Applications of Bioremediation for Environmental Management

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Abstract: *Contamination of soils, ground water, sediments, surface water and air with hazardous and toxic chemicals is one of the major environmental problems facing the industrialized world today. The need to remediate these sites has led to the development of new emerging technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal. Bioremediation, the use of microorganisms or microbial processes to degrade environmental contaminants, is among these new technologies. Bioremediation has numerous applications, including clean-up of ground water, soils, lagoons, sludge and process-waste streams. Microbes are very helpful to remediate the contaminated environment, number of microbes including aerobes, anaerobes and fungi are involved in this process. The success of the technique depends on interdisciplinary approach involving such disciplines as biotechnology, microbiology, engineering, ecology, geology and chemistry. It is the most effective management tool to manage the polluted environment and recover contaminated soils. In this paper an attempt has been made to evaluate the potential of bioremediation techniques for environmental management. The article also summarizes interaction of microbes and microbial gene with plants in pollution remediation by using genetic engineering approaches. Genetic engineering allows introduction of desired traits into cells to improve the genome of wild plant and thereby improve the efficiency and versatility of bioremediation.*

Keywords*: Bioremediation, Environmental Management, Hazardous Chemicals*

1. Introduction:

Intensification of agricultural and manufacturing industries has resulted in an increased release of a wide range of compounds into the environment. Excess loading of hazardous waste has led to the scarcity of clean water and disturbances of soil. Due to rapid expansion of the industries, health care and vehicles etc. causing growing pressure on our natural resources which hinders our proper environmental management. The problem is worldwide, and the estimated number of contaminated site is significant. Therefore it is widely recognized that contaminated land is potential threat to human health and its continual discovery over recent years has lead to international efforts to remedy many of these sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use [1].

Types of contaminants that environmental biotechnology investigators have expertise with include chlorinated solvents, petroleum hydrocarbons, ketones, TNT, inorganic nitrogen (NO_3,NH_4), Tt, Pu, Cr, U and other heavy metals. Some technologies that have been used are high temperature incineration and various types of chemical decomposition (e.g. base catalyzed dechlorination, UV oxidation). They can be very effective at reducing levels of a range of contaminants, but have several drawbacks, principally their technological complexity, the cost for small scale application and lack of public acceptance, especially for incineration that may increase the exposure to contaminants for both the worker at the site and nearby residents.

Bioremediation is recently developing as a versatile tool for remedy of contaminated soil and ground water. Bioremediation uses biological agents, mainly microorganisms that is Yeast, Fungi and Bacteria to clean up contaminated soil and water [2]. The bioremediation and natural attenuation area has both basic research and field application foci for the environmental biotechnology. The basic research foci are co-metabolism, bio-treatbility, biotransformation kinetics and modeling biogeochemical processes. The field application foci are co metabolic techniques, biogeochemical assessment techniques and modeling of attenuation and environmental fate [3]. Bioremediation utilizes the application of genetic engineering to improve the efficiency and cost, which are key factors in the future widespread exploitation of microorganisms to reduce the environmental burden of toxic substances. Genetic engineering is the changing and improving of an organism's genome using biotechnological approaches (genome isolation, recombination and gene transformation). Genetic engineering allows introduction of desired traits into cells for their application in pollutant cleanup techniques. Bang et alengineered E.coli to produce sulphide by heterologous expression of thiosulphate reductase gene from *Salmonella enteritica*, which enhanced protein synthesis leading to precipitation of cadmium sulphide [4]. Genetic engineering is also being used in transformation of the microbial gene (e.g. merA & mer B) in to the chloroplast genome of plants , resulted in high level of tolerance to the pollutants (e.g. organomercurial compound) and such transformation also prevents the escape of transgene via pollens to the related crops [5].

Recent studies have revealed that, while conventional cleanup technologies have prevented the contamination problem from spreading, in most cases they are incapable of restoring the water to meet health-based standards in a reasonable time frame. Notably accomplishment of these studies include clean up of polluted water and land areas.

