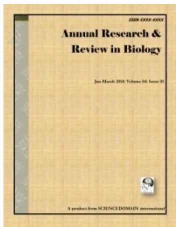

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Bioremediation: Step towards Improving Human Welfare

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Authors' contributions

This work was carried out in collaboration between both authors. Author LP designed the study, and managed the analyses of the study. Author PK managed the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The term "bioremediation" refers to the process by which toxic contaminants such as xenobiotics are reduced into non-toxic by-products (carbon dioxide and water or organic acids and methane) with the help of biological agents. Most of the organisms' especially human beings are continuously compromising their health with environmental contamination, which is increasing rapidly because of increasing population, industrialization and urbanization. Due to scarcity of resources and simultaneous advances in Science and Technology- human beings have started to exploit more natural resources thereby causing damage to the environment. An ideal solution to get rid of environmental contamination is through Bioremediation has become the most effective innovative low cost technology to come along that uses biological systems for the treatment of polluted environment. This technology includes both *in situ* (occurs at the site of contamination) and *ex-situ* (contaminant is taken out of the site of contamination and treated somewhere else) strategies. This paper provides an overview on environmental problems related to xenobiotics control strategies, its limitations and varieties of approaches of bioremediation.

Keywords: Bioremediation; biotechnology; in situ; environment.

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1. INTRODUCTION

In early times, land and resources were in abundance. Today, due to neglect and careless activities by man, it has resulted in increased release of a wide range of xenobiotic compounds to the environment. The rapid growth of different industries in the past century has extremely increased the amount of toxic waste products in to water bodies including ground water. Environmental problems caused by the release of wide range of pollutants from industries are creating imbalance to the ecosystem, causing changes in climatic conditions, reduction of water to worrying level in the ground, as well as in oceans, the melting of icecaps and glaciers, global warming due to excess of green house gasses, ozone layer depletion due to photochemical oxidation etc. Due to these worrying issues Ecologists are focusing more on remedial techniques to reduce the impacts of pollution [1]. The increasing need for remediation of contaminated sites has led to the development of new technologies like bioremediation that emphasize on the biological detoxification and destruction of the organic-contaminants by using micro-organisms.

In earlier days conventional treatment methods (physical, chemical & thermal reassembling) were used to decontaminate the site. By this the estimated cost risen up to US $0.6–2.5million for $1m^3$ from 1 acre contaminated [2]. Heavy costs are expected to be used to treat all sites polluted with xenobiotics.

The term bioremediation is defined as the process of using biological agents such as fungus and bacteria to remove toxic waste from environment. It is the most effective tool to manage the contaminated environment and recover contaminated soil eco-friendly and economically. Bioremediation is an effective and result oriented cleaning technique to manage the polluted environment and to recover contaminated soil [3]. Bioremediation is a process that involves detoxification and mineralization as it destroys or renders harmless various contaminants, using the biological activity of certain microorganisms [4]. The process of Bioremediation involves the use of effective plants or microorganisms (natural or genetically modified) to treat toxic contaminants (heavy metals) with organic molecules that are difficult to break down. These heavy metals are not completely degraded but they are transformed into substances with negligible or no toxicity [5]. Bioremediation technology uses micro-organisms which are intended to degrade hazardous organic pollutants up to environmentally safe levels. Bioremediation enhances the rate of the natural microbial degradation process of contaminants by supplementing the working microorganisms with carbon sources, nutrients, or electron donors. This can be done by using indigenous micro-organisms or by an enriched culture of micro-organisms that have specific characteristics to degrade the desired contaminant at a faster rate [6]. Bioremediation enhances the possibility of destroying or rendering harmless various contaminants using natural biological activity; As such it uses relatively low-cost and low technology techniques, which generally have a very high public acceptance and can be carried out on site. So bioremediation seems to be a good alternative to general clean-up treatment technologies [7]. Bioremediation technologies can be classified into two general categories: *in situ* (which occurs at the site of contamination) or *ex situ* (in which the contaminant is taken out of the site of contamination and treated somewhere else) [4]. One of the types of *in situ* method is “Intrinsic bioremediation” in which the indigenous subsurface bacteria are stimulated by injecting compounds to provide food and energy. The stimulated bacteria break down the target contaminants into less harmful substances [8]. Overall, bioremediation seems to be a very promising and reliable technology with great potential to deal with different types of contaminated sites. The bioremediation technology offer many advantages as the technique is not costly like other treatment methods, eco-friendly and alternative to conventional treatments, which rely on incinerations, volatilization or immobilization of the pollutants. The conventional treatment technologies do not completely remove the pollutants but simply create a new waste.

