Chapter

Phytoremediation: A Synergistic Interaction between Plants and Microbes for Removal of Petroleum Hydrocarbons

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Abstract

Rapid industrialization leads to the deterioration of quality of life and the environment. Petroleum hydrocarbon pollution is one of the contributing factors to that. Petroleum hydrocarbons (PHCs) are natural products, and under high temperature and pressure, they are produced by the anaerobic conversion of biomass. Excessive use of PHCs leads to pollution in the agriculturally important soils and the ultimate source of potability of water, that is, groundwater which is gaining significant attention throughout the world. The fortuitous release of PHCs such as gasoline, diesel, and heating oil are common sources of groundwater contamination. The PHC concentrations in groundwater are often above drinking water standards and bioremediation actions have to be taken. Due to their organic nature, PHCs are difficult to degrade as unavailable for microbial action. Due to this, PHCs are the most widespread environmental contaminants. Plant-microbe synergistic association for remediation of PHCs is comprehensive and it is an effective tool for reclamation of soil and environment from these kinds of undesirable materials. In addition to providing plant growth promotion, microbes can degrade PHCs effectively.

Keywords: petroleum hydrocarbon, biosurfactants, PGPR, biodegradation

1. Introduction

Different types of petroleum hydrocarbons exist, which include saturates, aromatics, asphaltenes, and resins (i.e., pyridines, quinolines, carbazoles, sulfoxides, and amides). Microbes degrade these PHCs to different extents due to their different bioavailability to microbial action. Soils are complex, highly dynamic systems that are the product results from interactions between abiotic and biotic processes that have taken place over billions of years. The result is a spatially complex environment that leads to the spatial and temporal heterogeneity of microbial activity and their diversity. The role of plants and the soil-living microbes remains, to a large extent, unexplored. However, the action of microbes to degrade organic contaminants into harmless compounds has been explored to treat contaminated environments. This approach is referred to as phytoremediation. Phytoremediation is a term that describes the application of plants to reduce the contaminant and its mobility or toxicity in soil, groundwater, or other media [1]. Phytoremediation has been increasingly considered as an appropriate strategy to restore hydrocarbon-polluted soils in ecologically protected areas and agricultural fields. In addition to this, the bioaugmented bacterial species in ecologically protected areas, even if for bioremediation purposes, remains technically questionable because soils usually contain indigenous microbime capable of metabolizing hydrocarbons [2, 3]. In addition to phytoremediation, the use of hydrocarbon-utilizing microorganisms reduces contaminant toxicity by excretion of variety of biosurfactants which are biodegradable and consequently environmentally safe for the reclamation of polluted environments. Different kinds of microorganisms have been found to produce this surfactant, including plant growth-promoting rhizobacteria (PGPR). These PGPRs which are established bio-enhancers and biocontrol agents, due to the possession of properties to solubilize phosphate, produce IAA (indole acetic acid), and sequester iron under stress conditions through production of siderophores, will be best if used for remediation of oil-contaminated sites as this will serve two purposes. On the one hand, they will promote plant growth, and second, by producing biosurfactants that will enhance the process of remediation of oil-contaminated sites.

2. Petroleum hydrocarbon phytotoxicity

Soil physicochemical and biological properties are majorly deteriorated by petroleum hydrocarbon pollution resulting in deleterious effects to plant health and the environment.

The availability of different nutrients like exchangeable iron, phosphorous, sulfate, soil water, and soil air and such changes affect plants adversely.

Hydrostatic anaerobic conditions interfere with the soil plant water relations and seriously harm to plants [4, 5].

The effects of different contaminants on soil plant and microbes depend on the extent and the type of contamination [6].

Contamination of soil results in deterioration of soil properties leads to the damage of crop and the soil may remain not suitable for plant health for several months or years. The soil microflora and its fertility are drastically reduced by undesirable contamination [7].

Extensive damages of soil due to contamination may be for long term. Diesel fuel kills plants cells on contact but it is not a systemic killer. During taking up water and other nutrients diesel fuels reached to the plant roots and damage the roots, and this restrict the plant from uptake of essential nutrients. It can also create imbalance in soil, plant and water relationship [8].

3. Remediation approach

According to Langbehn and Steinhart [9], various approaches significantly treat the problem soil including thermal treatment (physico-chemical techniques), the extraction of gases or liquid matter, soil washing, solidification, stabilization *etc.* However, these techniques require very heavy equipment, require huge amount of energy and are very expensive. According to Rahman et al. [10], efficient removal of petroleum HCs contamination in the soil remains a challenge.

3.1 Phytoremediation

Bio-phytoremediation or the synergistic association between plants and their rhizosphere microbiome for removal of contaminants from the environment has recently become an area of huge possibilities and the intense experimentation [11].

The environmental contaminants mainly caused threat to the plant development germination and root elongation, and these are the two critical stages that are sensitive for contaminant [12].

Some plants which are tolerant to the contaminants show successful germination and root elongation.

