

Review Article

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Rhizoremediation – Plant Microbe Interactions in the Removal of Pollutants

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ABSTRACT

Rhizoremediation is an in-situ remediation approach involving microorganisms for the biodegradation of organic pollutants and various other contaminants in the root zone. Plant roots provide a rich niche for the microorganisms to grow at the expense of the root exudates and in turn microbes act as biocatalysts to remove the pollutants. The harmful pollutants such as: polycyclic aromatic hydrocarbons (PAHs)- pesticides, herbicides etc. are converted to degradable compounds, while heavy metals such as zinc, copper, lead, tin, cadmium etc. are transformed from one oxidation state or organic complex to another. Various mechanisms employed are: producing bio-surfactants which are amphiphilic molecules that form spherical or lamellar micelles, thereby solubilizing hydrophobic contaminants in their core and enhancing their bacterial degradation to simple harmless compounds, producing metal chelating siderophores for heavy metal acquisition, increased humification, biofilm production, acid production etc. The process is affected by various-chemical, physical, biological factors i temperature, pH, soil conditions, nature of the pollutant, indigenous microflora etc. Plant -bacteria interactions play a key role in the process and are characterized by: Colonization of the roots by bacteria, maintenance of the catabolic activity and effect of the external environment conditions on the interaction. Technological advancements and increased insight into sequencing techniques can make rhizoremediation a promising and fertile future technology.

Keywords

Rhizoremediation,
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Introduction

The ever increasing pollution levels have become a major environmental concern. The rapid technological advancements, industrialization, massive use of fertilizers and pesticides etc. have added on to the baggage of pollutants. These pollutants include: - hydrocarbons, heavy metals, dioxins, chlorinated compounds etc. (Zhuang

et al., 2007) various conventional physico-chemical techniques like composting, land forming have been used to combat this problem. These are all invasive, time consuming, lead to generation of more toxic substances and even result in emission of greenhouse gases. Therefore, biological remediation of these pollutants is a viable option. Bioremediation involves the use of living organisms to neutralize these pollutants

or to get rid of them. One such bio remedial technique which has come into light is rhizoremediation. Over the past few years intensive studies are being carried to investigate various microorganisms as bio remedial agents. Rhizoremediation is one such technique involving the use of rhizobial microflora mainly rhizobacteria. It is a technique trending these days and is ecofriendly and even cost effective and offers impactful future prospects.

Rhizoremediation

Rhizosphere is nutrient rich area or zone surrounding the plant roots. It is rich in microorganisms mainly characterized as plant growth promoting organisms which enhance plant growth directly or indirectly. These rhizobial microbes show immense interactions with the plants and are also involved in the remediating of the harmful pollutants found in the soil as poly-aromatic hydrocarbons, polychlorinated biphenyls, halogenated compounds, pesticides like atrazine, heavy metals like –cadmium, mercury and many more (Zhuang *et al.*, 2007). This clean-up of the hazardous wastes and chemicals brought about by rhizo-microflora is known as rhizoremediation.

Rhizoremediation is a specific subset of phytoremediation. Its cost effectiveness, convenience with little or no side effects have made it a growing technology. It is a clean and green phenomenon (Corgie *et al.*, 2003). Although, it can be time consuming, yet its end results are phenomenal as the pollutant is completely destroyed or it is converted to harmless form. It is suitable for large or small sites with low to moderate pollutant levels. It is a symbiotic relationship between the plant roots and the microorganisms where plant roots provide rich niche for the growth of the microbes (which grow at the expense of root exudates) and in turn microbes as the

biocatalyst that remove the pollutants. Even the utilization of non-rhizospheric or ex-situ microbes can help in remediating the polluted sites but these organisms face certain shortcomings compared to rhizospheric microflora.

The shortcomings include- lack of nutrients in soil, soil surface properties, toxicity or reduced bioavailability of the contaminants, competition with the indigenous microflora and failure to express required catabolic functions etc., (Amora-Lacazano *et al.*, 2010). Moreover, the number of microbes in the rhizosphere outnumber the microbes in bulk soil. Increased degradation of the pollutants in the rhizosphere is beneficial to the plant and result in improved plant growth on contaminated soil.

