Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; SP1: 373-377

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(Special Issue- 1)
2nd International Conference
"Food Security, Nutrition and Sustainable Agriculture - Emerging Technologies"
(February 14-16, 2019)

Developments, current practices and perspectives of Bioremediation

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Abstract
Environmental contamination due to anthropogenic and natural sources is increasing day by day because of increase in population, industrialization and urbanization. The enigma for the public, scientists, academicians and politicians is how to tackle the contaminants that jeopardize the environment. Advances in science and technology, since industrial revolution has also increasingly enabled humans to exploit natural resources and cause damage to the environment. The ideal solution for pollution abatement is Bioremediation, the most effective innovative technology to come along that uses biological systems for treatment of contaminants.
Although, this novel and recent technology is a multidisciplinary approach, its central thrust depends on microbiology. This technology includes biostimulation (stimulating viable native microbial population), bio augmentation (artificial introduction of viable population), bioaccumulation (live cells), biosorption (dead microbial biomass), phytoremediation (plants) and rhizoremediation (plant and microbe interaction). Rhizoremediation, which is the most evolved process of bioremediation, involves the removal of specific contaminants from waste product of contaminated sites by mutual interaction of plant roots and suitable microbial flora. This paper represents an exhaustive evaluation with respect to developments, current practices and perspectives of a variety of approaches of bioremediation.

Keywords: Bioremediation, phytoremediation, polyaromatic hydrocarbons, rhizoremediation

Introduction

Vast number of pollutants and waste materials containing heavy metals are disposed into the environment per annum. According to Third World Network reports, more than one billion pounds (450 million kilograms) of toxins are released globally in air and water. The contaminants causing ecological problems leading to imbalance in nature is of global concern. The environmentalists around the world are trying to overcome it by several means. However, they are raising their voices at international platforms regarding the depletion of natural resources; little attention is given to their words and continues to use them without caring the adverse consequences. Usually the contaminated sites are treated with traditional methods like physical, chemical and resembling excavation and transportation. By this method, the cost of removal of 1 m3 soil from a 1-acre contaminated site is estimated as US $0.6–2.5 million [1]. Billions of dollars are expected to be used to clean up all sites polluted with polycyclic aromatic hydrocarbon (PAHs) in coming decades [2]. The bioremediation technology is cost effective, eco-friendly and alternative to conventional treatments, which rely on incinerations, volatilization or immobilization of the pollutants. The conventional treatment technologies simply transfer the pollutants, creating a new waste such as incineration residues and not eliminate the problem. 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 [3]. Compared to other methods, bioremediation is a more promising and less expensive way for cleaning up contaminated soil and water [4]. Bioremediation uses biological agents, mainly microorganisms, e.g. yeast, fungi or bacteria to clean up contaminated soil and water [5].

Bioremediation, i.e. the use of living organisms to control or remediate polluted soils, is an emerging technology. It is defined as the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes. Some tests make an exhaustive examination of the literature of bioremediation of organic and inorganic pollutants [6, 7], and another test takes a look at pertinent field application case histories [8]. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules. Most important parameters for bioremediation are i) the nature of pollutants, ii) the soil structure, pH, Moisture contents and hydrogeology, iii) the nutritional state, microbial diversity of the site and iv)Temperature and oxidation-reduction (redox- Potential) [9]. In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources [10]. Bioremediation activity through microbe is stimulated by supplementing nutrients (nitrogen and phosphorus), electron acceptors (oxygen), and substrates (methane, phenol, and toluene), or by introducing microorganisms with desired catalytic capabilities [11, 12]. Plant and soil microbes develop a rhizospheric zone (highly complex symbiotic and synergistic relationships) which is also used as a tool for accelerating the rate of degradation or to remove contaminants. Groundwater is one of the most vital sources of drinking water on earth. However, in the past few decades, it has been contaminated with petroleum hydrocarbons, which leaked from underground storage tanks. These organic compounds have caused serious public concern because benzene, toluene, ethylbenzene, and xylene (BTEX) are ubiquitous pollutants hazardous to human health [13]. *In situ* bioremediation technology is a widely used technology that can clean up BTEX-contaminated sites, indigenous microorganisms to enhance biodegradation of organic constituents in the subsurface. Bacteria have huge catabolic possibility for remediating wastes; however, the interactions between bacteria and pollutants are complex and suitable remediation does not always take place. Hence, molecular approaches are being applied to enhance bioremediation. The recent developments are taking place in bioremediation by utilizing rhizoremediation, protein engineering, metabolic engineering, whole-transcriptome profiling, and proteomics for the degradation of recalcitrant pollutants such as chlorinated aliphatic and polychlorinated biphenyl as well as for binding heavy metals [14]. Cell surface expression of specific proteins allows the engineered microorganisms to transport, bio accumulate and/or detoxify heavy metals as well as to degrade xenobiotics [15].