For the removal of oil from contaminated soil plants, including grasses and legumes, proved with higher potential as compared to other plants [13, 14] due to the higher root surface area, root elongation, and better soil compaction [13].

According to Wiltse et al. [15], crude oil contamination reduces in the rhizosphere by 33–56% compared to control by using various strains of alfalfa (*Medico sativa* L.), whereas after 8 weeks, 80% of diesel fuel degraded with an alfalfa treatment (Komisar and Park [16]), and 46% of crude oil was removed in 12 weeks of interval with broad bean (*Vicia faba*) as compared to 33% without plants.

Reilley et al. [17] evaluated switchgrass as an independent species for PHCs remediation.

According to Jordahl et al. [18], for bengene, toluene, xylenes (BTX) phytoremediation, the hybrid poplar trees (*Populus deltoides x nigra*) rhizosphere showed the potential results.

Wild and Jones [19] investigated that carrot peels were accumulated with PAHs to a maximum value of 200 μg total PAHs kg^{-1} dry weight in laboratory condition.

4. Mechanism of action by plants

4.1 Phytodegradation/transformation

The degradation of contaminants by using plant-produced enzymes release of into the soil or through metabolic processes can be divided into components including absorption, translocation, and contaminants metabolism by the plant and the root exudates-mediated degradation of contaminant.

4.1.1 Fate of contaminants by the action of plants

The ability of a plant metabolize organic pollutant is generally dependent on the bioavailability of the pollutant, and it is reflected by the octanol-water partition coefficient, Kow, of the pollutant [20, 21].

For the plant action for the contaminant, the type of plants and contaminant also affect the bioavailability of contaminant [22].

Cunningham and Berti [20] explained depending factors of plant to absorb, translocate, and metabolize the contaminant (Kow values in log scale) as described below.

i. With log Kow ≤ 1, plants are able to absorb, translocate, and metabolize hydrophilic contaminants.

   As these contaminants are with high bioavailability, their absorption is controlled by water influx into the plant and they may cause groundwater contamination.

ii. With log Kow values between 1 and 4, plants are able to absorb, translocate, and metabolize the contaminants. According to Briggs et al. [23], the highest contaminant concentration translocated at shoots part with log Kow of 1.8, with declining concentrations at higher and lower values of log Kow.

iii. With log Kow values larger than 4 plants are generally unable to absorb, translocate, and metobilize contaminants due to high hydrophobicity and the contaminant adsorbs to lipids on the root surface of the plant [20–22].

The contaminant absorbed by the plant may be translocated to different plant parts where it is metabolize partially or completely or incorporated into cellular constituents and volatilized [22, 24]. This whole process that includes absorption, translocation, and volatilization called as phytovolatilization.

4.1.2 Significance of root exudates

Role of root exudates may aid remediation direct degradation of contaminants. Root exudates increase the access of the pollutant, soil lubrication, and acting as co-metabolites with PHCs.

According to Schnoor et al. [22], few plant-based enzymes are able to remediate 2,4,6-trinitrotoluene (TNT) and trichloroethylene (TCE).

The bioavailability of the contaminant is the extent to which a pollutant is accessible for to microbial activity [25].

Root exudates (organic acids in nature) may enhance the pollutant bioavailability by competing with the original pollutant for absorption/adsorption sites in the soil due to structural similarity.

Roots are also release lipids and sterols that have been found to increase the bioavailability of contaminants and making them available for microbial degradation. The root passages of plants are also facilitating by lipids and sterols.

Root exudates may act as co-metabolites with the pollutant as root exudates are structurally similar with PHCs [26]. Root exudates and pollutant that are structurally similar showed in **Figure 1**.

4.2 Phytovolatilization

According to Farrell and Germida [25], there is pollutant movement from the soil or groundwater and into the plants and then to the atmosphere, which is called phytovolatilization. This happened when the pollutant is absorbed by the roots, translocated to the plant, and volatilized into the atmosphere.

4.3 Phytostabilization

According to Farrell and Germida [25], plants use to restrict the pollutant in the soil or groundwater called "phytostabilization" and it can be explained by three mechanisms: (1) absorption, (2) root accumulation, and (3) surfaces adsorption by root and entering into humic matter in the plant rhizosphere. All three mechanisms are dependent on the value of Kow as explained above.

4.3.1 Absorption by root and accumulation

In absorption and accumulation, contaminant remains restricted in the roots of the plant. The translocation of the contaminants is not possible into the rest of the plant and are therefore not degraded, incorporated into the cell structure, or volatilized. This may happen for contaminants with a log Kow value between 1 and 4 [20].

