10. Biotechnological interventions in preserving environment through bioremediation

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Introduction

Environment is an essential component for survival of life. Preserving the environment is imperative to ensure the survival of future generations. However, the list of chemical pollutants released into the environment by human activities is rapidly increasing. The pollutants include petroleum hydrocarbons, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phthalate esters, nitroaromatic compounds, dyes, radionuclides, industrial solvents, pesticides and metals. Biotechnology harnesses cellular and biomolecular processes to develop technologies and products that help improve our lives and the health of our planet. With the advent of biotechnology coupled with the use of plants and microorganisms, the concentration of toxicity in the air, water and environment can be reduced to a greater extent and coined by the term 'bioremediation'.

Bioremediation is described as the process where plants and other organisms are used to clean the soil and the water, seeking to remove the pollutants from environment. Using biotechnology, the hazardous wastes can be degraded or removed from the environment using beneficial microbes and plants that have the ability to accumulate and or degrade such wastes. Using genetic engineering, microorganisms and plants that have the ability to degrade hazardous wastes have been employed successfully and are described in this article. The role of environmental biotechnology needs a special mention as in the recent decades it has played a significant role by integrating plants and microbes to address the issues related to environmental management and sustainable development.

Common pollutants in environment

Among the pollutants the major wastes arise from the agricultural and industrial applications. Agricultural wastes include agrochemical residues (thiamethoxam), animal and vegetal wastes from farms, slaughterhouses and poultry houses as well as harvest waste, fertilizer remnants and the pesticides that enter into soil, water and air. Industrial wastes comprise the major pollutants which include metallic wastes, mineral wastes, wood wastes, paper and cardboard wastes and combustion wastes. The major biodegradable pollutants are following:-

Hydrocarbons are organic compounds whose structures consist of hydrogen and carbon. They are linear linked, branched or cyclic molecules and are observed as aromatic or aliphatic hydrocarbons. The first one has benzene (C6H6) in its structure, while the aliphatic one is seen in three forms: alkanes, alkenes and alkynes.

Polycyclic aromatic hydrocarbons (PAHs): are important pollutants class of hydrophobic organic contaminants (HOCs) widely found in air, soil and sediments. The major source of PAH pollution is industrial production. They have been studied with increasing interest for more than twenty years because of more findings about their toxicity, environmental persistence and prevalence (OKERE & SEMPLE, 2012). PAHs can absorb to organic-rich soils and sediments, accumulate in fish and other aquatic organisms, and may be transferred to humans through seafood consumption. The biodegradation of PAHs can be considered on one hand to be part of the normal processes of the carbon cycle, and on the other as the removal of man-made pollutants from the environment. The use of microorganisms for bioremediation of PAH-contaminated environments seems to be an attractive technology for restoration of polluted sites.

Polychlorinated biphenyls (PCBs) are mixtures of synthetic organic chemicals. Due to their non-flammability, chemical stability, high boiling point and electrical insulating properties, PCBs were used in hundreds of industrial and commercial applications including electrical, heat transfer, and hydraulic equipment- as plasticizers in paints, plastics, and rubber products; in pigments, dyes, carbonless copy paper and many other industrial applications. Consequently, PCBs are toxic compounds that could act as endocrine disrupters and cause cancer. Therefore, environmental pollution with PCBs is of increasing concern (SEEGER *et al.,* 2010).

Pesticides are substances intended for preventing, destroying, repelling or mitigating any pest. Those that are rapidly degraded are called nonpersistent while those which resist degradation are termed persistent. The most common type of degradation is carried out in the soil by microorganisms, especially fungi and bacteria that use pesticides as food source.

Dyes are widely used in the textile, rubber product, paper, printing, color photography, pharmaceuticals, cosmetics and many other industries. Azo dyes, which are aromatic compounds with one or more (–N=N–) groups, are the most important and largest class of synthetic dyes used in commercial applications. These dyes are poorly biodegradable because of their structures and treatment of wastewater containing dyes usually involves physical and /or chemical methods such as adsorption, coagulation-flocculation, oxidation, filtration and electrochemical methods (VERMA & MADAMWAR, 2003). The success of a biological process for color removal from a given effluent depends in part on the utilization of microorganisms that effectively decolorize synthetic dyes of different chemical structures.

