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Rhizoremediation: A Promising Rhizosphere Technology

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1. Introduction

An increasingly urban population and industrialized global economy over the last century have serious consequences on the environment. Understanding the sources, pathways and contaminants in the urban environment is essential for making informed management decisions. Urban areas are major concentrators, repositories and emitters of a myriad of chemicals because of the wide range and intensity of human and anthropogenic activities. Common contaminants include petroleum hydrocarbons (PHCs), polycyclic aromatic hydrocarbons (PAHs), halogenated hydrocarbons, pesticides, solvents, metals, salt and the resulting stresses on human and ecosystem health are well documented [1]. Polycyclic aromatic hydrocarbons are a class of complex organic chemicals consisting of over hundred different organic compounds. PAHs are unique contaminants in the environment because they are generated continuously by incomplete combustion of organic matter, for instance in forest fires, home heating, traffic, and waste incineration [2]. PAHs are hydrophobic compounds and their persistence in the environment is chiefly due to their low water solubility [3]. Generally, solubility of PAHs decreases and hydrophobicity increases with an increase in number of fused benzene rings. In addition, volatility decreases with an increasing number of fused rings [4]. The major source of PAHs is from the combustion of organic material [5]. PAHs are formed naturally during thermal geologic production and during burning of vegetation in forest and bush fires [6]. PAHs and their alkyl homologous may also be derived from biogenic precursors during early diagenesis [7]. However, anthropogenic sources, particularly from fuel combustion, pyrolytic processes, spillage of petroleum products, waste incinerators and domestic heaters [8] are significant sources of PAHs in the environment. At depth 90-135 cm, only

phenanthrene (1.4 mg/kg), pyrene (4.0 mg/kg), chrysene (0.9 mg/kg) and dibenzoanthracene (0.8 mg/kg) were found [9]. The concentration of PAHs in the environment varies widely, depending on the level of industrial development, proximity of the contaminated sites to the production source and the mode of PAH transport. Kanaly and Harayama [10] reported that in soil and sediment, PAHs concentrations vary from 1 µg/kg to over 300 g/kg.

PAHs have been detected in a wide variety of environmental samples, including air [8], soil [11], sediments [19], water [12], oils, tars [13] and foodstuffs [14]. PAHs contamination on industrial sites is commonly associated with spills and leaks from storage tanks and with the conveyance, processing, use and disposal of these fuel/oil products [4]. PAHs are also a major constituent of creosote (approximately 85% PAH by weight) and anthracene oil, which are commonly used pesticides for wood treatment [15]. The main route for PAH transport is through the atmosphere. Results from ambient air monitoring programs have shown that PAH concentrations are usually of the order of a few nano-grams per cubic metre of air [16]. However, PAH concentrations may vary from season to season depending on emissions arising from the combustion of home heating products. Motor vehicles, including spark emission and diesel automobiles, trucks and buses, also contribute to atmospheric PAHs pollution through exhaust condensate and particulates, tyre particles and lubricating oils and greases [17]. During the combustion of fossil fuels, diesel powered vehicles are the major sources of lighter PAHs to the atmosphere, whereas gasoline vehicles are the dominant source of higher molecular weight PAHs [18]. The persistence of PAHs in the environment depends on the physical and chemical characteristics of the PAHs. PAHs are degraded by photo-oxidation and chemical oxidation [19], but biological transformation is probably the prevailing route of PAH loss [20]. The microbial metabolism of PAHs containing up to three rings (naphthalene, phenanthrene, anthracene, fluorene) has been studied extensively.

Heavy metals such as lead, mercury, and cadmium are ranked second, third, and seventh, respectively, on the 2003 Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA, commonly known as Superfund) priority list for hazardous substances because they are toxic widespread pollutants. Soil contamination is a particularly serious environmental concern, as the majority of superfund sites are highly contaminated with heavy metals [21].

Therefore, for remediation of contaminated environmental soil, the traditional technologies routinely used are as excavation, transport to specialized landfills, incineration, stabilization and followed by coagulation filtration or ion exchange are expensive and disruptive to the sites [22]. However, there has been much interest in bioremediation technologies which use plants and microorganisms (including bacteria) to degrade toxic contaminants in environmental soil into less toxic and/or non-toxic substances [23]. 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 [24].