A number of bioremediation strategies have been developed to treat contaminated wastes and sites. Selecting the most appropriate strategy to treat a specific site can be guided by considering three basic principles: the amenability of the pollutant to biological transformation to less toxic products (biochemistry), the accessibility of the contaminant to microorganisms {bioavailability} and the opportunity for optimization of biological activity (bioactivity) [6].

Bioremediation is use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. The microorganisms may be indigenous to the contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Microbes metabolically transform the contaminant into less toxic innocuous state. Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are imported to a contaminated site to enhance degradation we have a process known as bioaugmentation. For bioremediation to be effective, microorganisms must enzymatically attack the pollutant and convert them to harmless product [7]. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at faster rate.

The microorganisms break down contaminants by using them as an energy source or co-metabolizing them with an energy source. More specifically, bioremediation involves the production of energy in a redox reaction within microbial cells. These reactions include respiration and other biological functions needed for cell maintenance and reproduction. A delivery system that provides one or more of the following is generally required: an energy source (electron donor), an electron acceptor, and nutrients. Different types of microbial electron acceptor classes can be involved in bioremediation, such as oxygen-, nitrate-, manganese-, iron (III)-, sulfate-, or carbon dioxide-reducing, and their corresponding redox potentials .

Most bioremediation are running under aerobic conditions but running a system under anaerobic conditions [8] may permit microbial organisms to degrade otherwise recalcitrant molecules.

2. Role of microbes in bioremediation:

Natural microorganisms, either indigenous or extraneous (introduced), are the prime agents used for remediation of environmental pollution [9]. The organisms that are utilized vary, depending on the chemical nature of the polluting agent, and are to be selected carefully as they only survive within a limited range of chemical contaminants [10]. The first patent for biological remediation agents was registered in 1974, being a strain of *Pseudomonas putida* that was able to degrade petroleum. About 70 microbial genera were reported to degrade petroleum compounds and equilibrium is maintained as equal number of microbes has been added to the list in successive last two decades [11]. We can subdivide these microorganisms into the following groups:

Aerobic - Microbes that can grow in the presence of oxygen, for instance *Pseudomonas, Alcaligenes, Rhodococcus, Mycobacterium and Sphingomonas.* These microbes use the contaminant as the sole source of carbon and energy.

Anaerobic - Microbes that can grow in the absence of oxygen, for instance Sulfate reducing bacteria. These bacteria used for the bioremediation Polychlorinated biphenyls (PCBs), dechlorination of solvent Trichloro ethylene (TCE) and chloroform.

Ligninolytic Fungi - Fungi have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutant. For instance White rots fungus *Phanaerochaete chrysosporium.*

Methylotrophs - Microbes that grow by utilizing methane for carbon and energy. These microbes have the enzyme, methane mono oxygenase that has a broad substrate range including the chlorinated aliphatic trichloroethylene and 1, 2-dichloroethane.

3. Types of bioremediation

In-situ and Ex-situ: In-situ bioremediation is defined as technique that is applied to soil and ground water at the site with minimal disturbance and Ex-situ technique is applied to soil and ground water at the site which has been removed from the site of excavation (soil) or pumping (water). Summary of bioremediation strategies is given in Table 1.

***Table 1**: Summary of bioremediation strategies*

Technology	Examples	Benefits	Limitations	Factors to consider
In-situ	In-situ bioremediation, Biosparging, Bioventing, Bioaugmentation	Cost efficient, Noninvasive, Natural attenuation Processes, Treats soil and water	Environmental constraint, Extended treatment Time, Monitoring difficulties	Biodegradative abilities of indegenious microorganisms, Presence of metal and other inorganic environmental parameters**,** Geological factors, Distribution of pollutants
Ex-situ	Land farming, Composting, Biopiles	Cost efficient, Low cost, Can be done on site	Space requirements, Extended treatment Time, Bioavalibity limitations, Mass transfer problems, Need to control abiotic loss	-do-
Bioreactors	Slurry reactor, Aqueous reactor	Rapid degradation kinetics, Optimizes environmental parameters, Enhance mass transfer, Effective use of inoculants and surfactants	Soil requires excavation, Relatively high capital cost, Relatively high operating cost	-do- Bioaugmentation, Toxicity of amendments, Toxic concentrations of contaminants