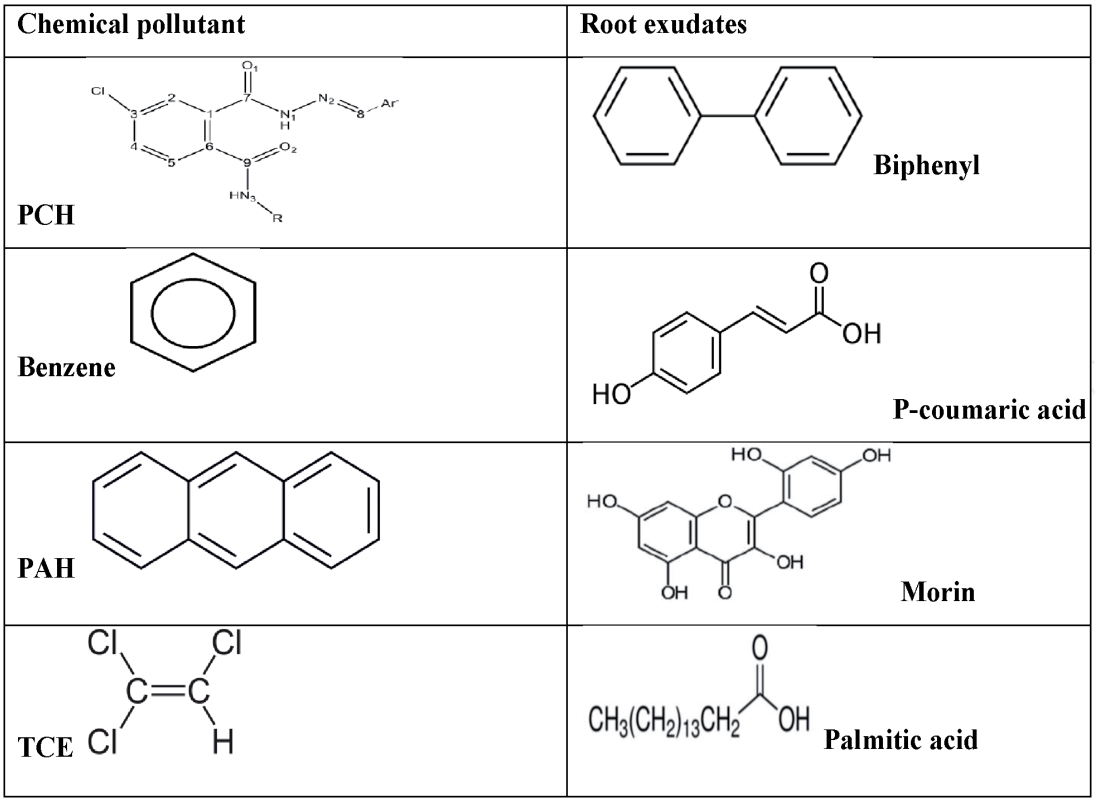

Figure 1.
Similar structures of root exudates and chemical pollutants.

4.3.2 Root adsorption

In these mechanisms, contaminants are immobilized by adsorption to lipids on the surface of the roots. This may happen for contaminants with a log Kow value above 4 [20–22].

5. Degradation of organic contaminants by microorganisms

The halogenated products of petrochemicals and mineral oil constituents are the most important classes of organic contaminants in the environment. Microbial degradation of organic contaminants normally occurs as a result of microorganisms act on the contaminant for their own metabolisms and production cellular constituents or reproduction. Organic contaminants used by microorganisms as a source of carbon and electrons that the organisms use to obtain energy [26].

According to Committee on *In Situ* Bioremediation in the year 1993, the microbial metabolism of contaminants involves aerobic respiration. Anaerobic respiration, co-metabolism, fermentation, reductive dehalogenation, and the use of inorganic compounds as electron donors: these are the variations in metabolism by microorganisms.

Interestingly, bacteria are capable of rapidly distributing genetic information to each other, thus allowing them to adapt rapidly to adverse environment, such as exposure to new pollutants [27].

The *dioxygenase* enzyme plays a significant role in aerobic biodegradation of PAHs by involving the incorporation of two atoms of molecular oxygen into the contaminant and production of less toxic compounds such as acids, alcohols, carbon dioxide, and water [28–30].

In contrast, biodegradation by eukaryotic fungi which is similar to the biodegradation mechanism found in mammals initially it involves the incorporation of only one atom of oxygen into the PAHs (polycyclic aromatic hydrocarbons) [29, 31–33].

Although most of the time fungal transformations result in compounds that are less toxic than the parent PAHs, while some of the minor metabolites are produced, they are more toxic compounds than the PAHs [33].

The microbial breakdown and removal of contaminants are interrelated processes that occur in the soil.

According to Lyman et al. [34], biodegradation is the microbial-mediated chemical transformation of organic compounds, while microbial uptake is the direct removal of the contaminant by adsorbing compounds to the membrane surface or by absorbing compounds through the membrane.

These two processes are interrelated in that the contaminant taken up may be the original contaminant or a biotransformation product.

That microbes are able to degrade and take up pollutants has been well studied, and it is the conceptual basis for other remediation techniques like air sparging, land farming, composting, bioreactors, intrinsic remediation, and others [35].