Radionuclides are atoms with unstable nuclei, characterized by excess energy available to be imparted either to a newly created radiation particle within the nucleus or via internal conversion. During this process, the radionuclide is said to undergo radioactive decay, resulting in the emission of gamma ray(s) and/or subatomic particles such as alpha or beta particles (LLOYD & LOVLEY, 2001).

Heavy metals cannot be destroyed, but must either be converted to a stable form or removed. Bioremediation of metals is achieved through biotransformation. Mechanisms by which microorganisms act on heavy metals include biosorption (metal sorption to cell surface by physicochemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilization through the formation of insoluble sulfides or polymeric complexes), intracellular accumulation, and enzyme-catalyzed transformation (redox reactions).

Role of microorganisms in bioremediation

Several microorganisms can be used in degrading the toxicity in the environment. Recently, Wood *et al.,* (2016) reviewed the uses of several bacteria involved in bioremediation of toxic chemicals. Some of them include *Microbacterium* spp. (Nickel), *Bacillus subtilis* (Copper) and *Pseudomonas fluorescens* (Cadmium) to name a few. *Bacillus, Corynebacterium, Staphylococcus, Streptococcus, Shigella, Alcaligenes, Acinetobacter, Escherichia, Klebsiella* and *Enterobacter*, are hydrocarbon degrading bacteria. Bacterial strains that are able to degrade aromatic hydrocarbons have been isolated from soil and characterized. These are usually gram negative bacteria most of them belonging to the genus *Pseudomonas*. The biodegradative pathways have also been reported in bacteria from the genera *Mycobacterium, Corynebacterium, Aeromonas, Rhodococcus* and *Bacillus* (MROZIK *et al.,* 2003).

Although many bacteria can metabolize organic pollutants, a single bacterium does not possess the enzymatic capability to degrade all or even most of the organic compounds in a polluted soil. Mixed microbial communities have higher biodegradative potential since genetic information of more than one bacterium is necessary to degrade the complex mixtures of organic compounds present in contaminated sites (FRITSCHE & HOFRICHTER, 2005). Both, anaerobic and aerobic bacteria are capable of biotransforming PCBs. Higher chlorinated PCBs are subjected to reductive dehalogenation by anaerobic microorganisms. Lower chlorinated biphenyls are oxidized by aerobic bacteria (SEEGER *et al.,* 2001). Research on aerobic bacteria isolated so far has mainly focused on gram-negative strains belonging to the genera *Pseudomonas, Burkholderia, Ralstonia, Achromobacter, Sphingomonas* and *Comamonas*. However, several reports about PCB-degrading activity and characterization of the genes that are involved in PCB degradation indicated PCB-degrading potential of some Gram-positive strains as well (genera *Rhodococcus, Janibacter, Bacillus, Paenibacillus* and *Microbacterium*) (PETRIC *et al.,* 2007). Successful removal of pesticides by the addition of bacteria had been reported earlier for many compounds, including atrazine (STRUTHERS *et al.,* 1998). Recent report has shown that chlorpyrifos could be degraded by *Providencia stuartii* isolated from agricultural soil (SUREKHA RANI *et al.,* 2008) and isolates *Bacillus, Staphylococcus* and *Stenotrophomonas* from soil can degrade dichlorodiphenyltrichloroethane (DDT) (KANADE *et al.,* 2012).

Moreover, bacterial strains are being harnessed for degrading the oil spills (*Alcanivorax borkumensis*), for removing hazardous wastes from water and to degrade plastics (*Ideonella sakaiensis* 201-F6). Biotechnological applications using engineered bacterial strains are rapidly increasing in the recent years with several examples. Bacterial species are assisted in the process of degradation by fungi (e.g., *Aspergillus* sp.), protozoa, and representatives of Archaea. Recently, Perpetuo *et al.* (2011) reviewed in detail about the advantages of genetic engineering of bacteria for bioremediation. They also highlighted the current situation pertaining to biosorbents, their mechanisms, pros and cons. In addition, the achievements and current status of biosorption technology, which exploits natural biodiversity and molecular tools, in order to engineer microorganisms, were discussed in detail.

