

Bioremediation Strategies for Environmental Management: A Review

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Abstract

Environmental contamination is increasing day-by-day due to rapid industrialization and growing population. The environmental contaminants are introduced into the environment either accidentally or deliberately as a result of human activities, and have the potential to cause deleterious effects on human health and other living creatures. Both domestic as well as industrial wastes have contributed in enhancing this contamination. The continuous and uncontrolled discharge of wastes into the environmental sink has become an issue of major global concern. Bioremediation is a unique, eco-friendly and effective method for removal of contaminants/pollutants from soil and/or water. This technology makes use of microorganisms and biological processes to remove or detoxify the pollutants. The purpose of bioremediation is to make environment free from pollution with help of environmental friendly microbes. This review highlights the salient features of bioremediation process, different strategies, its benefits and limitations, and recent developments in bioremediation management.

Keywords: bioremediation, biostimulation, contamination, genetically modified microorganisms, phytoremediation, waste management

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INTRODUCTION

Remediation of contaminated sites has become a serious issue due to the overexploitation of natural resources, and hazardous effects of pollution and unwanted wastes on the environment and human health. With the advent of rapid industrialization, vast quantities of hazardous wastes have been generated that has heightened the environmental problems by depleting and polluting the natural resources. Today, man is caught in the vicious circle of increasing demands, declining resources and increasing waste, generated by the industries and/or municipalities, thereby creating problems of enormous dimensions. Both domestic and industrial wastes have contributed in enhancing this problem.

Immediate steps are required to be taken in this regard; otherwise the consequences may be disastrous both for the ecosystem and human welfare.

Remediation involves monitoring and controlling the development, treatment, storage, and disposal of wastes. In other words, focus is laid on ensuring that wastes generated are managed and disposed in a manner protective of human health and the environment. The wastes can be in the form of solids, liquids, sludges, or contained gases, generated by chemical production, manufacturing, industrial discharge, and domestic activities. These can cause damage during inadequate storage, transportation, treatment, or disposal operations.

Improper storage or disposal of waste often causes contamination of surface and groundwater supplies. Those living at or near old and abandoned waste disposal sites may be in a particularly vulnerable position.^[1] This has increasingly led to the need of issuing strict norms and regulations related to storage, transportation, treatment, and disposal of solid and liquid waste, as well as remediation of contaminated sites. Stringent cleanup standards are being directed to protect soil, vegetation and ecosystem and to prevent contamination of the groundwater.^[2]

REMEDICATION TECHNOLOGIES

The existing remediation technologies for waste and other contaminants can be classified as either those having demonstrated full-scale applications and removal efficiencies or as emerging/innovative technologies that have few or no reported full-scale applications, but have shown pilot- and laboratory-scale removal efficiency.^[2] Several parameters are taken into consideration while selecting a specific remediation technology. This includes overall costs, cleanup time, reliability, and maintenance. Besides, other criteria such as the technique's ability to clean up to a desired level (minimum pollutant concentration achievable by the technology), community acceptability, applicability, post-treatment costs, soil quality required after the intervention (in case of soil pollution), environmental impacts, and risks of remediation activities/processes, are also taken into consideration.^[2] 'Bioremediation technology' has come-up as an innovative approach to degrade environmental pollutants using natural biological processes. This method degrades or detoxifies the substances including heavy metals that are otherwise hazardous to human health and the environment. The aim of the present review is to describe some generally accepted bioremediation

strategies; the benefits, limitations and challenges associated with this technique and subsequently the recent advancements being undertaken for improvising it.

Bioremediation

Bioremediation is the branch of biotechnology that uses biological organisms to solve environmental problems *i.e.*, cleaning the environment from pollutants and contaminants. Bioremediation makes use of biological processes and organisms to metabolize pollutants. In other words, instead of simply collecting the pollutant and storing it, bioremediation relies on catabolic activity of naturally occurring microbial living organisms to consume and break down the compound, turning it into harmless, natural substances such as carbon dioxide, water, inorganic salts, and microbial biomass.^[3-7]