Objectives of this Review

- Explore the current concepts of bioremediation.
- Provide an insight in to the role of various developed processes and major controls that may be used for their management in degradation of inorganic and organic soil pollutants.
- Highlight the limitations and challenges associated with the various developed processes of bioremediation.

Development of Bioremediation

Bioremediation techniques are divided into three categories; *in situ*, *ex situ* solid and *ex situ* slurry. With *in situ* techniques, the soil and associated ground water is treated in place without excavation, while it is excavated prior to treatment with *ex situ* applications. Selection of appropriate technology among the wide range of bioremediation strategies developed to treat contaminants depends on three basic principles *i.e.*, the amenability of the pollutant to biological transformation (Biochemistry), the accessibility of the contaminant to microorganisms (Bioavailability) and the opportunity for optimization of biological activity (Bioactivity) [16]. Simple hydrocarbons and petroleum fuels degradability decreases as molecular weight and degree of branching increase. Aromatic hydrocarbons one or two ring compounds degrade readily, higher molecular weight compounds less readily. Alcohols, esters, nitrobenzenes and ethers degrade slowly, chlorinated hydrocarbons decreasing within increasing chlorine substitution – highly chlorinated compounds like PCBs and chlorinated solvents do not appreciably degrade aerobically, Pesticides are not readily degraded. Few environmental conditions are required for the soil remediation (Table 1).

Table 1: Environmental factors and optimum condition for microbial activity for soil bioremediation [16].

Environmental Factor	Optimum conditions	Condition required for microbial Activity
Available soil moisture	25-85% water holding capacity	25-28% of water holding capacity
Oxygen	>0.2 mg/L DO, >10% air-filled pore space for aerobic Degradation	Aerobic, minimum air-filled pore space of 10%
Redox potential	Eh > 50 mill volts	-
Nutrients	C:N:P= 120:10:1 molar ratio	N and P for microbial growth
pH	6.5-8.0	5.5 to 8.5
Temperature	20-30 ºC	15-45ºC
Contaminants	Hydrocarbon weight of soil 5-10% Of dry	Not too toxic
Heavy metals	700ppm	Total content 2000ppm
Type of soil	-	Low clay or silt content

In-Situ Bioremediation

Bioventing

Bioventing encourages the in-situ biodegradation of POLs (petroleum-oil-lubricants) by providing oxygen to microorganisms in the soil. The system supplies oxygen by injecting air directly into the residual contamination. In contrast to soil vapor vacuum extraction (SVE), bioventing uses low airflow rates to provide only enough oxygen to keep up microbial activity. Optimal flow rates maximize biodegradation as vapors move slowly through biologically active soil while minimizing volatilization of contaminants. A basic bioventing system includes a well and a blower, which pumps air through the well and into the soil [17].

Biopiling

Bio pile treatment is a full-scale technology in which excavated soils are mixed with soil amendments, placed on a treatment area, and bio remediated using forced aeration. The contaminants are reduced to carbon dioxide and water. The basic bio pile system includes a treatment bed, an aeration system, an irrigation/nutrient system and a leach ate collection system. Moisture, heat, nutrients, oxygen, and pH are controlled to enhance biodegradation. The irrigation/nutrient system is buried under the soil to pass air and nutrients either by vacuum or positive pressure. Soil piles can be up to 20 feet high and may be covered with plastic to control runoff, evaporation and volatilization, and to promote solar heating. If volatile organic compounds (VOCs) in the soil volatilize into the air stream, the air leaving the soil may be treated to remove or destroy the VOCs before they are discharged into the atmosphere. Treatment time is typically 3 to 6 months [18].

Ex-Situ Bioremediation

Composting

Composting is a process by which organic wastes are degraded by microorganisms, typically at elevated temperatures. Typical compost temperatures are in the range of 55° to 65° C. The increased temperatures result from heat produced by microorganisms during the degradation of the organic material in the waste. Windrow composting has been demonstrated using the following basic steps. First, contaminated soils are excavated and screened to remove large rocks and debris [19, 20].

