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Bioremediation of heavy metals and organic pollutants through green technology

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Abstract
Human activities have increased pressure on the natural resources and become the source of a myriad of pollutants. Recent advancing in biotechnology, bioremediation has become one of the most rapidly developing fields of environmental restoration, utilizing microorganisms to reduce the concentration and toxicity of various chemical pollutants, such as organic and inorganic compounds, industrial solvents and pesticides etc. Currently, extensive research is being focused on finding an optimal microbial biomass that would be as cheap as possible for the removal of contaminating products from large volume of polluted area. Bioremediation is emerging as an effective and attractive management tool to treat and recover the environment, in an ecofriendly manner. Bioremediation has been used at a number of sites worldwide, with varying degrees of success. Bioremediation, both in situ and ex situ have also enjoyed strong scientific growth, in part due to the increased use of natural attenuation, since most natural attenuation is due to biodegradation. Bioremediation technology, which leads to degradation of pollutants, may be a lucrative as well as environmentally friendly alternative.

Keywords: pollutants, bioremediation, biodegradation, microbial biomass, ecofriendly

Introduction

Chemicals are part of our daily life. All living and inanimate matter is made up of chemicals and virtually every manufactured product involves the use of chemicals. Many chemicals can, when properly used, significantly contribute to the improvement of our quality of life, health and well-being. But other chemicals are highly hazardous and can negatively affect our health and environment when improperly managed. If the final product contains high heavy metals concentration it may be noxious to soil, plants and human health. Heavy metals uptake by plants and successive accumulation in human tissues and bio magnifications through the food chain causes both human health and environment concerns.

According to the Stockholm Convention on Persistent Organic Pollutants, 9 of the 12 persistent organic chemicals are pesticides. Classes of organic pesticides (consisting of organic molecules) include organochlorine, organophosphate, organometallic, pyrethroids, and carbamates among others (Stockholm, 2011) [12]. Most pesticides cause adverse effects when reaching organisms. The intensity of the toxic effect varies with time, dose, organism characteristics, environmental presence or pesticide characteristics. Their presence in environment determines the dose and time at which an organism is exposed and could represent a hazard for worldwide life due to their mobility. Hence, the persistence in the environment leads to a risk for life: the more persistent a pesticide is, the worse its environmental impact. Unlike organic pollutants, heavy metals do not decay and thus pose a different kind of challenge for remediation. Currently, plants or microorganisms are tentatively used to remove some heavy metals.

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site. Technologies can be generally classified as in situ and 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 looks at the whole system, the living soil communities and aims to restore the maximum health, diversity and life.

Research is underway at a number of facilities using exogenous, specialized microbes or genetically engineered microbes to optimize bioremediation (Hassan *et al.*, 2003) [5].

The process of biodegradation is a well-established and powerful technique for treating domestic and industrial effluents. A microbial population has an amazing and extensive capacity to degrade a variety of organic compounds (Manogari *et al*., 2008) [9].

A successful, cost effective, microbial bioremediation program is dependent on hydro geologic conditions, the contaminant, microbial ecology and other spatial and temporal factors that vary widely. Microbiological assays are carried out to assess microbial growth conditions, population densities and presences of enzymes capable of destroying contaminants of concern and microbes studies to evaluate bioremediation potential under controlled conditions.

During implementation of microbial bioremediation programs, performance monitoring plays a key role in evaluating treatment. Properly executed, microbial bioremediation is cost effective and expeditiously destroy or immobilize contaminants in a manner that human health and environment requires (Gadd, 2000) [3].

Bioremediation is an innovative technology that frequently being chosen for the cleanup of polluted sites. Recent research expanding the capabilities of these technologies, along with it generally lower cost, has led to bioremediation becoming an increasingly attractive cleanup technology. The process of bioremediation enhanced the rate of the natural microbial degradation of contaminants by supplementing these microorganisms with nutrient carbon sources is electron donor. This can be done by using indigenous microorganisms or by adding an enriched culture of microorganisms that have specific characteristics that allow them to degrade the desired contaminants at the quicker rate. Ideally, bioremediation results in the complete mineralization of contaminants to H_2O and CO_2 without the buildup of intermediates.

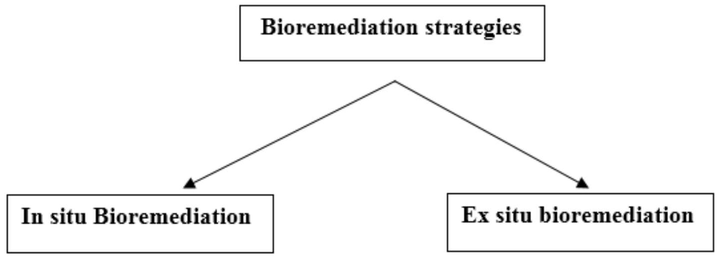

Fig 1

It may be done in situ or in a treatment cell and has been successfully used to remove large petroleum spills, wood-preserving wastes (PCP and creosote), coke wastes, and certain pesticides in the soil. The large requirement of space, proper management of leachates and prevention of volatile gases are some of the limitations associated with land farming. Table 1 summarizes the advantages and disadvantages of different types of bioremediation strategies.