Some of the example of in-situ and ex-situ bioremediation is given below:

1. Land farming: Solid phase treatment system for contaminated soil; may be done in-situ or ex-situ.
2. Composting: Aerobic thermophilic treatment process in which contaminated material is mixed with a bulking agent; can be done using static piles or aerated piles.
3. Bioreactors: Biodegradation in a container or reactor; may be used to treat liquids or slurries.
4. Bioventing: Method of treating contaminated soil by drawing oxygen through the soil to stimulate microbial activity.
5. Biofilters: Use of microbial stripping columns to treat air emissions.
6. Bioaugmentation: Addition of bacterial cultures to a contaminated medium; frequently used in both in situ and ex situ system.
7. Biostimulation: Stimulation of indigenous microbial populations in soil or ground water by providing necessary nutrients.
8. Pump and Treat: Pumping ground water to the surface, treating and reinjecting.

4. Genetic engineering of microbes for bioremediation:

Genetic engineering, also called genetic modification, is the direct manipulation of an organism's genome using biotechnology. New DNA may be inserted in the host genome by first isolating and copying the genetic material of interest using molecular cloning methods to generate a DNA sequence, or by synthesizing the DNA, and then inserting this construct into the host organism. Genes may be removed, or "knocked out", using a nuclease (Fig. 1).

Genetic engineering techniques have been applied in numerous fields including research, agriculture, industrial biotechnology, medicine and now this technique is used for the treatment of environmental pollutant by microorganisms. Engineered in-situ bioremediation accelerate the degradation process by enhancing the physic chemical condition to encourage the growth of microorganism.

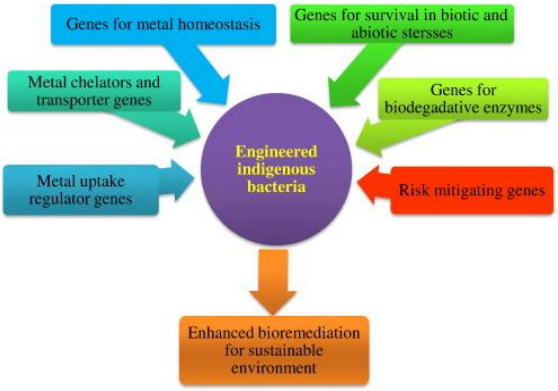


***Fig. 1**: Genetic engineered bacteria for the Bioremediation*

Genetically engineered microorganisms (GEMs) have shown potential for bioremediation of soil, ground water and activated sludge, exhibiting the enhanced degrading capabilities of a wide range of chemical contaminants as shown in [12]. It is used to construct cells for the bioremediation of pollutant, for example Krishnasamy and Wilson constructed an *E.coli* strain that accumulated Ni^{+2} by introducing the nixA gene (coding for a nickel transport system) from *Helicobacter pylori* into *E.coli JM109* that expressed a glutathione S-transferase pea metallothioneine fusion protein [5]. The recombinant E.coli strain accumulated four times more nickel than the wild type.

Microbial methylation plays an important role in the biogeochemical cycle of metals, because methylated compounds are often volatile i.e. selenium to volatile dimethyl selenide (Microorganism in Environmental Management: Microbes and Environment, 2012).

The potential for creating, through genetic manipulation, microbial strains able to degrade a variety of different types of hydrocarbons [13]. They successfully produced a multiplasmid-containing *Pseudomonas* strain capable of oxidizing aliphatic, aromatic, terpenic and polyaromatic hydrocarbons.

Superbug, *Pseudomonas putida* that contained the XYL and NAH plasmid as well as a hybrid plasmid derived by recombinating parts of CAM and OCT developed by conjugation could degrade camphor, octane, salicylate, and naphthalene [12] and could grew rapidly on crude oil because it was capable of metabolizing hydrocarbons more efficiently than any other single plasmid [13].