Need for Bioremediation

Contamination by xenobiotic compounds such as organic petroleum hydrocarbons, pesticides and other agrochemicals, pharmaceutical products and heavy metals through human activity, causes serious problems that can lead to deleterious effects to the environment.^[8-12] Conventional methods to remove, reduce or remediate contaminated sites such as pump and treat systems, excavation and removal, soil vapor extraction, incineration, containment and other chemical treatments,^[13] are costly, time consuming, invasive, and often disruptive to natural habitats.^[10] Lately, with the emerging science and technology, bioremediation methodologies have become increasingly important as means of cleaning up environmental contaminants. This method takes advantage of natural biological processes to reduce or eliminate risks to health and the environment posed by toxic or hazardous substances. Bioremediation has been validated and is being used as an effective means of mitigating:

- hydrocarbons
- halogenated organic solvents
- halogenated organic compounds
- non-chlorinated pesticides and herbicides
- nitrogen compounds
- metals (lead, mercury, chromium)
- radionuclides

Working Strategy for Bioremediation

Bioremediation works when the micro-organisms come in contact with contaminants present in the soil and/or water such as oil, diesel, or gas. Optimal environmental conditions need to be maintained that promotes and enhances the degradation process. These conditions include a balance of temperature, surface pH, moisture, availability of nutrients, oxygen content etc.

To start the bioremediation process, microbes secrete enzymes to break down (catalyze) the contaminants into much smaller and simpler pieces. These broken-down contaminants serve as a source of food and energy for the microbes. During this process, the microbes release water, carbon dioxide and other non-harmful amino acids as by-products. If optimal environmental conditions and adequate food supply is obtained, the indigenous microbes reproduce and multiply, creating additional microbes to further aid in the removal of additional contaminants. The cycle is repeated until the food source is depleted. It usually takes weeks to several months for the microbes to clean up a site. However, the total time period depends upon several factors such as the amount of contaminants present at that site, number of microbes present, and availability of ideal environmental conditions.^[14]

Types of Bioremediation

Bioremediation techniques are broadly categorized as *in situ* and *ex situ*. The *in situ* bioremediation involves treating the

contaminated material at the site, while *ex situ* involves removing the contaminated material from the same site so as to be treated elsewhere. The former involves minimal disruption of sites and elimination of handling costs, requiring longer periods of treatment and extended monitoring. They can also be constrained by geological, hydrogeological and other environmental factors, resulting in a low efficiency of contaminant removal.^[10,15,16]

The latter involve the removal of materials by excavation, pumping or dredging, which allows greater process control though there will be some disruption to the site. Due to excavation and transportation of materials, the remediation costs is significantly increased in the *ex situ* method, leading to a preference for *in situ* techniques.^[7,16,17] Some examples of bioremediation technologies are biostimulation, bioaugmentation, bioventing, phytoremediation, landfarming, composting, biopiling, biosparging and bioreactor.^[18]

Biostimulation: Biostimulation involves modification of the environment to stimulate existing bacteria capable of bioremediation. This can be done by addition of various forms of limiting nutrients and oxygen either in liquid or gaseous form, electron acceptors such as phosphorus, nitrogen, oxygen, or carbon (e.g. in the form of molasses), which are otherwise available in quantities low enough to constrain microbial activity.^[18,19] The disappearance of contaminants is monitored to ensure that remediation occurs.

Bioaugmentation: It involves addition of micro-organisms that can clean up a particular contaminant, to the indigenous population for bioremediation. The rationale for this approach is that either the indigenous microbial population is not in adequate number to degrade the

contaminant, or they are not capable enough to degrade the wide range of potential substrates present in the complex mixtures. Speed of decontamination is a primary factor during the process of bioremediation. When the indigenous contaminant-degrading population is low, the lag period to start the bioremediation process gets reduced.

Bioaugmentation is usually carried out for contaminants removed from the original site, for example in municipal wastewater treatment facilities. To date, this method has not proved to be very successful when done at the site of the contamination since it is difficult to control site conditions for the optimal growth of the microorganisms added.