Bioremediation can be classified into two different types - *in situ* bioremediation which involves treating the contaminants at the site (Ex. oil spills) and *ex situ* bioremediation which involves the removal of the contaminated material to be treated in a different place (Ex. composting). The various steps involved in bioremediation are described in Figure 1.

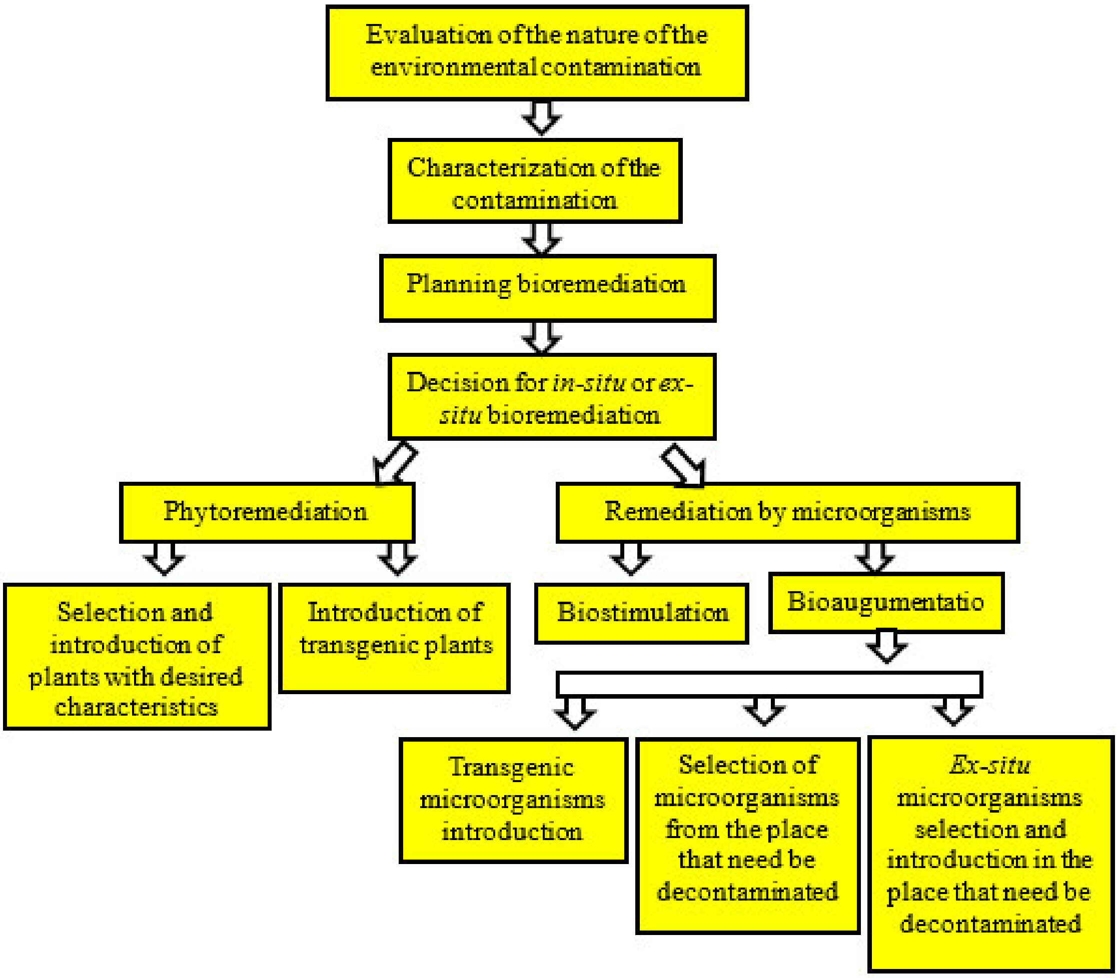

Figure 1. Steps involved in bioremediation process

Biotechnology and phytoremediation

With urbanization and population explosion, the increased human activities in the industrial and agricultural sectors have made a severe impact on the environment (REDONDO-GOMÈZ *et al.*, 2011) by the deposition of non-degradable wastes, which form a natural part of the biogeochemical cycle (BARBOSA *et al.*, 2015). The wastes constitute accumulation of heavy metals (arsenic, copper, lead, chromium, iron, manganese, zinc, etc.), petrochemical residues, sewage from industrial activities, hospital and residential activities, pesticides and fertilizers from agricultural use, explosives and ammunition from military use (LANGSTON, 2016) and other environmental pollutants (such as: benzene, chloroform, vinyl chloride, and carbon tetrachloride). To solve this problem it is not only necessary to remove the contaminants, but also to reuse and recycle these toxic products, transforming them from waste to wealth in a eco-friendly way.