1.1 Environmental Problems Related to Xenobiotics

The presence of an organic chemical compounds (xenobiotics) in the environment is always a risk for living organisms [9]. The major worldwide problem is pollution of groundwater and soil that can result in uptake and accumulation of toxic compounds in food chains and also harm both the plantation and animal life of affected habitats. The contamination of groundwater resources by xenobiotics is a major environmental problem, with an estimated 3 hundred thousand to 4 hundred thousand contaminated sites only in the USA alone [10]. Pollutants of the contaminated sites can constitute the risk to the biosphere. Though major release which includes a considerable number of known contaminated sites exist and new ones are continuously being discovered. Most of these sites threaten to pollute supplies of drinking water and therefore constitute a serious health hazard for current and future generations. To remedy this situation, numerous remediation techniques have been developed. Because of the cost and time consideration physical and chemical treatment processes are currently the extensively used remediation methods. The term Bioremediation refers to the process in which microbes break down the contaminants either through oxidative or reductive processes. Under favourable conditions, microbes can degrade organic contaminants completely into non-toxic by-products such as water, carbon dioxide or organic acids [11].

Xenobiotics are artificially made chemical compounds that are very difficult to degrade. These compounds are made by synthetic organic-chemicals and are not usually the part of the biosphere. They accumulate in the environment and cause harmful effects on the living organisms. A substance that is foreign to biological system is known as xenobiotic compound. Most of the xenobiotic compounds are degraded by microorganism may be defined as weak xenobiotic, however, few of them may persist longer in the environment and not easily degraded is known as recalcitrant compound [12]. Xenobiotics include chemically synthesized compounds such as pesticides, polystyrene, polyethylene and PVC. Some compounds are recalcitrant that are not easily biodegradable due to the extensive branching of the molecule or introduction of halogen, nitro or sulphonyl groups.

1.2 Sources of Xenobiotics

There are several sources of xenobiotics some known and others unknown. However, majority of them are from anthropogenic sources of human activity. For simplicity the sources of xenobiotics are going to be grouped into twelve broad groups which indeed might not cover all. The sources include: Agricultural practices, Cigarette smoking, Electronic waste, Energy generation resulting from burning of fuels and also leaks of transformer oils from electrical installations, Industry (Textile, Agro-chemical, paints, etc.), Mining of precious minerals, Natural emissions, Oil and gas production and processing, Pharmaceuticals and hospital effluents, Radioactive materials, Transportation and Others [12a].

1.3 Principles of Bioremediation

Bioremediation is defined as the process that uses biological agents (yeast, bacteria and fungi) to biologically degrade the environmental contaminants into less toxic forms [13].

Bioremediation is a more promising and less expensive way to clean-up contaminated soil and water [14]. As such, it uses relatively low-cost, low-technology techniques compared to other methods. This technique is considered to be effective, only when the microorganisms attack the pollutants enzymatically and renders the organic waste as harmless products. Maintenance of optimum environmental conditions is necessary to permit microbial growth and proper degradation to occur at faster rate and to ensure the effective bioremediation [7]. Table 1 summarizes the advantages and disadvantages of bioremediation.

Broadly there are three classes of bioremediation:

1. Biotransformation – Is the alteration of contaminant molecules into less hazardous molecules.
2. Biodegradation - Is the breakdown of organic substances into inorganic molecules.
3. Mineralization - Is the complete biodegradation of organic materials into inorganic constituents.

The above mentioned three classifications of bioremediation can occur either via *in situ* (which occurs at the site of contamination) or *ex situ* (in which the contaminant is taken out of the site of contamination and treated somewhere else) [6c]. *In situ* bioremediation technologies include: Bioventing, Biosparging and Bioaugmentation. *Ex situ* strategies involves the excavation of the contaminants from its original site and places them in a contained environment. This makes the process even faster by allowing the users easier monitoring and maintaining of conditions. However, the removal of the contaminant (Ex situ) from the contaminated site is more laborious, costly and potentially more harmful. *Ex situ* bioremediation technologies include: bioreactors, bio-filters, land farming, biopiling and some composting methods [6].

Soil washing [6a] is another method that can be used, where water is flushed through the contaminated region and then transferred to a bioreactor for treatment [6b] as shown in Fig. 1. Similarly, in soil venting air is flushed through the contaminated region and then transferred to a bioreactor for treatment. The method of contaminant extraction depends on the nature of the contaminant (whether it is gas, liquid or solid phase, its chemical properties, and its toxicity) [6c].

