IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 8, Issue 8 Ver. II (Aug. 2014), PP 23-27
www.iosrjournals.org

Rhizoremediation: A Promising Rhizosphere Technology

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Abstract: *Interactions between microorganisms and plants have undoubtedly had major effects on the development of civilization since humans began to rely extensively on cultivated crops for food. Positive and negative interactions take place not only between microbes but also between microbes and plants. The rhizosphere is a zone of predominantly commensal and mutualistic interactions between plants and microbes. 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. 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. Worldwide, contamination of soil and ground water is a severe problem. The negative effects of pollutants on the environment and on human health are diverse and depend on the nature of the pollution. Awareness about harms of pollution in the scientific world has aroused in 1990s but now even general public is highly concerned about this issue. The search for alternative methods for excavation and incineration to clean polluted sites resulted in the application of bioremediation techniques. A cost effective and highly ethical method Rhizoremediation which involves the breakdown of contaminants in soil resulting from microbial activity that is enhanced in rhizosphere. So, an effort is made to review the recent advancements on rhizoremediation-based abolition of heavy metals.*
Key words: *rhizosphere; rhizoremediation; heavy metals.*

I. Introduction

Intensification of agriculture and manufacturing industries has resulted in increased release of a wide range of xenobiotic compounds to the environment. Excess loading of hazardous waste has led to scarcity of clean water and disturbances of soil thus limiting crop production (Kamaludeen et al., 2003). The negative effects of pollutants on the environment and on human health are diverse and depend on the nature of the pollution. Common contaminants include petroleum hydrocarbons (PHCs), polycyclic aromatic hydrocarbons (PAHs), halogenated hydrocarbons, pesticides, solvents, metals, salt and the results in stresses on human and ecosystem health. The search for alternative methods for excavation and incineration to clean polluted sites resulted in the application of rhizoremediation techniques. Rhizosphere bioremediation or rhizodegradation is the enhanced biodegradation of recalcitrant organic pollutants by root-associated bacteria and fungi under the influence of selected plant species. Use of selected vegetation and sound plant management practices, increase the total proportion of pollutant degraders in numbers and activity in the rhizosphere, leading to enhanced rhizodegradation. The rhizosphere is the zone of soil around the root in which microbes are influenced by the root system forming a dynamic root-soil interface (Kuiper et al., 2004; Pilon-Smits, 2005; Barea et al., 2005). The use of microbial metabolic potential for eliminating soil pollutants provides a safe and economic alternative to other commonly used physico-chemical strategies (Vidali, 2001). Soil pollutants that are remediated by this method are generally organic compounds that cannot enter the plant because of their high hydrophobicity. Plants are generally not considered as the main mode of remediation in this technique. Plant-microbial interactions in the rhizosphere offer very useful means for remediating environments contaminated with recalcitrant organic compounds (Chaudhry et al., 2005). The root system of plants can help to spread bacteria through soil and help to penetrate otherwise impermeable soil layers. The plant creates a niche for rhizosphere microorganisms to do the degradation. Root exudates and root turnover can serve as substrates for microorganisms that perform pollutant degradation. Selection for organisms that may be useful in rhizoremediation has been attempted with good success. Studies of the most suitable plant species for rhizoremediation showed that various grass varieties and leguminous plants such as alfalfa are suitable (Kuiper et al. 2001; Qiu et al. 1994; Shann and Boyle 1994). This probably is due to their ability to harbor large numbers of bacteria on their highly branched root systems. When a site gets polluted, the composition of the indigenous microbial population in the soil and ground water will adapt to this new situation. Bacteria able to use the pollutant substrates as a nutrient source will be able to proliferate and may become dominant (Liu and Suflita 1993). The natural, non-engineered process of degradation of xenobiotics by the indigenous microbial population is referred to as natural attenuation, and is regarded as the simplest form of bioremediation. The Environmental Protection Agency (EPA,United States) defines natural attenuation, or intrinsic remediation, as a combination of degradation, dispersion, dilution, sorption, volatilization, and chemical and biochemical stabilization of contaminants. Bioaugmentation is a method to improve degradation and enhance the transformation rate of xenobiotics by the injection (seeding) of specific microbes, able to degrade the xenobiotics of interest. Many microbes are described to have the genetic tools to mineralize recalcitrant pollutants such as PAHs, chlorinated aliphatics and aromatics, nitroaromatics, and long-chain alkanes (Cerniglia 1993; Cerniglia and Heitkamp 1987; Grosser et al. 1991; Heidelberg et al. 2002; Kastner and Mahro 1996).In this review, some generally accepted rhizoremediations are described. The aim of present review is to provide improved understanding of mechanism of microbial interaction in rhizosphere, which will help to translate the results of simplified bench scale and pot experiments to the full complexity and heterogeneity of field experiments with predictable remedial success.