Conventional strategies of phytoremediation have been focused in the prospection of indicative species that are able to fix, accumulate and/or degrade different types of toxic waste from the environment. Excess toxic elements in the environment reduce plant growth and overall development by negatively affecting the biomass production (BARBOSA *et al.*, 2015). In addition, another major problem is the low ability of fixation and immobilization of toxic elements. In this sense, some initiatives have been done by testing the effect of growth regulators in reducing environmental stress and increase the fixation of toxic components having been verified that the brassinosteroids had a positive effect by increasing the photosynthetic capacity and biomass production in crop species (VÁZQUEZ *et al.*, 2013; COLL *et al.*, 2015).

Other studies have used biotechnological techniques to produce genetically modified plants with high capacity to extract toxic elements from soil and water to perform phytoremediation of contaminated environments. This latter approach has been known as environmental biotechnology which includes the integration of natural sciences and genetic engineering in order to achieve plants that are able to degrade environmental contaminants (NANEKAR & JUWARKAR, 2015). This technique has become promising in the remediation of contaminated soils, but its success depends on the ability of the selected species to produce high amount of biomass, and extract and accumulate the toxic compound present in the soil (REDONDO-GOMÈZ *et al.*, 2011).

According to Pilon-Smits (2005) phytoremediation is based on the use of plants and microbes associated in order to remove contaminants from the environment. There are different ways to start the cleaning processes (Figure 2):

1. Phytodegradation - refers to uptake, metabolism and degradation of the contaminant in the body of the plant;
2. Phytoextraction - is considered the best way to remove pollutants from the soil which implies in the uptake and accumulation of the contaminant in harvestable parts of the plant tissue;
3. Phytovolatilization - comprehends the uptake of the contaminant by the plant and later release in volatile degraded product;

4. Phytostabilization or phytoimmobilization - means the use of plants to control *in situ* contaminants by changing the chemical, physical or biological conditions of the soil;
5. Phytostimulation - refers to the process where chemical substances released by the roots of plants mimic pollutants that enhance microorganisms' activities in the rhizosphere. These chemical compounds may serve as energy sources to the microorganisms;
6. Rhyzofiltration - the process where plant roots remove contaminants by absorption, precipitation and concentration of pollutants.

Environmental biotechnology techniques have improved the fixing and storage capacity of several species. Examples of transgenic plants include *Populus tremula* L. x *Populus alba* L. with increased capacity of the cytochrome P450 E1 being able to absorb trichloroethylene, vinyl chloride, carbon tetrachloride, chloroform and benzene from air and hydroponic solutions (DOTY *et al*., 2008); *Agrostis stolonífera* L. and *Panicum virgatum* L. with two bacterial genes being able to absorb and utilize nitramide (popular as RDX - a toxic component from explosives) in the plant metabolism, where the best lineages could neutralize all toxic products from the soil in less than two weeks (LANGSTON, 2016); *Nicotiana glauca* Graham transformed with the phytochelatin gene TAPCS1 showing higher capacity in accumulating zinc, boron, cadmium, nickel and lead. The tolerance to heavy metals was also found in *Arabidopsis* Heynh., *Nicotiana tabacum* L., and *Brassica juncea* (L.) Czern. with the over-expression of genes that induce the phytochelatin formation (ISAA, 2006). Other examples of transgenic plants developed to extract toxic products from environment can be obtained from ISAA (www.isaa.org.kc).

