truly known and understood.

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Table 1. Soil and underground water bioremediation techniques

Contaminants	Technology	Description
CHC, BTEX	Bioventing	It accelerates the eliminating process of volatile organic compounds and induces *In situ* bioremediation
CHC, BTEX, PAH, MTBE	Air sparging (AIS)	It physically removes semi-volatile and volatile compounds and induces *In situ* bioremediation
CHC, BTEX, PAH, MTBE	Bio sparging	It accelerates the organic compounds biodegradation by helping native microflora within physical venting pocess.
CHC, BTEX, PAH,	Phytoremediation	Suitable plants have ability to degrade organic compounds and removal heavy metal are used.

Note: *CHC*: Chlorinated Hydrocarbon Compounds; *BTEX*: Petrol Hydrocarbons (Benzene, toluene, ethylbenzene, xylenes); *MTBE*: Methyl tert-butyl eter *PAH*: Polycyclic Aromatic Hydrocarbon.

2. Principles of bioremediation

Environmental biotechnology has been used for many centuries. Because of advancement of molecular biology and biotechnology, it can be used more efficient biological processes. The efficiency of cleaning up polluted water and land areas is getting increase as important achievement of these studies. Bioremediation is defined as organic wastes are biologically degraded under appropriate conditions. Bioremediation uses primarily microorganisms or microbial process to degrade and transform environmental contaminants into harmless and less toxic forms. İt uses bacteria, fungi and plants obtained from natural habitat. Microorganisms produce enzymes which are responsible attack the pollutants and convert them into harmless products. Environmental conditions must be appropriate for microbial growth and activity for bioremediation can be effective.

3. Bioremediation system and process

Bioremediation Technologies can be mainly classified as *ex situ* or in *situ*. *Ex situ* remediation techniques which involve the physical removal of the contaminated material for the treatment process. *Ex situ* remediation includes techniques such as land farming, biopiling, and processing by bioreactors during chemical, physical and thermal processes [3]. In contrast, in situ remediation include treatment of the contaminated material in place. Ex situ biological processes include: landfarming, composting, biopiling and the use of bioreactors. Several soil and underground water bioremediation techniques are shown in Table 1. [4].

Composting include in excavating the soil and then mixing with a bulking agent; to make it easier to deliver the optimum levels of air and water to the microorganisms such as hay, wood, corncobs and vegetative waste with the contaminated soil. After this process, the soil is covered to maintain optimum conditions for bacterial growth and to protect it from erosion. Composting is known to have faster remove contamination (approximately only a few weeks)and most effective for removing PAH, TNT and RDX [5, 6]. A brief of bioremediation strategies are used for the several charactheristic hazardous wastes is shown Table 2. [7].

Table 2. A brief of bioremediation strategies are used for the several charactheristic hazardous wastes (Adapted Brar, S. 2006)

Hazardous wastes	Examples	Bioremediation Strategies
Hydrocarbons	Dibenzothiophene Toluene Phenantherene Carbazole Octane etc.	Surfactans Fungi Composting Plants Fertilizers
Halogenated organic compounds	Carbontetrachloride Tetrachloroethylene Trichloroethylene PCBs DDT etc.	Aeration Nutrient application Bioaugmantation Phytoremediation
Metals	Iron Lead Chromium Mercury Copper etc.	Injection wells Constructed wetlands Phytoremediation

Land farming is a simple technique that involves the excavation of the contaminated soil and spreading it on thin surface and periodically tilled until pollutants are degraded. The supporting the growth of indigenous microorganisms, minerals and nutrients are also added. Landfarming has taken attention as a disposal technique because it reduces maintenance costs such as clean-up liabilities.

Biopiles methods are reorganized both landfarming and composting. It usually used for remove contamination petroleum hydrocarbons as controlling losses of the contaminant by volatilization and leaching. Biopiles involve a suitable environment for endemic aerobic and anaerobic microorganisms [8].

In situ Bioremediation is the technique cleaning up hazardous chemicals as a means of biological treatment in place avoiding excavation and transport of contaminant. This treatment is required that the soil can be effectively treated to diffuse oxygen throughout the soil. The most common land treatments are bioventing, biosparging, bioaugmentation and biopiling.

Bioventing involves nutrients and supplying air to stimulate the indigenous bacteria with the help of wells through the contaminated soil. Bioventing uses low oxygen flow necessary for the biodegradation. It degrades adsorbed fuel residues, simple hydrocarbons and volatile compounds where the contamination is under the surface [9].