Depending upon the microorganisms, a number of different microorganisms are able to degrade a number of different PHCs, and the specific catabolic pathway used is dependent on the microbe and pollutants.

In general, microbes degrade PHCs by adsorbing the contaminant to the membrane surface or absorbing the contaminant through the membrane and by using oxygenase enzymes, incorporating oxygen into, and cleaving the structure of the hydrocarbon.

Finally, oxidation of subsequent end products and incorporation into the Krebs cycle may result in the final degradation step and the release of CO_2, H_2O, and energy [35, 36] but the complete degradation does not always happen. Sometimes end products may be directly degraded by microbes and not degraded further or may be degraded to smaller, simpler, more stable intermediaries and then incorporated into the soil as humus or soluble acids, ketones, and alcohols [34].

The biodegradation of the contaminants are depends on the size of the contaminant and the types and geometry of its bonds. PHCs have bonds that microbes have difficulty breaking or are not able to break due to their ring structure and hydrophobic nature. For example, linear alkanes were found to be more readily degradable than branched alkanes or ring structure [35].

Diverse group of microbes able to degrade different contaminant depending on the sites/ locations of contamination. Not all microbes are able to directly take up all contaminants. This results in variations of PHCs and microbial population composition over time and space with the most rapidly degradable HCs and associated microbes being replaced by less degradable hydrocarbons and associated microbes. According to Riser-Roberts [35], one kind of microorganism is very rarely able to fully degrade any other specific contaminant. The diverse microbial populations are able to do effective remediation of the contaminants.

6. Plants and microbes synergy for PHCs biodegradation

Plants have been shown to facilitate organic contaminant degradation principally by providing optimal conditions for microbial proliferation in the root zone. The degradation processes are influenced not only by rhizospheric microorganisms but also by specific properties of the host plant [37]. If plants can be successfully acclimatized on polluted soils, then the plant-microbial interaction in the root zone (rhizosphere) may provide an economical method for enhancing microbial degradation of complex PHCs (**Figure 2**).

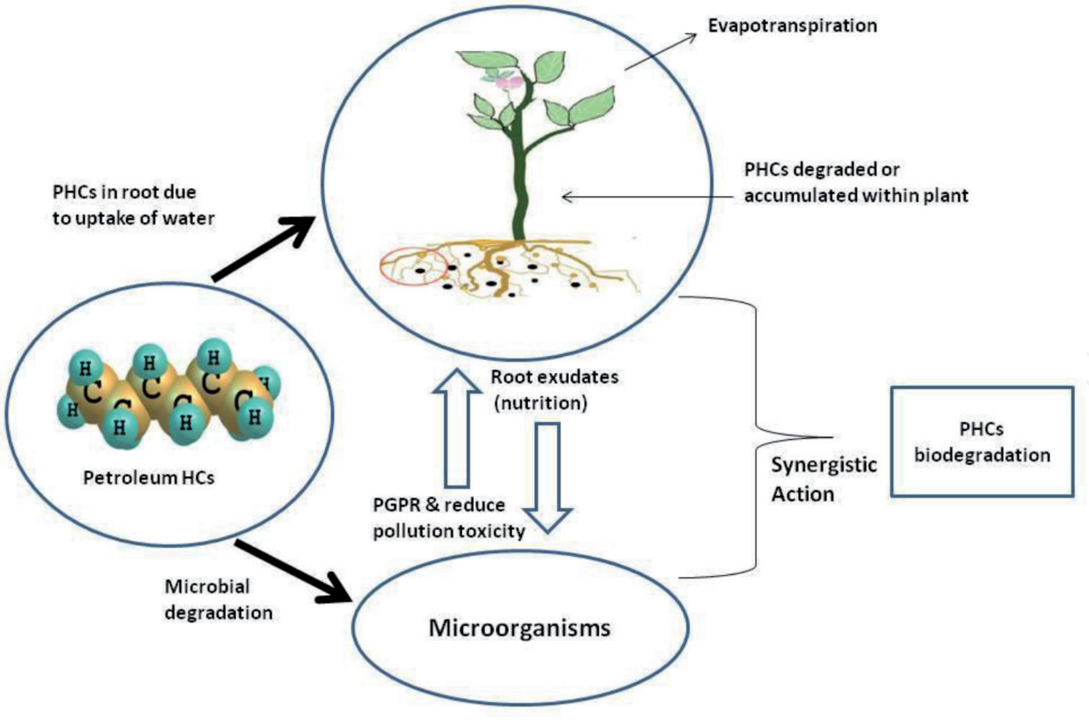

Figure 2.
Mechanism of plant microbe interaction for petroleum HCs pollutants remediation.