Biosorption (using microbially produced metallothioneins (MTs) and phytochelatins (PCs) having heavy metal binding affinities) and immobilization are major mechanisms utilized by animals and plants to limit the concentrations of internal reactive metal species [25].

Phytoremediation, the use of plants to degrade toxic contaminants in the environment involves a number of processes including phytoextraction, phytotransformation, phytostabilization, phytovolatilization and rhizofiltration [26]. Phytoextraction (or phytoaccumulation) involves the uptake and concentration of pollutants into harvestable biomass for sequestration or incineration. Phytotransformation involves enzymatic modification resulting in inactivation, degradation (phytodegradation), or immobilization (phytostabilization) of pollutants. Phytovolatilization involves the removal of pollutants from soil and their release through leaves via evapotranspiration processes and rhizofiltration involves the filtering of water through a mass of roots to remove pollutants. While some success has been reported using plants alone in bioremediation, the use of plants in conjunction with plant associated bacteria offers much potential for rhizoremediation [27].

The importance of plant microbe partnerships in the remediation of organic contaminants was confirmed in studies at the level of rhizosphere [28, 29], the phyllosphere and inside the plant [30, 31]. Rhizoremediation is considered as the most potential approach for PAHS remediation in soil [37]. Soil microflora play vitally important role during rhizoremediation of xenobiotics [32]. The interaction among microbial degrader, plant and PAHs in soil might be regulated through rhizosphere processes [39].

Rhizoremediation systems for PAHs rely on a synergistic relationship between suitable plants and their root associated microbial communities [32]. Degradation is facilitated through a rhizosphere effect where plants exude organic compounds through their roots and thereby increase the density and activity of potential hydrocarbon degrading microorganisms in the zone, surrounding the roots [35]. The biodegradation abilities of bacteria and the expression and maintenance of bacteria in the rhizosphere are extremely important for the effective removal of contaminants in rhizoremediation [36]. Thus, bioremediation, phytoremediation and rhizoremediation contribute significantly to the fate of hazardous waste and can be used to remove these unwanted compounds from the biosphere [37, 38].

Rhizoremediation is proposed as the most potential approach for PAHs remediation in soil [39]. Plant associated bacteria, such as rhizospheric bacteria have been shown to contribute to biodegradation of toxic organic compounds in contaminated soil and could have potential for improving phytoremediation [40]. The amalgamation of the activity of plant roots and rhizospheric microbial communities like secretion of root exudates (various organic acids and amino acids etc.), production of siderophores, HCN, phytohormone and phosphatases by plant growth promoting rhizobacteria (PGPR) are also effective for ecorestoration of polluted sites [41]. The plant growth-promoting capability of *B. aryabhattai* strains may be utilized as an environmentally friendly means of revegetating barren lands [41]. The valuable effects of some rhizobacteria on plant growth are well known, and the so called PGPR have been utilized for several decades, although their mechanisms of plant growth promotion have not been completely elucidated [42]. Some of the important mechanisms include direct phytohormonal action, increase of plant nutrient availability and the enhancement of other plant beneficial microorganisms [43]. When a suitable rhizospheric isolated strain is introduced together with a suitable plant, it inhabits on the root along with indigenous population, thereby enhancing the bioremediation process [44]. In addition,

such capability for root colonizing, pollutant degrading bacteria utilize the growing root system and hence this acts as an injection system to spread the bacteria through soil [45]. Plant root performs certain specialized roles, including the ability to synthesize, accumulate and secrete a diverse array of nutrient compound consequently no requirement of exogenous carbon source, roots may regulate the soil microbial community in their immediate vicinity, cope with herbivores, encourage beneficial symbioses, change the chemical and physical properties of the soil and inhibit the growth of competing plant species [46]. PGP bacteria may facilitate plant growth either directly or indirectly [47]. Though the rhizoremediation process takes place naturally, but it can be modified by premeditated exploitation of the well-equipped rhizospheric microorganisms whereas it can be proficient by using suitable plant microbe pairs.

Incorporation of plant and PGPR having the pollutant degrading activity may be performed. Similarly, Kuiper *et al.* [48] described the pair of a grass species with a naphthalene degrading microbe which protected the grass seed from the toxic effects of naphthalene and the growing roots exploited with the naphthalene degrading bacteria into soil.