In biosparging, air (oxygen) and nutrients (if needed) are injected to increase groundwater oxygen concentrations and biological activity of naturally occurring bacteria. This technique is an in situ remediation technology that uses indigenous microorganisms to biodegrade organic compounds like petroleum constituents.

Bioaugmentation is inoculated with indigenous and exogenous strains known to be capable of degrading the contaminants site to increase the active enzyme concentration to treat contaminated soil and water. It is used at sites soil and groundwater are contaminated with chlorinated ethenes, such as tetrachloroethylene and trichloroethylene. The chlorinated compounds are completly degraded to ethylene and chloride by *in situ* microorganisms. Bioreactors are used *ex situ* treatment of contaminated soil and water pumped up from contaminated plume. Bioreactors as well as slurry or aqueous reactors involves the processing of contaminated solid material or water. The aim of the solid phase treatment process is provided that the water, air, nutrients, pollutants and microorganisms are in persistent interaction. It is controlled environmental factors such that pH, temperature, air, rinsing and shaking rate for optimum growth microorganisms and biodegradetion by means of bioreactors. After the treatment, the slurry must be washed and then the water treated with standard wastewater techniques [10].

4. Advantages and disadvantages of bioremediation

The bioremediation methods depend on having the right organisms in the right ground with the environmental factors for optimum removal pollutant. The right organisms to degrade the pollutants efficiently are bacteria, fungi, and plants. Bioremediation provides several advantages compared with conventional techniques like that land filling or incineration. Bioremediation perceived by the public, because it is a natural process and acceptable waste treatment process. The residues are formed by a bioremediation procees usually harmless and include such water, and carbon dioxide. Bioremediationis often less expensive and site disruption is minimal and can be done on site.

Bioremediation has also its limitations. All compounds are not amenable and complete degradation, as an example, some chlorinated compounds, heavy metals, and radionuclides. Biological process requires the presence of metabolically capable microbial population, appropriate amount of nutrients and contaminants, and suitable environmental growth conditions.

Bioremediation takes longer than conventional treatments. Bioremediation is a scientifically intensive procedure, therefore some of the questions can be answered before biremediation process: Whether contaminant is biodegradable, biodegredation is occurring in the site naturally, environmental conditions are appropriate for biodegredation. As a consequence well educated scientific people are required to answer these questions.

5. Phytoremediation

Plants act as filters and metabolize substances in the natural ecosystem. Phytoremediation is a coming up technology that uses green plants and their biomass to degraded or remove contaminants from soil and water. Phytoremediation displays potential for accumulating, immobilizing, and rendering harmless environmental contaminants. Phytoremediation has several advantages such as acquiring energy mainly from sunlight, preserving the natural properties of soil and carrying on high levels of microbial biomass [11, 12]. There are approximately 400 plant species act as hyperaccumulator plants include the major families, for instance *Asteracea, Fabacea, Lamiacea* and *Brassicaea* showed the potential of accumulating Co, Cu, Cd/Zn, and Se [13].There are five types of phytoremidation techniques, classified based on the contaminant removal type: Phytodegradation, phytostabilization, phytotrans - formation, phytoextraction, and rhizofiltration. There are two approaches for phytoremediation mechanism: (1) organic pollutants can be taken up directly by plants and are degraded inside plants, (2) enzymes are secreted by plant can degrade organic pollutant [14, 15]. Phytoextraction or phytoaccumulation is the technique accumulating contaminants into the roots or leaves of plants. This process provides high efficiency and low remediation cost. Phytotransformation, also called phytodegradation, is the breakdown of organic contaminants taken up by plant through metabolic processes within the plant. Phytostabilization is the using exact plant species to reduce the mobility and prevent migration to the groundwater or air.

Rhizodegradation is the breakdown of contaminant in the rhizoshpere (soil surrounding the roots of plants) through microbial activity using microorganisms such that (bacteria, fungi, or yeast). Rhizodegradation is a symbiotic relationship that has acquired between microbes and plants. Microbes provide an appropriate soil environment, while the plants provide nutrients necessary for the microbes to flourish. Rhizofiltration is the absorbtion or precipitation onto plant roots (or absorbtion into the roots) of contaminants and also is a water remediation technique. Phytoremediation has considerable commer-cialization in the United States and Europe. This process is limited as a soil contaminant should not surpass an exact depth so that the roots of plant are in contact with the metal pollutants. Decontaminating a site takes a long time so it could be harvest several times [16].