II. Plants suitable for rhizoremediation

The first studies toward degradation of compounds in the rhizosphere mainly focused on the degradation of herbicides and pesticides (Hoagland et al. 1994; Jacobsen 1997; Zablotowicz et al. 1994). These studies suggested that plants are protected against these compounds by the degrading bacteria. Research on phytoremediation, through trial and error, has focused on densely rooted, fast growing grasses and plants, such as Brassica sp., with fine root systems. Mulberry *(Morus alba L.)* and poplar (*Populus deltoides*) trees have been used successfully in the phytoremediation of chlorophenols and chlorinated solvents such as trichloroethylene (TCE) (Stomp et al. 1993).Various grass varieties and leguminous plants have shown to be suitable for rhizoremediation (Kuiper et al., 2001, 2004).

Role of plants in rhizoremediation

The mucigel secreted by root cells, lost root cap cells, the starvation of root cells, or the decay of complete roots provides nutrients in the rhizosphere (Kuiper et al., 2004; Lynch & Whipps, 1990). In addition, plants release a variety of photosynthesis derived organic compounds (Pilon-Smits, 2005; Salt et al., 1998). These root exudates contain water soluble, insoluble, and volatile compounds including sugars, alcohols, amino acids, proteins, organic acids, nucleotides, flavonones, phenolic compounds and certain enzymes (Chaudhry et al., 2005; Pilon-Smits, 2005; Salt et al., 1998; Anderson et al., 1993). Plants may contribute to remediation in several ways, by reducing the leaching of contaminants, aerating soil, phytodegradation/transformation, phytovolatilization, evapotranspiration, and rhizoremediation (Amos & Younger, 2003; Chang et al., 2005; Cunningham et al., 1995). The selection of bioremediation or phytoremediation for cleanup of a contaminated site may depend upon prevailing conditions that support the application of microbes, plants, and/or both.

Factors affecting rhizoremediation:

The differing physical, chemical, and biological properties of the root-associated soil, compared with those of the bulk soil, are responsible for changes in microbial diversity and for increased numbers and metabolic activities of microorganisms in the rhizosphere microenvironment, the phenomenon called the rhizosphere effect (Barea et al., 2005; Kuiper et al., 2004; Pilon-Smits, 2005; Salt et al., 1998).The effects of soil moisture, temperature, aeration, pH, and organic matter content on the biodegradation of pesticides have been investigated in many studies (Bending et al., 2006; Charnay et al., 2005; Rasmussen et al., 2005).

The rate of exudation changes with the age of a plant, the availability of mineral nutrients and the presence of contaminants (Chaudhry et al., 2005). The nature and the quantity of root exudates, and the timing of exudation are crucial for a rhizoremediation process. The root exudates mediate acquisition of minerals by plants and stimulate microbial growth and activities in the rhizosphere in addition to changing some physicochemical conditions. Plants might respond to chemical stress in the soil by changing the composition of root exudates controlling, in turn, the metabolic activities of rhizosphere microorganisms (Chaudhry et al., 2005). Some organic compounds in root exudates may serve as carbon and nitrogen sources for the growth and long-term survival of microorganisms that are capable of degrading organic pollutants (Pilon-Smits, 2005; Salt et al., 1998; Anderson et al., 1993).

Soil Conditions:

The success of bioremediation depends on a number of soil physico-chemical factors such as moisture, redox conditions, temperature, pH, organic matter, nutrients and nature, and amount of clay that affect microbial activity and chemical diffusion in soils. Schroll et al. (2006) quantified the effect of soil moisture on the aerobic microbial mineralization of selected pesticides (isoproturon, benzolin-ethyl, and glyphosphate) in different soils. They found a linear correlation ($p < 0.0001$) between increasing soil moisture (within a soil water potential range of -20 and -0.015 MPa) and increased relative pesticide mineralization.