Previously, several researchers have also used this symbiotic relationship of plant and microbes for degradation of hazardous and xenobiotic compounds like PCBs, PAHs and TCE [49]. Mechanical injection of contaminated sites with pollutant degrading bacteria has been used to clean polluted sites in an inexpensive and less labor intensive way than the removal and/or combustion of polluted soils [50].

2. Bioremediation

Polycyclic aromatic hydrocarbons (PAHs) are of particular concern because of their toxic, mutagenic and carcinogenic properties [51]. There is thus a chief interest in studying microorganisms present in contaminated environments as a means for bioremediation. The fate of PAHs and other organic contaminants in the environment is associated with both abiotic and biotic processes, including volatilization, photooxidation, chemical oxidation, bioaccumulation and microbial transformation. Microbial activity has been deemed the most influential and significant cause of PAHs removal [3, 12]. PAHs may also be degraded by some microorganisms in the soil [52]. The term bioremediation refers to the use of living organisms to degrade environmental pollutants [53]. Bioremediation is generally considered to include natural attenuation, biostimulation or bioaugmentation, the deliberate addition of natural or engineered microorganisms to accelerate the desired catalytic capabilities. According to the Environmental Protection Agency in the United States [54], natural attenuation processes may reduce contaminant mass (through destructive processes such as biodegradation and chemical transformations), reduce contaminant concentrations (through simple dilution or dispersion). Eventually, even the contaminants bound to the soil particles gets biodegraded by the bacterial species present in the environment.

3. Bioaugmentation

Bioaugmentation is the introduction of microorganisms with specific catabolic abilities into the contaminated environment in order to supplement the indigenous population and to speed up or enable the degradation of pollutants [48, 55]

Bioaugmentation has proven successful for remediation of PAHs in sediments with poor or lacking intrinsic degradation potential [17], while other studies demonstrated that bioaugmentation did not enhance biodegradation significantly compared to natural attenuation [56]. One of the main problems in applying bioaugmentation is to ensure the survival and activity of the introduced organisms in the environment [55]. Bioaugmentation can be inhibited by a variety of factors including pH and redox, the presence of toxic contaminants, concentration and bioavailability of contaminants or the absence of key co-substrates [48]. However, the key factor for the success of bioaugmentation process is the selection of the appropriate bacterial strain. When selecting the strain for augmentation purposes, the kind of microbial communities present in the source habitat should be considered [57]. Bioaugmentation strategies may prove successful especially in the remediation of manmade contaminants, where specialized bacteria with the appropriate catabolic pathways may not be present in the contaminated habitat [55].

4. Phytoremediation

Some workers quantified and compared the responses of soil microbial communities during the phytoremediation of PAHs in a laboratory trial [15]. A recent publication of some workers describes the development of transgenic poplars (*Populus* sp.) over expressing a mammalian cytochrome P450, a family of enzymes commonly involved in the metabolism of toxic compounds. The engineered plants showed enhanced performance about the metabolism of trichloroethylene and the removal of a range of other toxic volatile organic pollutants, including vinyl chloride, carbon tetrachloride, chloroform and benzene. Some workers suggested that transgenic plants might be able to contribute to the wider and safer application of phytoremediation [58]. Widespread phytoremediation field trials research was performed *in vitro* condition and many of the works explored the effects of plants on removal of contaminants from spiked soil and soil excavated from contaminated sites [7] and most of these experiments provided valuable insights into the specific mechanisms of phytoremediation of organic contaminants [29]. Previously, numerous organic pollutants such as TCE (trichloroethylene), herbicides such as atrazine, explosives such as TNT (trinitrotoluene), PHC, BTEX (mono aromatic hydrocarbons) and PAHs, the fuel additive MTBE (methyl tertiary butyl ether), and PCBs (polychlorinated biphenyls) have been successfully phytoremediated. [59] Major advantages of phytoremediation *viz.*, cost of the phytoremediation is lower than that of traditional processes both *in situ* and *ex situ*, plants can be easily monitored, possibility of the recovery and re-use of valuable products, use of naturally occurring organisms and preservation the natural state of the environment, low cost of phytoremediation (up to 1000 times cheaper than excavation and reburial) [60].