The application of genetic engineering for heavy metals removal has aroused great interest. For example, *Alcaligenes eutrophus* AE104 (pEBZ141) was used for chromium removal from industrial wastewater and the recombinant photosynthetic bacterium, *Rhodopseudomonas palustris*, was constructed to simultaneously express mercury transport system and metallothionein for Hg^{2+} removal from heavy metal wastewater [14]. Summary of genetically engineered microbes for degradation of contaminants is given in Table 2.

***Table 2**: Genetic improvement of microbes for the degradation of toxic contaminant*

Class of contaminants	**Example**	**Microorganisms**	**Important Genes**	**Transgenic microbes**
Chlorinated solvent	Trichloroethylene,	*Burkholderia cepacia* $PR1_{23}(TOM_{23C})$	*tomA* $^{+}$ (toluene*o*-monooxygenase) genes	*G.E* - *Pseudomonas fluorescens* strain
Pesticide[15]	Parathion, Atrazine	Pseudomonas strain WBC -3	Mph (methyl parathion hydrolase gene)	G.E - *E.Coli*
Metal	Co, Cu, Cd, Hg, Pb	*Zooglea spp, Bacillus spp, Rhizopus arrhizus, Synechococcus*	MTs(Metallothioneins)	*G.E* - *E.coli* and *R. metallidurans*
Polyaromatic hydrocarbon[16]	Naphthalene, pyrene	*Pseudomonas aeruginosa, Mycobacterium spp, Cynobacteria*	Nah b, Nah c and Nah f	Superbug G.E microbes

*G.E Genetic engineered

4.1 Interaction of microbes and their gene with plants for bioremediation:

In plant - microbe interaction, Plant roots provide a rich niche for bacteria to grow at the expense of root exudates; in turn bacteria act as biocatalysts that remove pollutants [17]. Endophytic bacteria (non-pathogenic bacteria that occur naturally in plants) and rhizospheric bacteria (bacteria that live on and near the roots of plants), have been shown to contribute to biodegradation of toxic organic compounds in contaminated soil and could have potential for improving bioremediation [18]. Members of the genus *Rhodococcus*, *Luteibacter* and *Williamsia*, which suggest that biostimulation through rhizoremediation is a promising strategy for enhancing Polychlorinated bi phenyls degradation in situ [19].

4.2 Plant growth-promoting rhizobacteria (PGPR):

The free living nitrogen fixer *Azospirillum lipoferum* generally found in the rhizoplane of the crop plants was used for Malathion degradation which is one of the largest organo phosphorus insecticides in the world [20]. Toyama et al. Suggested that the accelerated removal of benzopyrene and pyrene contaminant is due to rhizospheric bacteria *Mycobacterium gilvum* strains, isolated from rhizosphere sediments of aquatic plant *Phragmites australis* [21]. *Phragmites australis* root exudates containing phenolic compounds supported growth as a carbon source for the Mycobacterium strain, and induced benzo[a] pyrene-degrading activity of the strain. So the Mycobacterium - root exudates interactions can accelerate biodegradation of pyrene and benzo[a]pyrene in *P. australis* rhizosphere sediments.

4.3 Transgenic plants:

Many bacteria in the rhizosphere show only limited ability in degrading organic pollutants. With the development of molecular biology, the genetically engineered rhizobacteria with the contaminant- degrading gene are constructed to conduct the rhizoremediation [22]. Examples about the molecular mechanisms involved in the degradation of some pollutants such as trichloroethylene [23] and PCBs has been studied as shown in Fig. 2.

Sriprang et al. introduced *Arabidopsis thaliana* gene for phytochelatin synthase (PCS; PCSAt) into *Mesorhizobium huakuii* subsp. *rengei* strain B3 and then established the symbiosis between *M. huakuii* subsp. *rengei* strain B3 and *Astragalus sinicus* [24]. The gene was expressed to produce phytochelatins and accumulate Cd^{2+}, under the control of bacteroid-specific promoter, the nifH gene [25].