7. Enhanced rhizosphere degradation

The enhanced rhizosphere degradation is the breakdown of pollutant in the soil as a result of microbial activity that is enhanced in the presence of the rhizosphere [25] and should include another process in plant-mediated remediation, that is, phytoremediation, the removal of contaminants from the soil. Enhanced microbial activity in the rhizosphere provides the health benefits to the plant and the entire phytoremediation system. Komisar and Park [38] showed that vegetated (alfalfa) soils with diesel contamination observed more microbial count and rapid removal of contaminant. Banks et al. [39] observed that viable counts in soil spiked with polyaromatic and aliphatic hydrocarbons were higher in the presence of alfalfa than in soil without plants. Root elongation and growth opens deeper soil to better water infiltration and oxygen diffusion [40]. Root surfaces provide adhesive zone for soil microorganisms, and roots can disrupt soil aggregates and increase biodegradation of entrapped hydrophobic contaminants [41]. These studies showed how root growth increases microbial activity. Gunther et al. [14] showed that rhizosphere microbial community was mainly responsible for enhanced hydrocarbon disappearance as compared to root free soil. Hou et al. [42] found that due to higher rye grass root intensity, an increase in the degradation of contaminant. Banks et al. [41] observed that the huge reduction in total petroleum hydrocarbon (TPH) concentrations occurred in period with the greatest root growth, but did not evaluate concurrent microbial activity. Gunther et al. [14] found that soil planted with ryegrass reduced a greater amount of a mixture of hydrocarbons than soil without plant. The mixture of hydrocarbon includes *n*-alkanes (C_{10}, C_{14} to C_{18}, C_{22}, and C_{24}), also pristane, hexadecane, phenanthrene, anthracene, fluoranthene, and pyrene. After 22 weeks, the initial hydrocarbon concentration of 4330 mg total hydrocarbon per kg soil decreased to nearly 120 mg per kg soil (97% reduction) in planted soils as compared to 790 mg per kg soil (82% reduction) in soil without plant.

8. Role of microorganisms in reducing phytotoxicity to plants

Other significant role played by microbes involves their ability to reduce the phytotoxicity of contaminants to the plants system and can facilitate plant to grow in adverse soil environment, thereby stimulating the degradation of phytotoxic and non-phytotoxic contaminants [26].

According to Walton et al. [37], the defenses of plants to contaminants may be supplemented rhizopheric microbial activity for degradation of contaminants. The plants and microbes have synergistically work together for dealing with phytotoxicity, where microorganisms benefit from the root exudates while the plants benefit from the ability of microorganisms to break down toxic chemicals and PGPR properties. Rasolomanana and Balandreau [43] observed that rice growth was improved in soil oil residues had been applied.

The authors hypothesized that the increased growth resulted from the co-metabolic action of bacterial species (genus *Bacillus*) by using root exudates in the rhizosphere polluted with the oil residues.

8.1 PGPR

Plant growth-promoting rhizobacteria (PGPR) were first described by Kloepper and Schroth [44] as the soil bacteria that colonize the roots zone of plants by inoculation onto seed and that enhance plant growth. The bacteria inhabiting plant roots and facilitate the plant growth by the mechanisms are referred to as plant growth-promoting rhizobacteria (PGPR).

The plant growth-promoting rhizobacteria (PGPR) facilitate plant growth either directly or indirectly. The direct benefits to the plants are provided by the production of plant growth regulators such as auxine and cytokinines and by increasing the plant uptake of some micro and macro elements in the rhizosphere [45] and indirectly, through the action of biological control of plant pathogens or induction of host defense mechanisms [46–48].

The synergistic action that exists between plants and microbes in the rhizosphere plays significant roles in enhancing the efficacy of phytoremediation [49–54]. Root exudates can stimulate the growth of PGPR, which in turn can alleviate plant stress by lowering stress ethylene, facilitating the nutrient uptake and/or by degrading/sequestering soil contaminants [55].

These microorganisms are nourished and carried through the soil by plant roots [50]. The soil with large volumes of roots results the microbial population can reach to the concentrations upto ~10^{12} microbes per gram of soil [56].

This leads to increase the microbial population to ~500 kg ha^{-1} [57] in the rhizosphere zone. With the PGPR association, plant root growth enhanced and potentially used volume of soil and accelerating salt remediation.

According to Glick [58, 59], among many PGPR properties, the ACC (1-aminocyclopropane-1-carboxylic acid) deaminase activity is the key characteristic because PGPR use the ACC as an N source. Ethylene synthesis significantly reduced by using ACC, the precursor in plants to ethylene. [58, 60, 61] showed that by inhibiting ethylene synthesis, tolerance to stress has been observed. Some PGPR also observed to produce auxin to facilitate root growth.

Many strains of genus *Pseudomonas* observed as potential PGPR due to poses PGPR properties like 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, indole acetic acid (IAA) and siderophore, P, Zn, K solubilization, etc. Due to *ACC deaminase* activity, ethylene level could not harm root growth and development. *Pseudomonas* spp. provides better root elongation, seedling survival, biocontrol properties, etc. The detrimental effects of PHCs are significantly reduced by the synergistic action of plant microbes' association.