5. Rhizoremediation

Rhizospheric microbes can degrade the majority of environmental pollutants and degradation process stops when the microbe is deprived of food. These microbes have access to the best food source available in soil, namely root exudates [61]. Researchers have described an enrichment method for the isolation of microbes [62], which combine the properties of degradation of a selected pollutant and excellent root colonization. They have termed this process 'rhizoremediation' instead of phytoremediation to emphasize the roles of the root exudates and the rhizosphere competent microbe. The high concentration of metals in soils and their uptake by plants harmfully influence the growth, symbiosis and consequently the yields of crops [63] by disintegrating cell organelles and disrupting the membranes, acting as genotoxic substance [64] disrupting the physiological process, such as, photosynthesis or by inactivating the respiration, protein synthesis and carbohydrate metabolism. *Pseudomonas putida* is a root colonizer of potential interest for the rhizoremediation of pollutants and the biological control of pests [65]. According to hypothesis when a suitable rhizosphere strain is inoculated together with a suitable plant (e.g., coating bacteria on plant seed), these well-equipped bacteria might settle on the root together with the normal indigenous population, thereby enhancing the bioremediation process. Pioneer work about degradation of compounds in the rhizosphere was mainly focused for herbicides and pesticides [66]. In the past two decades, a large number of publications on rhizodegradation of various organic toxicants using different plants and/or microbial inoculants have been published [37, 67-71].

Field contaminated soils that have undergone prolonged periods of ageing generally appear to be much less responsive to rhizodegradation than freshly spiked soil [72-74]. Wenzel [67] concluded that low bioavailability is a main cause of failure of rhizodegradation in field contaminated and aged spiked soils. This has important implications for the applicability of rhizodegradation as well as for the evaluation of data obtained on freshly or only shortly aged, spiked soil material. Other strategies to enhance rhizodegradation (e.g. inoculation of degrader strains) are likely to fail where low bioavailability is the main constraint.

Interestingly, microbial treatments appeared to be successful at the laboratory experiments [75] but failed when applied to long term contaminated soil on field experiments [76]. This indicates again the importance of the experimental scale and of bioavailability. In view of the still disappointing and controversial results of traditional inoculation [77], enhanced rhizodegradation requires more sophisticated approaches. Enhanced degradation capabilities of inoculated microorganisms may be obtained by induction of a nutritional bias towards the inoculated strains. Only recently, Narasimhan *et al.* [78] identified root exudate compounds (phenylpropanoids) that created a nutritional bias in favour of enhanced PCB degradation. A successful rhizoremediation process could depend on the highly branched root system of the plant species where a large number of bacteria harbor, establishment of primary and secondary metabolism, survival and ecological interactions with other organisms [48]. Plant roots can act as a substitute for the tilling of soil to incorporate additives (nutrients) and to improve aeration in soil [48]. Plants also release a variety of photosynthesis derived organic compounds (root exudates), which might help in

degradation of pollutants [59]. The root exudates consists of water soluble, insoluble, and volatile compounds including sugars, alcohols, amino acids, proteins, organic acids, nucleotides, flavonones, phenolic compounds and certain enzymes [68].

Normally a symbiotic relationship develops between plant and soil microbes in the rhizosphere, where plants provide nutrients necessary for the microbes to flourish, while the microbes provide a healthier soil environment where plant roots can grow. Specifically, plants loosen soil and transport oxygen and water into the rhizosphere [26]. In addition, plants exude specific phytochemicals (sugars, alcohols, carbohydrates, etc.) that are primary sources of food (carbon) for the specific soil organisms that aid in providing the healthier soil environment [79]. Alternatively, the exuded phytochemical may be an allelopathic agent meant to suppress other plants from growing in the same soil [26]. In return for exporting these phytochemicals, plants are protected from competition, soil pathogens, toxins and other chemicals that are naturally present or would otherwise be growing in the soil environment [26]. Microbial populations can be several orders of magnitude higher in a vegetated soil compared to an un-vegetated soil. Rhizodegradation, sometimes called phytostimulation, rhizosphere biodegradation or plant assisted bioremediation/degradation, is the enhanced breakdown of a contaminant by increasing the bioactivity using the plant rhizosphere environment to stimulate the microbial populations [26].