Role of plants in the process

Plants have a basal role in the process: As plant roots can be considered as a substitute for tilling the soil, to spread the root associated micro-organisms through the soil and to penetrate layers normally not accessible to bacteria or other microbes and to incorporate nutrients, bring oxygen and provide better redox conditions which help to stimulate and activate the rhizosphere microflora (Thijis and Vangronsveld 2014). Also, majority of the plants live in symbiotic relationship with mycorrhiza which act as web like extension of the root system enhancing the absorptive area of plants. Plants also offer an effective clean-up technology compared to conventional methods:

They provide their own solar energy by photosynthesis and pump up pollutants with water stream making it much cheaper.

It is in-situ technology.

It generates less or even no waste and instead creates economic benefits.

Plant-microbe interactions in the rhizosphere

For a successful rhizoremedial strategy there are two requirements which must be accomplished. Firstly, the microorganisms must be able to proliferate in the root system (necessary for multiplying the catalytic potential). Secondly, catabolic pathways for remediation of the pollutants must be operative (Segura *et al.*, 2009). The interaction between plant and microorganisms involves:

Root colonization

The bio-degradative microorganisms colonize the rhizosphere similar to other rhizospheric microbial populations. These can either be recruited from the soil reservoir during growth of the plants on contaminated soil or these originate from (bacterized) seeds. Microorganisms are attracted towards the root exudates (secretion of plant roots) by chemotactic movement and they subsequently spread through the soil during root emergence and growth. Using in-vivo expression technology (IVET), transcriptomics and mutants defective in motility, the mechanism of root colonization and identification of genes which are activated during rhizosphere colonization are being discovered. Successful rhizosphere colonization depends not only on the interactions between the plants and the microbes but also on the interactions with other rhizospheric microorganisms and the environment.

Maintenance and regulation of gene expression

Selection of the appropriate plant to stimulate contaminant degradation is a key aspect in the rhizoremediation strategy, however choice of the bio-degradative microflora is of major concern. Plant roots release compounds called

as root-exudates as- sugars, organic acids, fatty acids, secondary metabolites, nucleotides and also inorganic compounds that play an important role in establishing and determining the rhizosphere microbial population. These help in selection of the microbes which respond to the exudate buffet rapidly. These secretions help in determination and regulation of the concerned bio-degradative microorganisms either directly or indirectly.

Communication and dynamics

Plants and the microorganisms send and receive multiple signals responsible for recognition of microbes, recruitment of catalytic potential, mycorrhization, resistance to stresses and quorum sensing. Various biotic factors and abiotic factors including the growth stage of the plant, soil type, contaminant type, season, temperature and the density of other microorganisms etc. play a significant role in shaping this interaction. For e.g.- Siciliano *et al.*, (Wu *et al.*, 2006) demonstrated that catabolic alkane monooxygenase gene were more prevalent in rhizospheric microbial communities than in the bulk soil contaminated with hydrocarbons.

Factors affecting rhizoremediation

Rhizoremediation is affected by various physical, chemical and biological factors. These may include –temperature, pH, soil conditions, microbial diversity, aeration, organic matter content, rate of exudation, age of the plant, availability of nutrients and the presence of contaminants. (Kala S 2014) These factors control the effectiveness of the process in one way or the other as follows:

Soil conditions

The accomplishment of rhizoremediation depends on the soil physico-chemical

conditions properties such as moisture, redox conditions, temperature, pH, organic matter, nutrients and nature and the amount of clay etc. that affect the microbial activity as well as diffusion of the chemicals in the soil. The effect of soil moisture on the aerobic microbial mineralization of the selected pesticides was quantified by Schroll *et al.*, (2006). They found a linear relation between the soil moisture and relative pesticide mineralization.

Temperature

Temperature and pH are the principle factors affecting the bio-degradation of contaminants of various types in the soil. It not only has an impact on the rate of biochemical reactions but also has an impact on the microbial activity.