8.2 Evaluating phytoremediation as a potential remediation technology

According to DOE [62], previous remediation processes posed with risk in human health or ecological imbalance, while remedial process like phytoremediation must offer advantages of reduction of health risk or cost-effectiveness over excavation and landfilling of polluted material as compared to the traditional approach.

8.3 Benefits of phytoremediation

Various benefits of phytoremediation have been explained or established:

- Phytoremediation can be more eco-friendly than other technologies.
- Phytoremediation may be suitable for cost savings more than 50% over traditional technologies [63].
- Phytoremediation offers the restoration of ecosystems, habitat to animals, biodiversity conservation, and reduces anthropogenic activities [62–64].
- Phytoremediation provides better environment for sustainable life [64].
- Erosion caused by wind or water may be significantly reduced by vegetation [64].
- Trees plantation decreases energy consumption and provides shade to buildings [65].
- Sequesters carbon and facilitates as carbon sink.

9. Conclusion and recommendations

Due to their hydrophobic nature, PHCs are pollutants with higher priority as they are difficult for degradation. Retention of these pollutants poses threat to biodiversity and environmental health. The reclamation of environment from PHCs' contamination is a global problem. Bio-phytoremediation proved to be an economic and alternative approach as compared to the physico-chemical process. Many factors that influence the remediation process, which include nutrition, physical conditions, microbial diversity, contaminant bioavailability, etc., can play an important role in the bio-phytoremediation of PHCs. Due to plant and microbe synergy, both possess enzymes, root exudates, etc. for better interaction of plant microbes with the contaminant. Therefore, phytoremediation with the involvement of microorganisms can be considered a key process of PHCs' remediation. The increase in our understanding of the bio-phytoremediation and the mechanisms by which petroleum hydrocarbons biodegradation occur will prove helpful for predicting the environmental fate of these compounds and for developing practical PHCs' bioremediation strategies in the future. It is crucial to continue in developing a technology which is cost-effective, feasible, and can remediate PHCs and other environment contaminants. Further study could be conducted for scaling up this technology or approach.

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References

[1] McCutcheon SC, Schnoor JL. Overview of phytotransformation and control of wastes. In: McCutcheon S, Schnoor J, editors. Phytoremediation: Transformation and Control of Contaminants. Hoboken, NJ: John Wiley & Sons, Inc.; 2003

[2] Bento FM, Camargo FAO, Okeke BC, Frankenberger WT. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Bioresource Technology. 2005;**96**:1049-1055

[3] Macnaughton SJ, Stephen JR, Venosa AD, Davis GA, Chang YJ, White DC. Microbial population changes during bioremediation of an experimental oil spill. Applied and Environmental Microbiology. 1999;**65**:3566-3574

[4] Adams RS, Ellis R. Some physical and chemical changes in soil brought about by saturation with natural gas. Soil Science Society of America Proceedings. 1960;**24**:41-44

[5] Udo EJ, Fayemi AA. The effect of oil pollution on germination, growth and nutrient uptake of corn. Journal of Environmental Quality. 1975;**4**:5377-5540

[6] Boethling RS, Alexander M. Effect of concentration of organic chemicals on their biodegradation by natural microbial communities. Applied and Environmental Microbiology. 1979;**37**:1211-1216

[7] Torstenssen L, Mikaelpell O, Bostenberg C. Need of a strategy for evaluation of arable soil quality. Environmental Pollution. 1998;**27**:4-7

[8] McCown BH, Deneke FJ, Richard WE, Tierzen L. The response of Alaskan terrestrial plant communities to the presence of petroleum. Environmental Pollution. 1972;**1**:34-43

[9] Langbehn A, Steinhart H. Biodegradation studies of hydrocarbons in soils by analyzing metabolites formed. Chemosphere. 1995;**30**:855-868

[10] Rahman KSM, Banat IM, Thahira J, Thayumanavan T, Lakshmanaperumalsamy P. Bioremediation of gasoline contaminated soil by a bacterial consortium amended with poultry litter, coir pith and rhamnolipid biosurfactant. Bioresource Technology. 2002;**81**(1):25-32

[11] Cunningham SD, Anderson TA, Schwab AP, Hsu FC. Phytoremediation of soils contaminated with organic pollutants. Advances in Agronomy. 1996;**56**:55-114

[12] Baud-Grasset F, Baud-Grasset S, Saffernan SI. Evaluation of the bioremediation of a contaminated soil with phytotoxicity tests. Chemosphere. 1993;**26**:1365-1374

[13] Aprill W, Sims RC. Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil. Chemosphere. 1990;**20**:253-265

[14] Gunther T, Dornberger U, Fritsche W. Effects of ryegrass on biodegradation of hydrocarbons in soil. Chemosphere. 1996;**33**(2):203-216

[15] Wiltse CC, Rooney WL, Chen Z, Schwab AP, Banks MK. Greenhouse evaluation of agronomic and crude oil-phytoremediation potential among alfalfa genotypes. Journal of Environmental Quality. 1998;**27**:169-173