6. Electrobioremediation

Electrobioremediation is a hybrid technology of biremediation and elektrokinetics for the treatments of hydrophobic organic compound. Elektrokinetics is one of the recently developed remediation technologies. Electrokinetics responsible for direct electric current to provide the degradation of soil contaminant and the removal of metals by direct movement of pollutants [17]. This process recently is used as one practical engineering technique for remediation of contaminated soils. Besides it has been increasing interest in using electrokinetic process ro removal contaminant with organic compound which has low degradabilit, low solubility, and low mobility [18]. The main phenomena that constitute electrokinetic remediation are electrolysis, electroosmosis, electrophoresis, electromigration, and diffusion. This direct current emerged from electrokinetic transport the pollutant over large distances and has not negative effect on the soil bacterium. This process produces and hydroxyl ions at the cathode hydrogen ions at the anode and so become pH gradient.

Electrophoresis and electromigration provide movement of ion complex, ions, and charged particles toward the oppositely charged electrode while electroosmosis provide movement of soil moisture toward the cathode. There are several studies combining electrokinetic process and biremediation but these studies has a little data on the influence of electric current on soil microorganism [19-23]. This is an important issue because in situ remediation relies on microbial activity and community. In a study, it has been achieved a successful combination of electrokinetics and bioremediation, soil parameters, elektric current, electrode, and electrolyte.

In addition to this, there are limitations requiring to overcome [24], mass transfer of electron acceptors and nutrients to microorganisms responsible for biodegradation [25], limited bioaccessibility of contaminants for biodegradation [26], the ratio between the target and nontarget ion concentrations, requirement of a conducting pore fluid to mobilize pollutants, toxic electrode effects on microbial metabolism [27], and adaptation of the indigenous microorganisms for biodegradation of a particular contaminant [28].

7. Bioremediation organisms

The microorganisms are known as bioremediators have been widely used to clean up environmental pollutant in contaminated sites containing a wide variety of hazardous wastes. The main requirements of the microorganisms are an energy source and a carbon source and they enables to remove the pollutants in the contaminated sites [29-31]. Some of these microorganisms are, *Streptomyces* [32] that can be able to retain trace elements from polluted waters, *Bacillus* [33] and *Pseudomonas* [34] to absorb metals. Some species used in bioremediation process are following, *Achromobacter, Alcaligenes*, *Arthrobacter, Bacillus, Cinetobacter, Corynebacterium, Flavobacterium, Micrococcus*, *Mycobacterium*, *Norcardia, Pseudomonas*, *Vibrio*, *Rhodococcus and Sphingomonas* [31, 35-39]. Yeasts are able to resist under inadequate environment and accumulate many metals and metaloids. Morover, They are known for dye decolourization of the food industry by means of biosorption, biodegradation, and bioaccumulation [40]. Some of yeasts are used this process are *Candida*, *Clavispora*, *Debaryomyces*, *Leucosporidium*, *Pichia*, *Rhodosporidium*, *Rhodotorula*, *Sporidiobolus*, *Sporobolomyces*, *Stephanoascus*, *Trichosporon* and *Yarrowia* [41].

Microorganisms are also used to remove various hazardous chemicals produced by the burning of fossil fuels and hydrocarbons. It is assumed that many microorganisms such as yeast, fungi, and bacteria has used petroleum materisl for energy and other biosynthesis methabolisms [42].

Algae and fungi are also excellent agent for remediation of wastewater and controlling metal concentrations of lakes and oceans. Using micro and macro algae for the degradation or biotransformation of cantaminant is named as Phycoremediation. Besedies, algae can accumulate or degrade toxic heavy metals and organic pollutants such as pesticides, hydrocarbons, biphenyls and phenolics for the contaminated environment [43]. Using fungal mycelium to decontaminate and filter the toxic material from contaminated sites is named mycoremediation. Some fungi species (*Phanaerochaete chrysosporium* and *Polyporus* sp.) show the ability to degrade a highly various permanent or hazardous environmental pollutants such as polycyclic aromatic hydrocarbons (PAHs), explosives, petroleum hydrocarbons, polychlorinated biphenyls(PCBs), and orgaochlorine pesticides [44-46].

8. Using biomolecular engineering techniques for bioremediation

Many microorganisms acquire the ability to transform, degrade, and chelate diverse hazardous pollutants. However, this process is relatively slow, genetically engineered microorganism (GEM) with enhanced capabilities can accelerate the progress of natural transformation ability. When confronted with heavy metals, Many microorganisms respond by producing metal binding peptides such as metallothionein (MTs), and phytochelatins (PCs). These peptides names as thiol-rich peptides bind to a different heavy metals and reduce their toxicity by separation [47]. These peptides extremely used to improve the metal accumulation ability of microorganism by producing them in a different cell location. Several limitations with MTs or PCs are suppling of the precursor glutathione (GSH), inefficient metal uptake, nonselective binding to a variety of heavy metals [48, 49].