Temperature:

Temperature and pH are the major factors affecting the biodegradation of pesticides in soil. Temperature not only affects the rates of biochemical reactions, as all microbial activities depend on thermodynamics, but also has a direct impact on cell physiology-altering proteins and cell membrane permeability (Alberty, 2006; Guillot et al., 2000; Mastronicolis et al., 1998).

pH:

The biodegradation of a compound is dependent on specific enzymes secreted by microorganisms. These enzymes are largely pH-dependent and bacteria tend to have optimum pH between 6.5 and 7.5, which equals their intracellular pH. A *Pandoraea* sp. isolated from an enrichment culture (Okeke et al., 2002) degraded HCH isomers over a pH range of 4 to 9 (Siddique et al., 2002), but the optimum pH for growth and biodegradation of α- and γ -isomers of HCH in soil slurries was 9. Singh et al. (2006) also reported the similar results while studying the biodegradation of organophosphate pesticides in soil. Degradation rate was slower in lower pH soils in comparison with neutral and alkaline soils.

Soil organic matter:

Soil organic matter also affects biodegradation of pesticides in soil by providing nutrients for cell growth and controlling pesticide movement by adsorption/desorption processes. Perrin-Ganier et al. (2001) monitored biodegradation of isoproturon (herbicide) by adding sewage sludge, nitrogen (N), and phosphorus (P) separately and observed that N and P had the greatest effect on isoproturon degradation.

III. Recent Research Studies Implying Rhizoremediation

Plant species shown to facilitate microbial degradation

Plant rhizosphere	Pollutant	Reference
Sugarcane	2,4-D	Sandman and Loos, 1984
Rice	Benthiocarb	Sato, 1989
Corn	Atrazine	Seibert et al., 1981
Kochia	Atrazine, metolachlor, and trifluralin	Anderson et al.1994
Zinnia anguistifolia	Mefenoxam	Pai et al.(2001)
Rye grass	Chlorpyrifos	Korade and Fulekar,2010
Pennisetum pedicellatum	Chlorpyrifos Cypermethrin Fenvalerate	Dubey and Fulekar, 2011a
Rice (cv. Supriya)	Parathion	Reddy and Sethunathan 1983
Mixture of grass, legume, herb and pine	Trichloroethylene	Walton and Anderson 1990
Prairie grasses	Polycyclic aromatic hydrocarbons (PAHs)	Aprill and Sims 1990
Prairie grasses	Polycyclic aromatic hydrocarbons (PAHs)	Qiu et al. 1994
Grasses and alfalfa	Pyrene, anthracene,phenanthrene	Schwab et al. 1995
Sugar beet (cv. Rex)	Polychlorinated biphenyls (PCBs)	Brazil et al. 1995
Barley (Hordeum vulgare)	2,4-D	Jacobsen 1997
Alfalfa and alpine bluegrass	Hexadecane and PAHs	Nichols et al. 1997
Wheat (Triticum aestivum)	2,4-D	Kingsley et al. 1994

Poplar (Populus deltoides nigra)	1,4-dioxane	Schnoor et al. 1998
Wheat	Trichloroethylene	Yee et al. 1998
Oat, lupin, rape, dill, pepper, radish, pine	Pyrene	Liste and Alexander 2000
Reed (Phragmitis australies) Abd El	Fixed nitrogen	Haleem et al. 2000
Poplar root extract	1,4-dioxane	Kelley et al. 2001
Corn (Zea mays)	3-methylbenzoate	Ronchel et al. 2001
Astragalus sinicus	Cd	Sriprang et al. 2002
Fern (Azolla pinnata	Diesel fuel	Cohen et al. 2002

IV. Conclusion

Rhizoremediation technology uses plant roots and associated microbial consortium to degrade environmental pollutants/toxins from soil with an aim of restoring area sites to a condition useable for intended purpose. Rhizoremediation takes advantage of plant roots natural symbiosis with mycorrhiza and root associated natural microbial flora for the enhanced degradation of pollutants in the rhizosphere. Although the studies described above represent only one pollutant and for one plant–bacterium combination, it is clear that similar microbe–plant combinations can be selected for other pollutants. Therefore, further studies of the selection of suitable rhizosphere bacteria or communities, able to sustain and proliferate on the root system of a plant which is suitable for rhizoremediation or phytoremediation, can yield useful novel (engineered) systems. These systems then can be an interesting tool to further improve and develop bioremediation into a widely accepted technique.

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