6. Factor influencing PAH degradation

Several factors that influence the rate of rhizoremediation of PAHs in soil e.g. soil type, texture, particle size, nutrients and organic matter content which can limit the bioavailability of pollutants [67]. The conditions that increase the possibility of degradation include the presence of low molecular weight PAHs, relatively recent PAHs emission or deposition, moderate soil pH, the presence of appropriate PAHs degrading bacteria and plants to facilitate decomposition by virtue of large root surface area or uptake affinity [80]. Root microbe interactions are considered the primary process of PAHs phytoremediation [81]. Natural attenuation in vegetated settings is thought to degrade one, two and three chain PAHs in periods ranging from 16 to 126 days [82]. By-products of degradation are thought to be less toxic and may serve as an energy source for other soil organisms. Research suggests that PAHs with fewer benzene rings are more easily digested by soil microbes. Johnson *et al.* [32] suggests that microbial degradation of PAHs and other hydrophobic substrates is believed to be limited by the amounts dissolved in the water phase, with sorbed, crystalline and non-aqueous phase liquid dissolved PAHs being unavailable to PAH degrading organisms.

The main problem for soil bioremediation is the bioavailability of the pollutant. Most of organic pollutants are highly hydrophobic compounds that dissolve poorly in water and many of them can form complexes with soil particle, this lack of bioavailability often lowers removal efficiencies [26]. Bioavailability is a dynamic process, determined by the rate of substrate mass transfer to microbial cells relative to their intrinsic catabolic activity [26].

7. Bioavailability

Bioavailability refers to the fraction of a chemical that can be taken up or transformed by living organisms from the surrounding bio-influenced zone where organism mediated biochemical changes occur [83, 84]. The success of any rhizoremediation process depends on the bioavailability of the specific pollutant and root microbial modifications of their solubility, physiochemical properties of the pollutant, soil properties, environmental conditions, biological activity and chemical speciation in the rhizosphere [59, 67]. Bio surfactants increase the bioavailability of hydrocarbons resulting in enhanced growth and degradation of contaminants by hydrocarbon degrading bacteria present in polluted soil [85].

The important pollutant properties controlling their fate in the environment include the vapour pressure and the Henry's constant [67]. The vapour pressure indicates whether or not a pollutant is easily volatilised in dry soil conditions, the Henry's constant provides a better measure of the volatilisation potential in wet and flooded soil. As the residence time in soil of highly volatile compounds such as chloroethene will be short, they are not a primary target of rhizodegradation. The solubility of a pollutant is further modified by soil properties. Organic matter quality and content, clay content, mineral composition, type of mineral surface, pH and redox potential are known as important controls of organic pollutant solubility, with hydrophobic, nonpolar organic matter being of particular importance for binding organic pollutants [86]. Binding of organic pollutants to the soil matrix is known to progress as the contact time increases, rendering pollutants less bioavailable [67]. This phenomenon is known as "ageing" and is attributed to sorption onto minerals and organic matter in soil, and subsequent interparticle diffusion in minerals and entrapment within humic complexes, nano- and microspores [83, 86].

Apart from the absorption capability of the organisms (biology), the bioavailability of a pollutant in soil not only depends on its solubility (chemistry), but also its diffusion and mass transport (physics) towards the sites and niches where degrader populations are abundant [83]. It is well established that bioavailability is one of the most limiting factors in bioremediation of persistent organic pollutants in soil [37, 86]. In bioreactor systems this problem is often addressed by agitation and mixing and addition of surfactants [34]. In recent past several microbes have been reported to be chemotactic towards different organic pollutants, for example toluene acting as chemoattractant to *Pseudomonas putida* [87]. Chemotactic bacteria might be more competent for bioremediation than their non-chemotactic counterparts [87].

8. Biodegradation of PAH

Many bacterial, fungal and algal strains have been shown to degrade a wide variety of PAHs [88]. The most commonly reported bacterial species include *Acinetobacter calcoaceticus*, *Alcaligenes denitrificans*, *Mycobacterium* sp., *Pseudomonas putida*, *Pseudomonas fluorescens*, *Pseudomonas vesicularis*, *Pseudomonas cepacia*, *Rhodococcus* sp., *Corynebacterium renale*, *Moraxella* sp., *Bacillus cereus*, *Beijerinckia* sp., *Micrococcus* sp., *Pseudomonas paucimobilis* and *Sphingomonas* sp. [89].