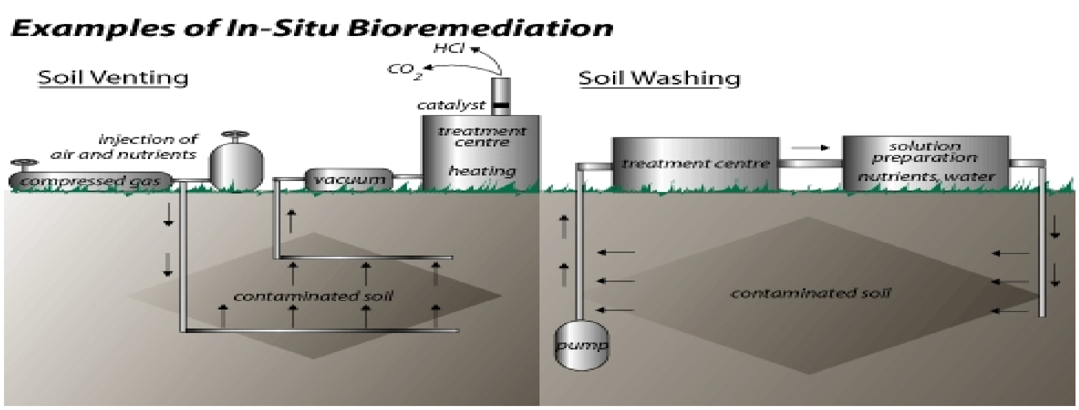

Fig. 1. In situ Bioremediation

Table 1. Developmental methods applied in Bioremediation

Technology	Examples	Benefits	Limitations	Applications	References
In situ	Biosparging	Most cost efficient and Non-invasive.	Environmental constraints.	Biodegradative abilities of indigenous microorganisms.	[15,16,17]
	Bioventing	Relatively passive	Extended treatment time	Presence of metals and other inorganics. Environmental parameters	
	Bioaugmentation	Natural attenuation processes Treats soil and water	Monitoring difficulties	Biodegradability of pollutants Chemical solubility Geological factors Distribution of pollutants	
Ex situ	Land farming (Solid-phase treatment system)	Cost efficient Simple procedure, Inexpensive, Self-heating.	Space requirements Slow degradation rates, Long incubation periods.	Surface application, aerobic process, application of organic materials to natural soils followed by irrigation and tilling.	[18]
	Composting (Anaerobic, convert's solid organic wastes into humus-like material)	Low cost Rapid reaction rate, Inexpensive, self heating	Extended treatment time Requires nitrogen supplementation, incubation periods months to years	To make plants healthier good alternative to land filling or incinerating practical and convenient	
	Biopiles	Can be done on site	Need to control abiotic loss Mass transfer problem Bioavailability limitation	Surface application, agricultural to municipal waste	
Bioreactors	Slurry reactors	Rapid degradation kinetic Optimized environmental Parameters	Soil requires excavation	Bioaugmentation Toxicity of amendments	[19]
	Aqueous reactors	Enhances mass transfer Effective use of inoculants and surfactant	Relatively high cost capital Relatively high operating Cost	Toxic concentrations of Contaminants	
Precipitation or Flocculation	Non-directed physico-chemical complex -ation reaction between dissolved contaminants and charged cellular components	Cost-effective	Yet to be exploited commercially	Removal of heavy Metals	[20]
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	
Electrodialysis	Uses cation and anion exchange membrane pairs	Withstand high temperature and can be reused	Yet to be exploited Commercially	Removal of dissolved solids efficiently	

1.4 Bioremediation of Pesticide: Cypermethrin

Cypermethrin [(+/)-a-cyano-3-phenoxybenzyl (+/)-cis, trans-3(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate] is among the synthetic pyrethroid pesticides, which is widely used to control pests. Bioremediation is considered to be as the most significant process for the removal of contaminants. Many efforts have been undertaken to isolate Cypermethrin degrading microbes from soil and polluted water and a lot of pyrethroid-degrading microorganisms have been isolated such as *Micrococcus sp.* [21], *Pseudomonas sp*. [22], and *Serratia sp*. [23]. Many fungal species such as *Mucor sp, Aspergillus carbonaruius, Aspergillus niger, Rhizopus sp, Saccharomyces cerevisiae, Botrytis cinerea, Neurospora crossa* and *Phanerochaete chrysosporium* have been extensively studied for the remediation of heavy metal [23a]. Several other microbial isolates that help in the degradation of toxic and recalcitrant xenobiotic compounds are listed in Table 2.