It is essential that contaminated sites be at the optimum temperature for bioremediation to progress successfully, since excessively high or low temperatures sometimes inhibit microbial metabolism. In addition, the solubility and bioavailability of a contaminant will increase as temperature increases, and oxygen solubility will be reduced, which will leave less oxygen available for microbial metabolism. Until recently, most research has focused upon the biodegradation of organic compounds at mesophilic temperatures; however, evidence suggests that the indigenous microbial communities of a contaminated site will adapt to the in situ temperature, such as those in Arctic and Antarctic soils. For example, a selection of *Rhodococcus* species that were isolated from an Antarctic soil were able to successfully degrade a number of alkanes at -2°C but were severely inhibited at a higher temperature. In addition, the PAHs naphthalene and phenanthrene were successfully degraded from crude oil in seawater at temperatures as low as 0°C.

pH

The biodegradation of the contaminant is dependent on specific enzymes secreted by the microorganisms. As the enzymes are pH dependent therefore, all microbes require an optimum pH for their growth viz., – bacteria grow at the optimum pH value of 6.5-7.5. Singh *et al.*, (2006) found that biodegradation of the organophosphate pesticides was slower in lower pH soils compared to neutral and alkaline soils.

Soil organic matter

It has a considerable effect on the degradation of the contaminants in soil as the soil organic matter acts as rich source of nutrients for growth of bio-degradative microflora and it also controls the movement of contaminants by adsorption and desorption processes. Perrin-Ganier observed that biodegradation of isoproturon (herbicide) was enhanced by adding sewage sludge (source of organic matter).

Microbial diversity

Rhizospheric microbial populations enhance the process of rhizoremediation by increasing the humification of organic pollutants i.e. a microbial consortium degrades the pollutants much more effectively than a single strain/species because of the presence of partners, which use various intermediates of degradation pathway more efficiently (Kuiper *et al.*,). Many such microbes colonizing the plant roots have been identified as *Pseudomonas spp.*, *Bacillus spp.*, *Azotobacter* and many more.

Plants

The age of plant influences the nature of root exudates which in turn characterizes the microbial flora residing in rhizosphere and

bringing about the process of rhizoremediation. Also, plant roots have major role and help in determination of microbial diversity around them.

Bioavailability of pollutants present in soil

The nature of pollutants present in the soil also governs the microbial populations found in it, thereby affecting the rate of process. Bioavailability is also extremely important for the biodegradation of organic pollutants. It is frequently observed that the rate of removal of compounds from soils is very low even though the compounds are biodegradable. However, the substrates in these instances may not be in a form that is readily available to the microorganisms. Biodegradation of hydrophobic pollutants may take place only in the aqueous phase; for example, naphthalene is utilized by pure cultures of bacteria only in the dissolved state. Bouchez *et al.*, (1995) similarly showed that phenanthrene biodegradation occurs only in the aqueous phase. The many observations of linear growth of bacteria and yeasts on slightly soluble substrates may be explained by the need for the substrates to be in the aqueous phase. In the case of the PAHs, it has been shown that linear growth is due to mass transfer limitation from the solid phase to the liquid phase.

All above factors individually and collectively control the process of rhizoremediation.

Mechanisms involved in the process

The microorganisms carry out the process of biodegradation by various mechanisms:

Bio-surfactant production

Bio-surfactants are amphiphilic molecules (both hydrophobic and hydrophilic) which form micelles with the hydrophobic

compounds as poly-aromatic hydrocarbons and thereby increase their bioavailability for the purpose of bioremediation. The micelles are formed when the surfactant concentration exceeds a certain critical value i.e. specific for the compound. The micelle formation enhances the solubility of hydrophobic contaminants and thus their degradation by microbes. Kuiper *et al.*, (2004) isolated *Pseudomonas putida* from plant roots at a site polluted with PAHs that produced two lipopeptide bio surfactants.

Biofilm formation

Biofilm is the aggregate of microorganisms in which cells are frequently embedded within a self-produced matrix (of exopolysaccharide). The microflora in biofilm have better chances of survival as they are protected by matrix and thus help in immobilization and degradation of the contaminants. (Segura *et al.*, 2009)

Organic acid production

Organic acids as phytic acid, gluconic acid etc. secreted by various rhizobial microflora lower the pH of soil, thereby increasing the solubility of contaminants and enhancing their degradation.

Various other mechanisms are involved in rhizoremedial action of microorganisms and may include siderophore production, release of enzymes like oxidoreductases, ACC deaminase production and many more (Wu *et al.*, 2006).