Bioventing: It is the most common *in situ* bioremediation method that is used to degrade compounds under aerobic conditions. In this technique, the oxygen and nutrients like nitrogen and phosphorus is injected *via* wells to the contaminated site to stimulate the indigenous bacteria. This method employs low airflow rates and provides only that much amount of oxygen required for biodegradation while minimizing volatilization and release of contaminants into the atmosphere. Bioventing is used for removal of simple hydrocarbons such as gasoline, oil, and petroleum. This method is more effective where the contamination is deep under the surface and has high temperature.^[18,20]

Phytoremediation: This technique uses the plant parts such as roots, stems, leaves, and tissues, to remove, transfer, stabilize, or destroy the contaminants. This method makes use of natural ability of certain plants called hyper accumulators and the processes associated with them (biological, chemical and/or physical) to bioaccumulate, degrade, or render harmless contaminants in soils, water, or air.^[18,21,22] Several contaminants including metals, pesticides, solvents, explosives,

crude oil and their derivatives, have been mitigated through phytoremediation projects across the world. For example, radioactive cesium and strontium were removed from Chernobyl site using sunflowers, arsenic contamination in water supplies of Bangladesh was removed with the help of water hyacinths.^[18] Other plants that have proven to be effective at hyper accumulating contaminants at toxic waste sites include mustard, alpine pennycress, hemp, and pigweed.

Landfarming: In this technique, contaminated soil is excavated and spread over a prepared bed made up of clean soil and a clay and concrete. In other words, a sandwich layer of excavated soil is prepared, with clean soil at the bottom and concrete layer at topmost. The excavated soil layer is left for natural degradation or slightly tilled periodically until the contaminants get degraded. The aim is to stimulate indigenous biodegradative microorganisms and promote degradation of contaminants aerobically. In general, the practice is limited to the treatment of superficial 10–35 cm of soil. This technique has received special attention as a disposal alternative due to reduced monitoring and maintenance costs, as well as clean-up liabilities.^[18,20]

Composting: In composting, contaminated soil is mixed with non-hazardous organic manure or other agricultural wastes. The presence of organic materials promotes the growth of microbial population that degrades the wastes at an elevated temperature ranging from 55–65°C. During degradation, the microbes release heat that raises the temperature, thereby increasing solubility of wastes and higher metabolic activity in composts.^[20]

Biopiling: It is a full-scale ex-situ bioremediation technology that combines the strategies of both landfarming and composting. The simplest biopile system includes a treatment bed, an aeration

system, an irrigation/nutrient system and a leachate collection system.^[20] In this method, polluted excavated soils are stockpiled into a layer within the treatment bed in order to prevent further contamination. Controlled parameters that enhance biodegradation of the contaminants include moisture, heat, nutrients, oxygen, and pH. This treatment process reduces the contaminants to carbon dioxide and water, a process that usually takes 3-6 months. Compared to other methods, biopiling provides a favorable environment for both indigenous aerobic and anaerobic microorganisms, with relatively small land-space needed for the procedure. This method is typically used for the treatment of surface contamination with petroleum hydrocarbons, as well as of capturing and treating volatile organic compounds.^[18,20]

Biosparging: It involves injection of air under pressure below the ground water to increase the concentration of oxygen and enhance the rate of aerobic degradation and volatilization of contaminants by the naturally occurring bacteria. Biosparging enhances mixing in the saturated zone, thereby increasing contact between soil and groundwater. This method involves low installation costs and allows great flexibility in the design and manufacture of the system.^[18,23]

Biosparging is usually used at sites contaminated with small-weight and/or medium-weight petroleum products such as gasoline and diesel fuel, jet fuel respectively. Heavy-weight products like lubricating oils take longer time for biodegradation, and because of this, biosparging is a less preferred choice of bioremediation for such products.^[20]

Bioreactor: This type of bioremediation makes use of slurry or aqueous reactors for *ex situ* treatment of contaminated sites. It

involves processing of contaminated soil, sediment or sludge and water *via* an engineered containment system.^[20,24] The rate and extent of biodegradation is greater in a bioreactor system as the environment inside the reactor can be easily controlled and predicted in comparison to the *in situ* methods. However, despite the benefits, there are certain disadvantages associated with this system. The contaminated soil requires pre-treatment such as excavation, soil washing, or vacuum extraction prior to placing inside the bioreactor.^[18]