1.5 Mechanism of Degradation of Cypermethrin

Micro-organisms play a significant role in the degradation of cypermethrin. Many microbes have been isolated which are able to utilize cypermethrin as their sole source of carbon simultaneously degrading it into various by products of it [21,22,23]. Photo-degradation is mainly the process which is involved in the degradation of the product on the uppermost surfaces of leaves and water [24]. In soil hydrolysis and photolysis play an important role in the degradation of cypermethrin. In soil degradation occurs primarily through cleavage of the ester linkage to give two of its major byproducts i.e., cyclopropane-carboxylic acid (CPA), 3-phenoxybenzoic acid (PBA), and carbon dioxide (CO_2) [25]. Some amount of CO_2 is also formed under oxidative conditions during the cleavage of both the cyclopropyl and phenyl rings [26]. The Requirements for microbial growth in bioremediation process are listed in Table 3.

Table 2. List of Xenobiotic compounds including microbes responsible for their degradation

Target compounds	Bacteria degrading the Compounds	References
	Pesticides	
Endosulfan compounds	*Mycobacterium sp.*	[27]
HCH	*Pseudomonas putida*	[28]
2,4-D	*Alcaligenes eutrophus*	[29]
DDT	*Dehalospirilum multivorans*	[30]
Halogenated organic compounds		
Vinylchloride	*Dehalococcoides sp.*	[31]
Atrazine	*Pseudomonas sp.*	[32]
PCE	*Dehalococcoides ethenogenes195*	[33]
PAH compounds		
Napthalene	*Pseudomonas putida*	[34]
PCP	*Psedomonas sp*	[35]
3CBA	*Arthrobacter sp.*	[36]
1,4DCB	*Alcaligenes sp.*	[29]
2,3,4-chloroaniline	*Pseudomonas sp.*	[37]
2,4,5-T	*Pseudomonas sp.*	[38]

Fluoranthrene	*Pseudomonas cepacia AC1100*	[39]
Pyrene	*MycobacteriumPYR-1*	[40]
	Sphingomonas paucimobilis	[34]
Phthalate compounds		
Phthalate	*Burkholderiacepacia*DBO1	[41]
Other compounds		
PCB	*RhodococcusRHA1*	[42]
Dioxins	*Dehalococcoides sp*	[43]
RDX	*Desulfovibrio sp.*	[44]
Benzene	*Dechloromonas sp*	[45]
Petroleum products		
Petroleum products	*Achromobacter sp.* *Acinetobacter sp.* *Micrococcus sp.* *Nocardia sp.* *Bacillus sp.* *Flavobacterium sp.*	[46]
	Bacillus sp.	[47]
	Pseudomonas sp.	[48]
Azo dyes	*Sphingomonas sp.*	[48]
Pyrethroid pesticides		
Bifenthrin	*Enterobacter aerogenes*	[49]
Cypermethrin	*Pseudomonas aeruginosa*	[50]
Deltamethrin	*Sphingobium* sp.	[51]
Organophosphorous pesticides		
Endosulfan	*Arthrobacter sp.*	[52]
Chlorpyrifos	*Bacillus pumilus*	[53]
Diazinon	*Serratia liquefaciens*	[54]

Fungus

Fungal isolates	**Pesticide**	**Place of Isolation**	**Reference**
Aspergillus niger	Endosulfan	Soil	[55]
Ganoderma austral	Lindane	*Pinus pinea*stump	[56]
Trichosporon sp.	Chlorpyrifos	Sewagesludge	[57]
Verticillium sp	Chlorpyrifos	Soi	[58]
T. versicolor (R26)	Atrazine	Soil	[59]
Aspergillus sydowii, *Bionectria* sp., *Penicillium miczynskii, Trichoderma* sp.	DDD	Marine Sponge	[60]

Table 3. Requirements for microbial growth in bioremediation process [61]

Factors	Condition required
Microorganisms	Aerobic or Anaerobic
Natural Biological processes of microorganisms	Catabolism
Environmental Factors	Temperature, pH ,Oxygen content, Electron acceptor/donor
Nutrients	Carbon ,Nitrogen ,Oxygen etc
Soil Moisture	25-28% of water holding capacity
Type of soil	Low clay or silt content

1.6 Development of Phytoremediation

Genetic modification can be used to enhance not only the microbes but also plants as well for bioremediatory purposes. Bioremediation by using plants is called phytoremediation. Phytoremediation is an emerging technology that uses various green plants or higher terrestrial plants for treating chemically polluted soils, reducing the amount of hazardous compounds by degrading or through the immobilization of contaminants from soil and water [62]. Using green plants as weapons, phytoremediation is emerging as innovative, one of most eco-friendly and cost effective technique than the earlier established treatment methods to target the organic and inorganic pollutants in the water, soil and air simultaneously [63]. Phytoremediation uses plants to remediate sites contaminated with organic and inorganic pollutants [50]. Phytoremediation can be classified in to subcategories depending up on the type of remediation (Fig. 3). Many different types of phytoremediation techniques are used now days for the treatment of contaminants which is tabulated as below (Table 4).