[16] Komisar SJ, Park J. Phytoremediation of diesel contaminated soil using alfalfa. In:

Fourth International In Situ and On-Site Bioremediation Symposium; 28 April-1 May 1997; New Orleans, LA. Vol. 3. 1997. pp. 331-336

[17] Reilley KA, Banks MK, Schwab AP. Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. Journal of Environmental Quality. 1996;**25**:212-219

[18] Jordahl JL, Foster L, Schnoor JL, Alvarez PJJ. Effect of hybrid poplar trees on microbial populations important to hazardous waste bioremediation. Environmental Toxicology and Chemistry. 1997;**16**:1318

[19] Wild SR, Jones KC. Polynuclear aromatic hydrocarbons uptake by carrots grown in sludge-amended soil. Journal of Environmental Quality. 1992;**21**:217-225

[20] Cunningham SD, Berti WR. Remediation of contaminated soils with green plants: An overview. In Vitro Cellular & Developmental Biology-Plant. 1993;**29**:207-212

[21] Alkorta I, Garbisu C. Phytoremediation of organic contaminants in soils. Bioresource Technology. 2001;**79**:273-276

[22] Schnoor JL, Light LA, McCutcheon SC, Wolfe NL, Carreia LH. Phytoremediation of organic and nutrient contaminants. Environmental Science & Technology. 1995;**29**:318A-323A

[23] Briggs GG, Bromilow RH, Evans AA. Relationship between lipophilicity and root uptake and translocation of non-ionized chemicals by barley. Pesticide Science. 1982;**13**:495-504

[24] Salt DE, Smith RD, Raskin I. Phytoremediation. Annual Review of Plant Physiology and Plant Molecular Biology. 1998;**49**:643-668

[25] Farrell RE, Germida JJ. Phytotechnologies: Plant Based System for the Remediation of Oil Impacted Soils. Saskatoon, SK, Canada: Department of Soil Science, University of Saskatchewan; 2000

[26] Siciliano SD, Germida JJ. Mechanisms of phytoremediation: Biochemical and ecological interactions between plants and bacteria. Environmental Reviews. 1998;**6**:65-79

[27] Bollag JM, Mertz T, Otjen L. Role of microorganisms in soil remediation. In: Anderson TA, Coats JR, editors. Bioremediation through Rhizosphere Technology. ACS Symposium Series 563. American Chemical Society. York, PA: Maple Press; 1994. pp. 2-10

[28] Gibson DT, Subramanian V. Microbial degradation of aromatic hydrocarbons. In: Gibson DT, editor. Microbial Degradation of Organic Compounds. New York: Marcel Dekker; 1984. pp. 181-252

[29] Pothuluri JV, Cerniglia CE. Microbial metabolism of polycyclic aromatic hydrocarbons. In: Chaudry GR, editor. Biological Degradation and Remediation of Toxic Chemicals. Porland, Oregon: Dioscorides Press; 1994. pp. 92-124

[30] Ewies JB, Erges SJ, Chang DPY, Schroeder ED. Bioremediation Principles. Toronto: McGraw-Hill, Inc.; 1998

[31] Cerniglia CE, Gibson DT, Van Baden C. Algal oxidation of aromatic hydrocarbons: Formation of 1-napthol from naphthalene from *Agmenellum quadruplicatum*, strain PR-6. Biochemical and Biophysical Research Communications. 1979;**88**:50-58

[32] Cerniglia, C.E., Kelly, D.W., Freeman, J.P., Miller, D.W. 1986.

Microbial metabolism of pyrene. Chemical ± biological interactions 57, 203-216.

[33] Sutherland JB. Detoxification of polycyclic aromatic hydrocarbons by fungi. Journal of Industrial Microbiology. 1992;**9**:53-62

[34] Lyman WJ, Reidy PJ, Levy B. Mobility and Degradation of Organic Contaminants in Subsurface Environments. Beltsville, Maryland and Washington, D.C., United States: C. K. Somley, Inc; 1992. p. 395

[35] Riser-Roberts E. Remediation of Petroleum Contaminated Soils: Biological Physical and Chemical Processes. Boca Raton, FL: Lewis Publishers; 1998. p. 542

[36] Chapelle FH. Ground Water Microbiology and Geochemistry. U.S. Geological Survey in Columbia, South Carolina: John Willey and Sons, Inc; 1992. p. 424

[37] Walton BT, Holyman AM, Perez MM, Anderson TA, Johnson TR, Christman RF. Rhizosphere microbial communities as a plant defense against toxic substances in soils. In: Anderson TA, Coats JR, editors. Bioremidiation through Rhizosphere Technology. Washington D.C.: American Chemical Society; 1994. pp. 42-92

[38] Komsar SJ, Park J. Phytoremediation of Diesel-Contaminated Soil Using Alfalfa. Germany: TIB, Leibniz Information Centre for Science and Technology; 1997. pp. 331-336. (ISBN: 1574770314, 1574770284)