Pseudomonas putida is a good candidate for metabolic engineering and genetic manipulation applications for expression of genes encoding several degradative enzymes [88]. Therefore, a *P. putida* strain was engineered to increase the efficiency of degradation of naphthalene and salicylate [88]. Similarly, another study demonstrated the efficiency of the naphthalene degradation process performed by different microbial strains of the genera *Pseudomonas* and *Burkholderia* in soil model systems [90]. Previous, studies have shown that bacteria can degrade BaP when grown on an alternative carbon source in liquid culture experiments [91]. Sor-khohet *al.* [93] isolated spore forming PAHs degrading bacteria and reported their subsequent genetic studies of their degradation pathways which may lead to the discovery of novel genes involved. Various studies showed the significance of the rhizospheric effect on degradation of organic pollutant molecules [94].

In recent past, Bisht *et al.* [44] reported four bacteria from non-contaminated rhizosphere of *P. deltoides* which were able to degrade 80-90% degradation of anthracene and naphthalene within 6 days. The maximum degradation pathways were reported from *Mycobacterium vanbaalenii* because of its exceptional ability to degrade a great variety of low and high molecular weight PAHs oxidatively in soil versatility of this species makes it probable inoculants in the remediation of PAHs contaminated sites [95].

Calotropis sp. as a dominant and common desert plants that grows widely in warm and urbanizing regions and has a high capacity for taking heavy metals into its tissues due to their abilities to absorb and tolerate heavy metals [96]. The use of the leaf biomass of *Calotropis procera* can be employed as good bio-sorbent for the removal of Cr (III) from aqueous solutions and as an alternative method of their removal from industrial effluent [97].

9. Rate of PAH Biodegradation

The rate and degree of PCB degradation decreases with the increase of chlorination degree. For example, 62% 2-Cl-PCBs, 28% 3-Cl-PCBs, 24% 4-Cl-PCBs, and 18% 5-Cl-PCBs were degraded during the two-month active treatment phase [98]. The reversibly sorbed PCBs will be bio stabilized within 5, 6, 12 and 15 years, respectively, during the passive phase. The rate of biodegradation of PAHs is highly erratic and is dependent not only on PAH structure, but also on the physicochemical parameters of the site as well as the number and types of micro-organism present. The rate and degree of PAH degradation decreases with the increase of number of benzene ring. PAHs sorb to organic matter in solid and sediments, and the rate of their sorption strongly controls the rate of which microorganism can degrade the pollutant. Much of the current PAH research focuses on techniques to enhance the bioability and therefore, the degradation rates of PAHs at polluted site. The sequential active passive biotreatment approach is an effective scheme for degradation of both PAHs and PCBs in the land treatment systems. The quantitative model, together with laboratory and field testing, can be a useful tool for the plan, design and operation of similar land treatment systems.

10. Microbial enzymes involved in PAHs degradation process

As bacteria initiate PAHs degradation by the action of intracellular dioxygenases, oxygenase, dehydrogenase, phosphatases, dehalogenases, nitrilases, nitroreductases and lignolytic enzymes [33] (Table 1). Aromatic ring dioxygenases are multicomponent enzymes which consist of an electron transport chain containing a ferredoxin and a reductase and a terminal dioxygenase [99]. The best studied PAHs dioxygenase is naphthalene dioxygenase from *Pseudomonas putida* encoded by the NAH plasmid pDTG1 [100].

Enzyme	Target pollutant
Aromatic dehalogenase	Chlorinated aromatics (DDT, PCBs etc.)
Carboxyl esterases	Xenobiotics
Cytochrome P450	Xenobiotics (PCBs)
Dehalogenase	Chlorinated solvents and Ethylene
Glutathione s-transferase	Xenobiotics
Peroxygenases	Xenobiotics
Peroxidases	Xenobiotics
Laccase	Oxidative step in degradation of explosives
N-glucosyl transferases	Xenobiotics
Nitrilase	Herbicides
Nitroreductase	Explosives (RDX and TNT)
N-malonyl transferases	Xenobiotics
O-demethylase	Alachlor, metalachor
O-glucosyl transferases	Xenobiotics
O-malonyl transferases	Xenobiotics
Peroxdase	Phenols
Phosphatase	Organophosphates

Table 1. Some important enzymes associated with bioremediation [26].