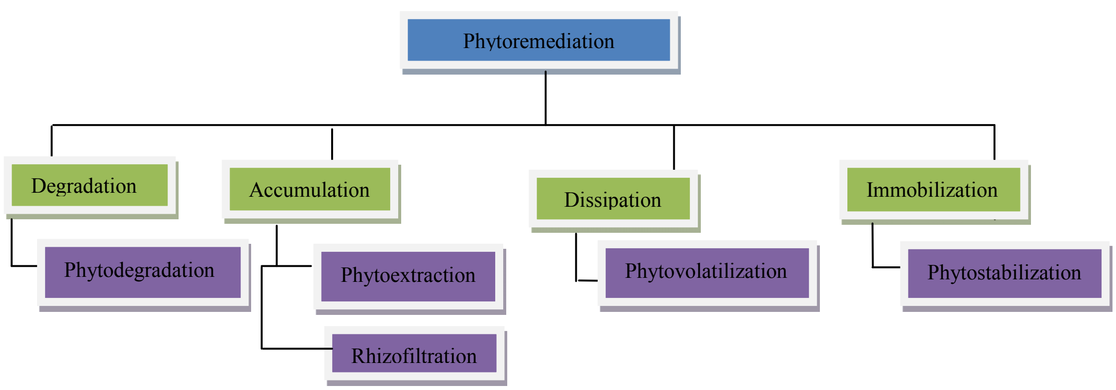

Fig. 3. Phytoremediation techniques for xenobiotic degradation [1]

Table 4. Types of Phytoremediation techniques

Process	Function	Pollutant	Medium	Plants	References
Phytodegradation	Plants and associated microorganisms degrade organic pollutants	DDT, Expolsives, waste and Nitrates	Groundwater	*Elodea Canadensis, Pueraria*	[64] [65]
Phytoextraction	Remove metals & organic pollutants that accumulate in plants.	Cd, Pb, As, Petroleum, Hydrocarbons & Radionuclides	Soil & Groundwater	*Viola baoshanensis, Sedum alfredii,*	[66] [67]
Phytostabilization (Immobilization)	Use of plants to reduce the bioavailability of pollutants in the environment	Cu, Cd, Cr, Ni, Pb, Zn	Soil	*Anthyllis vulneraria, Festuca arvernensis*	[68]
Phytotranformation	Plant uptake and degradation of organic Compounds	Cu, Cd, Cr, Ni, Pb, Zn Xenobiotic compounds	Soil	*Anthyllis vulneraria, Festuca arvernensis Cannas*	[69]
Phytovolatilization	capable of absorbing elemental forms of metals from the soil, biologically converting them into gaseous species	As, Hg & Se	Soil	*Pteris vittata*	[70] [71] [72]
Rhizofiltration	Roots absorb and Zn, Pb, Cd, As Groundwater adsorb pollutants, mainly metals, from water and aqueous waste streams	Zn, Pb, Cd, As	Groundwater	*Brassica juncea*	[73] [74]

2. CONCLUSION

Bioremediation provides a technique for cleaning up pollution by enhancing the natural biodegradation processes. The main aim of this paper is to provide the scientific understanding about the need of the bioremediation process to make the environment ecofriendly. This technology has the ability to clean the contaminated environments effectively. However, the rapid advances in the last few years have helped us in the understanding of process of bioremediation. This technology offers an efficient and cost effective way to treat contaminated ground water and soil. Its advantages generally outweigh the disadvantages. Environmental problems are the main concern to focus on. It is due to the caused by the industrial effluents which are responsible for the accumulation of pollutants and other fragmented compounds, which in turn form into other substitutes (natural or manmade), finally forming a xenobiont. There is a quick need to degrade these xenobiotic compounds in an eco-friendly way. Various techniques like microbial remediation, phytoremediation its subtypes have been discussed. Phytoremediation, a novel equipment based technique which is rapid as it uses plants for the bioremediation. Although slow, on the whole microbial bioremediation was found to cover wide range of recalcitrant contaminants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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