[39] Banks MK, Lee E, Schwab AP. Evaluation of dissipation mechanisms for benzo[a]pyrene in the rhizosphere of tall fescue. Journal of Environmental Quality. 1999;**28**:294-298

[40] Singer AC, Crowley DE, Thompson IP. Secondary plant metabolites in phytoremediation and biotransformation. Trends in Biotechnology. 2003;**21**(3):123-130

[41] Banks MK, Mallede H, Rathbone K. Rhizosphere microbial characterization in petroleum-contaminated soil. Soil and Sediment Contamination. 2003;**12**(3):371-385

[42] Hou FS, Milke MW, Leung DW, MacPherson DJ. Variations in phytoremediation performance with diesel-contaminated soil. Environmental Technology. 2001;**22**:215-222

[43] Rasolomanana JL, Balandreau J. Role de la rhizosphere dans la biodegradation decomposes recalcitrants: cas d'une riziere polluee par des residus petroliers. Revue D' Ecologie et de Biologie du sol. 1987;**24**(3):443-457

[44] Kloepper JW, Schroth MN. Proceedings of the 4th International Conference on Plant Pathogenic Bacteria. Vol. 2. Station de Pathoglogie Vegetale et Phytobacteriologie; 1978. pp. 879-882

[45] Glick BR. The enhancement of plant growth by free living bacteria. Canadian Journal of Microbiology. 1995;**41**:109-117

[46] O'Sullivan DJ, O'Gara F. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant pathogens. Microbiological Reviews. 1992:662-676

[47] Thomashow LS, Weller DM. Current concepts in the use of introduced bacteria for biological disease control: Mechanisms and antifungal metabolites. In: Stacey G, Keen NT, editors. Plant-Microbe Interactions. Vol. 1. New York: Chapman and Hall; 1996. pp. 187-227

[48] Van Loon LC, Bakker PAHM, Pieterse CMJ. Systemic resistance induced by rhizosphere bacteria.

Annual Review of Phytopathology. 1998;**36**:453-485

[49] Glick BR. Phytoremediation: Synergistic use of plants and bacteria to clean up the environment. Biotechnology Advances. 2003;**21**:383-393

[50] Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ. Rhizoremediation: A beneficial plant microbe interaction. Molecular Plant-Microbe Interactions. 2004;**17**:6-15

[51] Huang XD, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM. A multi-process phytoremediation system for removal of polycyclic aromatic hydrocarbons from contaminated soils. Environmental Pollution. 2004;**130**:465-476

[52] Pilon-Smith WAH. Phytoremediation. Annual Reviews in Plant Biology. 2005;**56**:15-39

[53] Choudhary NK, Fletcher RH, Somurel SB. Systematic review: The relationship between clinical experience and quality of health care. Annals of International Medical and Dental Research. 2005;**142**:260-273

[54] Sinha S, Mukherjee SK. Cadmium-induced siderophore production by a high Cd-resistant bacterial strain relieved Cd toxicity in plants through root colonization. Current Microbiology. 2008;**56**:55-60

[55] Hontzeas N. Expression and characterization of 1-ACC deaminase from the rhizobacterium *Pseudomonas pudta* UW4: A key enzyme in bacterial plant growth promotion. Biochimica et Biophysica Acta. 2004;**1703**:11-19

[56] Whipps JM. Carbon economy. The Rhizosphere. 1990:59-97

[57] Metting J. Soil Microbial Ecology: Applications in Agricultural and Environmental Management. England, U.K.: Taylor and Francis; 1992

[58] Glick BR. Bacterial ACC deaminase and the alleviation of the plant stress. Advances in Applied Microbiology. 2004;**56**:291-312

[59] Glick BR. Using soil bacteria to facilitate phytoremediation. Biotechnology Advances. 2010;**28**(3): 367-374

[60] Mayak S, Tirosh T, Glick BR. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiology and Biochemistry. 2004;**42**:565-572

[61] Sergeeva E, Shah S, Glick BR. Tolerance of transgenic canola expressing a bacterial ACC deaminase gene to high concentration of salt. World Journal of Microbiology and Biotechnology. 2006;**22**:277-282

[62] Department of Energy (DOE) U.S. A Framework for Net Environmental Benefit Analysis for Remediation or Restoration of Petroleum-Contaminated Sites. ORNL/TM-2003/17. National Petroleum Technology Office; 2003

[63] Environmental Protection Agency. The Quality of Our Nations Waters: A Summary of the National Water Quality Inventory: 1998 Report to Congress. EPA 84100001. Office of Water, U. S. Environmental Protection Agency; 2000

[64] Wilson MA. Ecosystem Services at Superfund Redevelopment Sites. Prepared for U.S. EPA, Office of Solid Waste and Emergency Response, Policy Analysis and Regulatory Management Staff; 2004

[65] Nowak DJ, Crane DE. Carbon storage and sequestration by urban trees in the USA. Environmental Pollution. 2002;**116**:381-389