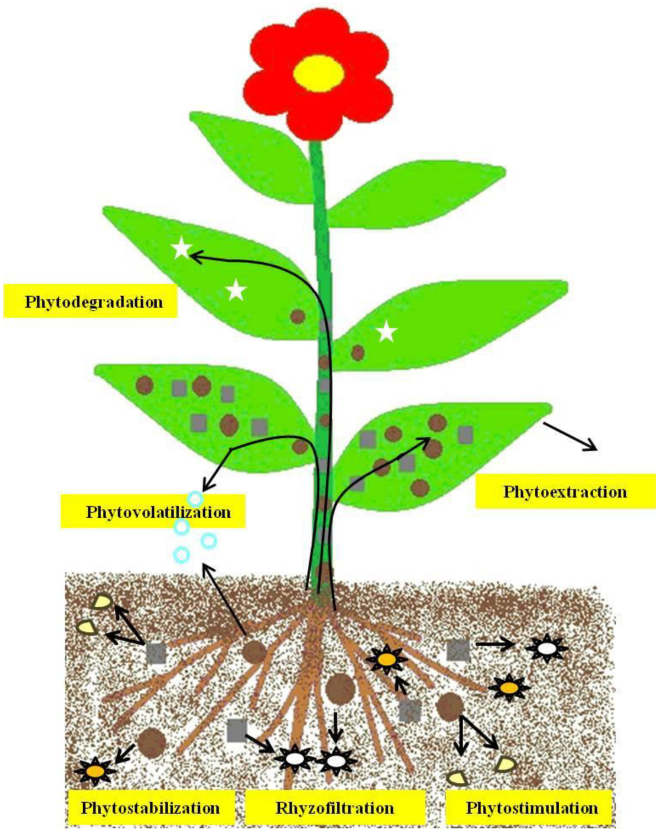

Figure 2. Different methods of phytoremediation (Adapted from Pilon-Smits, 2005 and Sharma & Juwakar, 2015)

Phytoremediation: Merits and demerits

Though phytoremediation is more advantageous, there exist few limitations too. It is rather a slow process and the contaminated material must be in proximity to the plant. Recently, Sharma & Juwarkar (2015) reviewed various advantages and limitations of phytoremediation which are briefly described below:

Merits

- Bound environmental disturbance and cost effective;
- Well studied in large contaminated area;
- Exploit the competence of the environment restore itself;
- Plants might be used as indicators of contaminated and phytoremediated area;
- Help stop the dissemination of the contamination, keeping them within or near the roots;
- Some plants that growing in wetland are able to transfer oxygen to the rhizosphere region allowing aerobic degradation of the contaminants by microorganisms;
- Improve the soil structure and quality;
- Increase soil porosity and water absorption that will accelerate nutrient cycling and increase the organic carbon in the soil;
- Prevent soil erosion;
- Avoid secondary wastes in the water and the air;
- Powerful in reducing $CO_{2;}$
- Help to improve the floral diversity by the green belt formation.

Demerits

- Is not so fast as the other *ex situ* methods to remove contaminants from the soil;
- Need a long time to clean up the environment;
- No indication in human or environmental conditions where contamination needs to be immediately removed;
- Contamination fixed on the soil or organic matter is not available to be degraded by plants;
- Environmental conditions should be within the plant's proximity.

Phytoremediation is thus a really useful process which when coupled with biotechnological tools is certainly a boon to environment. As far as limitations are concerned, the approach is under research and constant steps are being taken up by the research community to improvise the methods and reduce the limitations. One of the best examples is the development of transgenic *Arabidopsis thaliana* carrying the enzyme mercuric reductase from bacteria and is widely used in the biodegradation of mercury in soil.

Conclusions

To summarize, biotechnology is undoubtedly a wonderful approach for environmental cleaning. However, there are some challenges and risks which would be overcome in the near future. According to ISAA, although the use of biotechnology in developing transgenic plants aids in environmental cleaning, several challenges still remain to be overcome and are mentioned below

- A better understanding of the molecular mechanisms involved in degradation of the pollutants, and discovery of suitable genes for phytoremediation;
- Phytoremediation is only available for restricted number of pollutants and several contaminated patches exist with different pollutants and need to be resolved;
- The environmental biotechnology approaches are still in infancy. Field testing for phytoremediation is limited due to problems related to biosafety and gene escape.

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