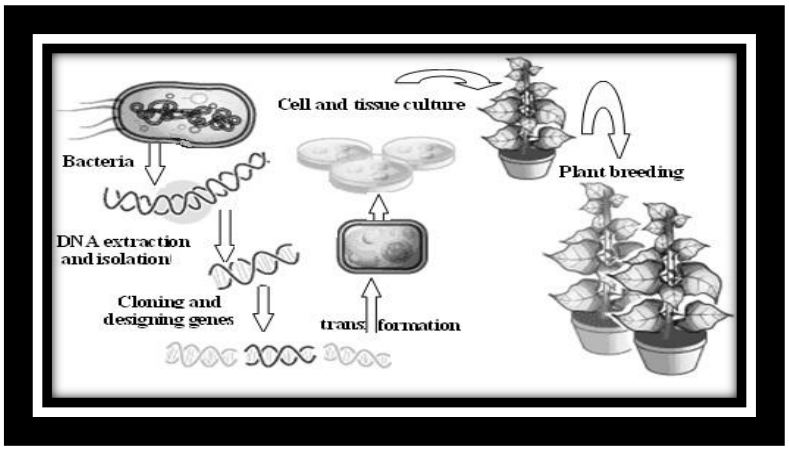


Fig. 2*: Transformation of microbial gene in plants for bioremediation*

Atrazine is one of the most widely used herbicides in the USA. Atrazine chlorohydrolase (AtzA), the first enzyme in a six-step pathway leading to the mineralization of atrazine in Gram-negative soil bacteria, catalyses the hydrolytic dechlorination and detoxification of atrazine to hydroxyatrazine. According to Bang et al. the use of transgenic plants expressing *atzA* to take up, dechlorinate and detoxify atrazine [26]. Alfalfa, *Arabidopsis thaliana* and tobacco were transformed with a modified bacterial *atzA* gene, p-*atzA*, under the control of the cassava vein mosaic virus promoter. All transgenic plant species actively expressed p-*atzA* and grew over a wide range of atrazine concentrations. It concludes that *in planta* expression of p-*atzA* is useful for the development of plants for the remediation of atrazine-contaminated soils and soil water, and as a marker gene to select for the integration of exogenous DNA into the plant genome.

Zeyaullah et al. developed the transgenic Nicotiana plant for the degradation of mercury. E.coli, have , a periplasmic protein, MerP, an inner- membrane transport protein, MerT, and a cytoplasmic enzyme, mercuric reductase (the MerA protein), are responsible for the transport of mercuric ions into cell and their reduction to elemental mercury, Hg0. PCR products of putative merA genes from environmental E. coli strains were purified and cloned into a plant expression vector pRT100. The construct will be transformed in calli of Nicotiana plants [27].

5. Conclusion:

The examples reviewed above indicate that remediation technologies using microorganisms are feasible alternatives to the physical cleansing of soils or the concentration of metals in polluted waters by physical or chemical means. The advantages of biological approaches include a higher specificity than the physical and chemical methods, their suitability to in-situ methodologies (e.g. the avoidance of high energy or toxic chemical addition), and the potential for improvement by genetic engineering.

Due to a whole range of factors: competition with microorganisms, insufficient supply with essential substrates, unfavorable external conditions (aeration, moisture, pH, temperature), and low bioavailability of the pollutant, biodegradation in natural conditions is lesser. So, environmental biotechnology has the objective of tackling and solving these problems so as to permit the use of microorganisms in bioremediation technologies. For this reason, it is necessary to support the activities of the indigenous microorganisms in polluted biotopes and to enhance their degradative abilities by bioaugmentation or biostimulation.

Genetic engineering is also used to improve the biodegradation capabilities of microorganisms. Plant-microbe interaction is also an inexpensive alternative for the removal of contaminants as it is widely applicable at those places where the action is less urgent, the level of contamination is not too high and when contaminated areas are large.

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