BENEFITS OF BIOREMEDIATION

- (1) It can be used for a variety of organic and inorganic compounds.
- (2) It requires very less effort and can be carried out on-site without disturbing the ongoing activities. This eliminates the transportation costs and efforts needed for transporting large quantities of wastes over long distances.
- (3) It can also be done off-site. This minimizes the potential threat to human health and the environment at the specific or near-by area.
- (4) It is a natural process, easy to implement and maintain.
- (5) Bioremediation is a cost-effective strategy as compared to other conventional methods that are used for clean-up of hazardous waste.
- (6) It is environment-friendly, devoid of use of any dangerous chemicals and aesthetically pleasing. Nutrients that are added for the growth of microbes are usually fertilizers used in gardens and fields. Also, the by-products released during the degradation process are harmless gases and water.
- (7) It helps in complete destruction of the contaminants and/or pollutants, and reduces the amount of wastes to be land-filled. It also eliminates the chance of future liability associated with treatment and disposal of contaminated material.

LIMITATIONS OF BIOREMEDIATION

- (1) It may take longer time *i.e.*, several months to years than other treatment options to remediate. This includes excavation and removal of soil and incineration.
- (2) It is limited to biodegradable compounds. Not all compounds are susceptible to rapid and complete degradation.
- (3) It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.
- (4) It depends on environmental conditions and controlled parameters.
- (5) Improved bioremediation technologies, appropriate for sites with complex mixtures of contaminants are required to be developed.
- (6) It may have possible harmful effects on the food chain, thereby disturbing the ecological balance.

RECENT STRATEGIES FOR BIOREMEDIATION

With the advent of genetic engineering technology, development of Genetically Modified Microorganisms (GMMs) is gaining special attention, due to their ability to utilize specific contaminants that include various hydrocarbons and pesticides. The important aspect of using GMMs as compared to the native organisms in bioremediation is the development of novel strains with desirable properties through pathway construction and modification of enzyme specificity and affinity. The technique of genetic engineering explores the ability of an organism to metabolize a xenobiotic by detecting the presence of degradative genes and transforming them into appropriate hosts through a suitable vector within a controlled setting.

In an initial study, The University of Tennessee in collaboration with Oak Ridge National Laboratory showed field based bioremediation using the genetically

modified microorganism *Pseudomonas fluorescens* strain designated HK44. This modified *P. fluorescens* HK44 was generated after insertion of the naphthalene catabolic plasmid (PUTK21). It was reported that introduction of the modified organism to naphthalene, increased the catabolic gene expression, naphthalene degradation and a coincident bioluminescent response as compared to that by the original parental strain. With the well-established tools from metabolic engineering and biochemistry, it is feasible to infuse different pathways into a 'designer' microbe. This technique is a powerful approach to enhance petroleum hydrocarbon biodegradation. Often, these pathways are combined with existing pathways to allow complete biodegradation. The construction of a hybrid strain which is capable of mineralizing components of a mixture of benzene, toluene and p-xylene simultaneously was attempted by redesigning the metabolic pathway of *Pseudomonas putida*.^[19] However, there are certain constraints associated with the use of GMMs. These can be listed as follows:

- (1) In using GMM, it can be problematic distinguishing between GMM specific degradation and biodegradation due to the presence of indigenous microbial consortia.
- (2) Inability to statistically conclude on bioremediation efficiency because of the highly heterogeneous distribution of the contaminants.
- (3) Sample-sample chemical analyses can typically vary by up to 200% making valid conclusions blurred.
- (4) Statistical models that can incorporate chemical heterogeneity kinetics into the entire design are required before valid efficacy assessments can be obtained.

CONCLUSION

Bioremediation is a new and natural technique that has shown remarkable

results with few setbacks. It is taking a leap in tackling environmental problems associated with different categories of wastes using variety of micro-organisms and biological processes. It is less expensive and destroys the contaminants without any major side-effects. However, before carrying out bioremediation treatment procedure, a thorough investigation of the environment where the contaminant exists, including the microbiology, geochemistry, mineralogy, geophysics, and hydrology of the system should be conducted. Also, more investigations are required to be done to understand the kinetics of degradation. In order to be widely used, the safety and efficacy of bioremediation techniques need to be first thoroughly validated and afterwards communicated well to the public.

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