Table 1: Advantages and disadvantages of different types of bioremediation strategies

Techniques	Example	Advantage	Disadvantage
In-situ	In situ bioremediation Bioventing Biostimulation Bioaugmentation	Most cost efficient Noninvasive Relatively passive Natural attenuation Treats soil and water	Environmental constraints Extended treatment times Monitoring difficulties
Ex-situ	Land farming Composting Biopiles /Biocells	Cost-efficient Can be done on site	Extended treatment time Need to control abiotic loss Mass transfer problem Bioavailability limitation
Bioreactor	Slurry/aqueous reactors	Rapid degradation kinetics Optimized environmental parameters Enhances mass transfer Effective use of inoculants and surfactants	Requires excavation Relatively high capital cost & operating cost

Source: M. Vidali Bioremediation. An overview Pure Appl. Chem., 73, 1163–1172, 2001

Features of Bioremediation

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

Factors affecting Bioremediation

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

Table 2: Factors affecting bioremediation

Factors	Condition required
Microorganism	Aerobic or anaerobic
Natural Biological processes of microorganism	Catabolism and Anabolism
Environmental Factors	Temperature, pH, Oxygen content, Electron acceptor/donor
Nutrients	Carbon, Nitrogen, Oxygen etc

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

We can subdivide these microorganisms into the following groups:

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

Anaerobic: In the absence of oxygen. Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE), and chloroform. Ligninolytic fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs. Methylotrophs aerobic bacteria that grows utilizing methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatic trichloroethylene and 1,2-dichloroethane.

Some common microorganism used in the process of remediation are *Acromobacter*, *Alcaligenes*, *Arthrobacter, Bacillus, Cinetobacter, Corneybacterium, Flavobacterium, Micrococcus, Mycobacterium, Nocardia, Pseudomonas, Vibrio, Rhodococcus and Sphingomonas* species (Gupta *et al.*, 2001, Kim *et al*, 2007, Jayashree, 2012) [4, 7, 6].

The main species involved in effective waste water treatment include lactic acid bacteria-*Lactobacillus plantarum, L. casei* and *Streptococcus lacti* and Photosynthetic bacteria *Rhodopseudomonas palustrus*, *Rhodobacter spaeroide*, etc. (Narmatha and Kavitha, 2012) [10].

White-rot fungi produce lignin peroxidase, manganeseperoxidase and laccase that degrade many aromatic compounds due to their nonspecific enzyme systems (Toh, Y C *et. al.,* 2003) [13]. In the previous type, the cells create enzymes such as laccase, Manganese peroxidase and lignin peroxidase to mineralize the dyes (Reghukumar C *et. al.,* 1996, Fu Y *et. al.,* 2001) [2].

Limitations of Bioremediation

- Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
- There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound. Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.
- Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids, and gases.
- Bioremediation often takes longer 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.

Factors affecting bioremediation of pollutants

Ecosystems are active environments with variable abiotic conditions, like pH, temperature, presence of oxygen, metals, salts, etc. Optimization of such abiotic conditions will greatly help in the development of industrial-scale bioreactors for bioremediation.

1. **pH:** In general, fungi show better biodegradation activities at acidic or neural pH whereas bacteria at neutral or basic pH. The pH plays a major upshot on the efficiency of degradation. Adaptation of microorganisms to varying pH enhances the process of effluent treatment.
2. **Temperature:** Temperature is an important environmental factor and the biodegradation activities of microorganisms are affected by changes in temperature. The rate of degradation increases with increasing initial temperature. The degradation activities of the microorganisms decrease because of slow growth, decreased reproduction rate and deactivation of enzymes responsible for degradation (Bizuneh Adinew, 2012) [1].
3. **Initial concentration of pollutants**: The effect of concentration of pollutants strongly influences the rate of degradation and also impacts the toxicity of particular molecule. Percentage of degradation increased with increase in time irrespective of initial concentration for bacteria.
4. **Effect of Nutrients**: Nutrients plays a significant role in degradation process, superior amount of nutrients significantly influences the growth of micro-organism and boost the degradation of contaminants in aqueous solution. Pseudomonas sp. BSP-4 isolated from azo dye contaminated soil capable to decolorize azo dye Black E by utilizing it as nitrogen source up to 300 ppm in 36 hours (Sudhakar P *et. al.,* 2002) [11].

Conclusion

Bioremediation is the microbial clean up approach on the front line and priority research area in the environmental biotechnology. This field has recent origin and grown exponentially over the last two decades. In this system microbes can acclimatize themselves to toxic wastes and new resistant strains develop naturally, which can transform various toxic chemicals to less harmful forms. The mechanism behind the biodegradation of recalcitrant compounds in the microbial system is because of the biotransformation enzymes.

Several reports suggest the degradation of complex organic substances, which can be brought about by an enzymatic mechanism like: laccase, tyrosinase, hexane-oxidase and aminopyrine-N-demethylase etc. A number of biotechnological approaches have been suggested by recent research as of potential interest towards combating this pollution source in an eco-efficient manner; mainly the use of bacteria and often in combination with physicochemical processes. Dyes constitute the largest class used in industries, which are xenobiotic in nature and found to be recalcitrant to biodegradation.

The isolation of new strains or the adaptation of existing ones to the decomposition of contaminant will probably increase the efficacy of bioremediation of those contaminants in the near future. The use of microbial or enzymatic treatment method for the complete remediation of an industrial or textile effluent process has following considerable advantages-

1. Eco-friendly nature
2. Cost-competitive
3. Less sludge producing properties
4. The end products with complete mineralization or non-toxic products, and
5. Could help to reduce the enormous water consumption compared to physicochemical methods.

Moreover the effectiveness of the microbial activity of remediation depends upon the adaptability and the activity of selected microorganisms. Large number of species has been tested for the removal and mineralization of various organic or inorganic pollutants and steadily increasing in recent years. The isolation of potent species and there by remediation is one of the interest in biological aspect of effluents treatment. A wide variety of microorganisms are capable to remove a wide range of contaminants using wide range of microorganisms including bacteria, fungi, yeasts, actinomycetes, algae and plants (Phytoremediation) capable to decolorize and even completely mineralize many pollutants under certain environmental conditions.

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