Improving rhizoremediation

Presence of bio-degradative strains, the expression of the catalytic genes and maintenance are all crucial factors which determine the success of rhizoremediation. However, under natural conditions, the

phenomenon can be slow, indicating that some of these factors or others are limiting the removal of pollutants (Thijis and Vangronsveld 2014). Therefore, technologies for improving the efficiency of the process were developed which include:

Bio-stimulation

It is the modification of the environment to stimulate the existing micro-organisms for carrying out the rhizoremedial mechanism. It involves- addition of compost as fertilizer, addition of minerals like phosphorus and nitrogen as bait for the microbes, improving physico-chemical properties of the soil by using additives as bio-surfactants for oil degradation etc.

Bio-augmentation

It is defined as the introduction of microbial strain or consortium into the soil to enhance degradation process. Delivery methods may involve seed coating, soil drenching, root dipping etc.

Genetically engineered microorganisms

For certain pollutants no catabolic pathways exist among the microbes to promote their degradation. To counter such pollutants genetically engineered microbes are being used. Barea *et al.*, (2005) reported the improved remediation of toluene by using genetically engineered bacteria. They transferred pTOM plasmid which encodes toluene degradation genes via conjugation from *B. cepacia* G4 to *B. cepacia* L S 2 4.

Rhizoengineering

It involves the use of transgenic plants which secrete more root exudates which all indirectly have a significant effect on rhizosphere microflora. All these techniques help to improve rhizoremediation thereby

making it more promising future technology. Currently, the PAH remediation is brought about by the process as stated in the following case studies:

Enhanced degradation of a mixture of three herbicides in the rhizosphere of an herbicide-tolerant plant

Anderson *et al.*, (1993) characterized a pesticide contaminated site and brought it into laboratory for degradation experiment. Three major herbicides were identified in soil- atrazine, metalachlor and trifluralin. Although their concentration was very high but still some herbicide tolerant plants were found growing in the soil. Initial number of microbes was determined from the soil and it was then incubated for 14 days. The herbicide degradation was enhanced in the soil both rhizospheric and edaphospheric. However, the number of the degradation rate was comparatively more in the rhizospheric soil.

Bioremediation of poly-aromatic hydrocarbons using rhizosphere technology

Bisht *et al.*, (2015) reported that rhizoremediation is a specific type of phytoremediation involving both plants and their associated rhizospheric microbes. Various microbes include: *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Mycobacterium spp.*, *Rhodococcus spp.*, *Paenibacillus spp.* are involved in PAHs degradation. They also explored the molecular communication between the plants and microbes and how this can be exploited for eliminating the contaminants.

Heavy metal degradation by soil rhizobacteria

Heavy metals are highly toxic when accumulated in excess, therefore these must be degraded. Although their complete

destruction is not possible but these can be converted to harmless oxidation state. Wu *et al.*, (2006) have engineered certain plant-microbe symbiosis for rhizoremediation of heavy metals. They demonstrated that the expression of the plasmid EC20 in *Pseudomonas putida* 06901 improves both the cell growth and its cadmium binding property in the presence of high levels of cadmium, thereby countering excessive level of cadmium in the polluted soils.

Rhizospheric degradation of phenanthrene is a function of proximity to roots

Corgie *et al.*, (2003) found that phenanthrene degradation is enhanced in rhizospheric zones compared to the bulk soil. They investigated the influence of the proximity to ryegrass roots on microbial population and their biodegradation of phenanthrene using compartment pots. The experiment carried out by them clearly proved the effectiveness of the rhizosphere and its microflora in degrading harmful PAH as phenanthrene.

Rhizoremediation of contaminated soils by comparing six root species

Al-Ameeri and Al-Sarawi (2017) carried out this experiment in Kuwait where samples were procured and were divided into two categories. From the chemical analysis it was found that rhizoremediation is a unique process for decreasing the level of soil contaminants as hydrocarbons and heavy metals. The most effective plant part in accumulating hydrocarbons and heavy metals were the roots.

The process of rhizoremediation is an emerging bio-remediating technology which if utilized well can help us to get clean and green environment by exploiting the natural resources in the best possible way. Also, in the field of agriculture where the

accumulation of chemical fertilizers and pesticides has led to the generation of barren land, many health hazards this technique can offer a solution to enhance biochemical yield, reclaim those barren lands in the best possible way. Thus, rhizoremediation can emerge as an eco-friendly cleanup technology.

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