11. Improvement in rhizoremediation

Rhizoremediation process can be designed to improve in several aspects like bioavailability of contaminant molecules, expression and maintenance of genetically engineered plantmicro-bial systems and root exudates for the effectiveness of the process.

Selection of bacteria, which are able to produce biosurfactants in the rhizosphere of the plants, is an interesting alternative to improve the removal efficiency [85]. In this context Kuiper *et*

al. [48] identified bacteria growing in a PAHs contaminated area that produces biosurfactants that facilitate the solubilisation of PAHs and hence biodegradation by microbes. This property is also of interest because a number of biodegradative microbes exhibit positive chemotaxis towards the pollutants [44]. Therefore, the combined action of biosurfactant and chemotaxis can contribute to bacterial proliferation and to microbial spread in polluted soils, in order that more ample zones can be cleaned [28].

Microbial degradation of contaminants in the rhizosphere provides a positive effect for the plant; the pollutant concentration is decreased in the area near the roots and the plant can grow better than those in contaminated areas [101]. Because of this mutual benefit it has been proposed that plants can select specific genotypes to be present in their roots. Experiments performed by Siciliano *et al.* [102] demonstrated that the presence of the alkane monooxygenase gene was more prevalent in endophytic and rhizosphere microbial communities than in those present in bulk soil contaminated with hydrocarbons. However, the results obtained when they studied the prevalence of the xylene monooxygenase or naphthalene dioxygenase genes were just the opposite, their presence was higher in bulk soil microbial communities than those near or on the plant. This suggests that if plants are influencing the rhizosphere, this effect is dependent on the contaminant. Some researchers also concluded that the effect depended on the type of the plant. This has led to the hypothesis that the effectiveness of rhizoremediation strategies is related to the selection of the best plant bacterium pair in each case.

In a case study it was found that rhizospheric *Pseudomonas* sp. of *Calotropis* plant a good degrader for naphthalene (78.44 %) and anthracene (63.53 %) as determined by HPLC analysis. Thus, it can be concluded that rhizosphere of *Calotropis* sp. is a source of *Pseudomonas* sp. possessing potent PGP attributes, PAH degradation and biocontrol activities against phytopathogenic fungi. Further studies are under- way to confirm their effectiveness in field conditions [103].

A number of scientists established chemotaxis of PAHs degrading rhizosphere bacteria (*P. alcaligenes*, *P. stutzeri* and *P. putida*) to naphthalene, phenanthrene and root exudates [104]. Fascinatingly, the bacteria were repelled by anthracene and pyrene. The attraction of competent bacteria to the root zone may improve bioavailability and increase PAHs degradation in the rhizosphere. Subjugation of the phenanthrene degrading activity of *P. putida* following exposure to root extracts and exudates recommended that enzyme induction may not occur during rhizodegradation of PAHs [92]. Genetically engineered plant microbial systems to improve the rhizoremediation techniques, in which the gene cloning of plants containing bacterial gene for the degradation of organic pollutants and of recombinant, root-colonizing bacteria (e.g. *P. fluorescens*) expressing degradative enzymes e.g. orthomonooxygenase for toluene degradation [25].

The studies pertaining to the rhizospheric microorganism associated with specific plant are still missing in the available literature even though a lot of work has been reported on bioremediation. Many researchers have carried out work on plant growth promoting (PGP) activity of rhizosphere of different plants, but no information about rhizosphere community of specific plant, its molecular characterization and utilization in sustainable agriculture, biofertilization and ecorestoration is reported in the literature. Rhizoremediation can be

successfully used for restoration of contaminated sites by choosing right type of plant cultivar with right rhizobacteria or by inoculating efficient rhizobacterial strains on plant seeds [34]. Bacteria inhabiting the rhizosphere of a suitable plant may be used as 'bacterial injection system' in soils for effective growth promotion and rhizoremediation.

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