The other major environmental issue is a radionuclide contamination result from nuclear plant leaks and nuclear weapons. Bacteria (such as *Deinococcus geothemalis and P. aeruginosa*) expose radiation in nature has extremely resistant to radiation so they are the best metabolic engineering candidates for removal radionuclide [50-52]. In agriculture, increasing the use of organophosphates (OPs) as pesticides has caused critical environmental pollution. OPs (parathion, paraoxon, or methyl parathion) mainly used insecticides are the ester forms of phosphoric acid. The bacteria in soil can degrade OPs by means of the enzyme as named organophosphate hydrolase (OPH or phosphotriesterase), so many researchers concentrate on OPs hydrolysis by OPH [53]. To improve the catalytic activity, substrat specificity of OPH and enhance biodetoxification efficiency genetic engineering techniques such as error prone PCR, DNA shuffling, or site-directed mutagenesis have been utilized. With applying these techniques OPH activity has increased 25-725 fold [54].

In recent years, rational design and directed evolution strategies have been used in bioremediation applications. The rational design approach for bioremediation mainly includes construction of a single microorganism has required enzymes or biodegradation ability. The using rational design to successfully modify an enzyme has provided very much information on the mechanistic, structural, and dynamic of the protein as will be used. Unlike rational design, directed evolution does not need a knowledge of protein structure and can describe the mutation affect enzyme activity. Directed evolution includes the constitution of a various library of gene variants by means of in vivo DNA shuffling, and random mutagenesis (such as gene recombination techniques or error prone PCR, in vitro staggered extension process (StEP) recombination) [55, 56].
Biomolecular engineering is successfully used to enhance the ability of enzyme or microorganism activity in bioremediation system. On the other hand, there are several challenges such as production of the desired enzyme has novel functions, constraints regarding genetically engineered microorganism (GEM) application, and surviving problem owing to extra energy demands for the presence of novel genetic material in the cell.

Bioremediation is an attractive alternative to traditional physico-chemical techniques for the cleaning up various pollutants in contaminated sites and it has also low cost and it can remove particularly contaminants without damaging its natural habitats. However, altough being more cost-effective solution to the polluted area, bioremediation strategies have had limited application because of the several challenges like that requirement of substrate, unadequate microorganism in naturally occurring which has appropriate biodegradative potential. Recently, advancing in biomolecular engineering shortened the process of pollutant degradation and therefore it will increase to focus on this exciting field in the next years.

9. Conclusion

Bioremediation is an interdisciplinary technology, including chemistry, microbiology, ecology, geology, and engineering. Microorganisms can facilitate environmental decontamination by volatilizing, binding, oxidizing, immobilizing or on the other hand degradating contaminants. Because of advantages such as is to be simpler, cheaper and more commonly used, it has been increased interest microbially mediated bioremediation. Various genetic, molecular, and metabolic engineering tools have accelerated the bioremediation progress and give rise to specifically designed microorganism for different removal process via using organism. As increasing our knowledge about enzymes responsible for biodegradation structural, pathway, and functional processes, our improvement in the field of the new designed pathway of bioremediation will further expand. Understanding the genetics and physiology of such organisms will be very effective to evaluate and enhance bioremediation. For this purpose, up to date having a database that collects the results of environmental molecular evaluations of contaminated and bioremediatied sites should increase.

The bioremediation process is influenced by different factors such as environmental factors (temperature, pH, electron acceptors, oxygen, nutrients, and soil type), spesific microorganism population biodegradability of contaminant. Bioremediation can change the oxidation state, helping in adsorption, accumulation in micro- or macro-organisms, though bioremediation not completely remove inorganic pollutants (radionuclides and metals). Consequently, bioremediation processes are having more and more importance with increased demand of ecofriendly bioremediation applications.

In this review, we focused on bioremediation principles, system and process, microorganisms, various applications, and biomolecular engineering techniques. Expanding the range of microbial biodiversity is the key environment friendly "green" technologies and increasing usefulness. It is vital that we have an understanding of a organism's degradative potential under different conditions, its molecular biology and its biochemical systems to enlarge the potential profits of microbial community in encountering pollution problems.

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