The soil is transported to a composting pad with a temporary structure to provide containment and protection from weather extremes. Amendments (straw, alfalfa, manure, agricultural wastes and wood chips) are used for bulking agents and as a supplemental carbon source. Soil and amendments are layered into long piles, known as windrows. The windrow is thoroughly mixed by turning with a commercially available windrow turning machine. Moisture, pH, temperature, and explosives concentration are monitored. At the completion of the composting period, the windrows would be disassembled and the compost is taken to the final disposal area.

Table 2: Developmental methods applied in bioremediation [20].

Technology	Examples	Benefits	Limitations	Applications	References
In situ	Biosparging Bioventing Bio augmentation	Most cost efficient Non-invasive Relatively passive Natural attenuation processes Treats soil and water	Environmental constraints Extended treatment time Monitoring difficulties	Biodegradative abilities of indigenous microorganisms Presence of metals and other inorganic Environmental parameters Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants	[21, 22, 23].
Ex situ	Land farming (Solid-phase treatment system) Composting (Anaerobic, convert's solid organic wastes into humus-like material) Biopiles	Cost efficient Simple procedure, Inexpensive, self-heating Low cost Rapid reaction rate, Inexpensive, self-heating Can be done on site	Space requirements Slow degradation rates, Long incubation periods Extended treatment time Requires nitrogen supplementation, incubation periods months to years Need to control abiotic loss Mass transfer problem Bioavailability limitation	Surface application, aerobic process, application of organic materials to natural soils followed by irrigation and tilling To make plants healthier good alternative to land filling or incinerating practical and convenient Surface application, agricultural to municipal waste	[21, 22].
Bioreactors	Slurry reactors Aqueous reactors	Rapid degradation kinetic Optimized environmental parameters Enhances mass transfer Effective use of inoculants and surfactant	Soil requires excavation Relatively high cost capital Relatively high operating cost	Bio augmentation Toxicity of amendments Toxic concentrations of contaminants	[24].
Precipitation or Flocculation	Non-directed physico-chemical complex -ation reaction between dissolved contamin-ants and charged cellular components (dead biomass)	Cost-effective	Yet to be exploited commercially	Removal of heavy Metals	[25].
Microfiltration	Microfiltration membranes are used at a constant pressure	Remove dissolved solids rapidly	Yet to be exploited Commercially	Waste water treatment; recovery and reuse of more than 90% of original waste water	-
Electro dialysis	Uses cation and anion exchange membrane pairs	Withstand high temperature and can be reused	Yet to be exploited commercially	Removal of dissolved solids efficiently	-

Advantages of bioremediation

- Bioremediation is a 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 con- taminant increase in numbers when the contaminant is present; when the contaminant is degrad- ed, 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 contam- inants. Many compounds that are legally considered to be hazardous can be transformed to harm- less products. This eliminates the chance of future liability associated with treatment and dispos- al 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 of nor- mal activities. This also eliminates the need to transport quantities of waste off site and the poten- tial 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.

Disadvantages of bioremediation
- Bioremediation is limited to those compounds that are biodegradable. Not all compounds are sus- ceptible 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 con- ditions, 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 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.

Conclusions

The main aim of this paper is to provide the scientific understanding needed to harness natural processes and to develop methods to accelerate these processes for the bioremediation of contaminated environments. Except few limiting factors, this technology has the ability to rejuvenate the contaminated environments effectively. However, rapid advances in the last few years has helped us in the understanding of process of bioremediation. The use of culture independent molecular techniques has definitely helped us to understand the microbial community dynamics, structure and assisted in providing the insight in to details of bioremediation which has surely facilitated to make the technology safer and reliable. In this context, bioremediation in relation to process optimization, validation and its impact on the ecosystem can be performed and by judicious use of the models that can predict the activity of microorganisms that are involved in bioremediation with existing geochemical and hydrological models, transformation of bioremediation from a mere practice into a science is now a reality. With the exciting new development in this field and focus on interdisciplinary research and using it on gaining the fundamental knowledge necessary to overcome the obstacles facing current technologies and also with respect to ethical, legal, and social issues involved this technology will go a long way in cleaning the environment in near future.

Acknowledgement

Authors are grateful to Baba farid group of institutions Batihnda (Punjab) India, for providing necessary facilities for the execution of the present work.

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