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REMOVAL OF POLY AROMATIC HYDROCARBONS – BIOREMEDIATION TECHNIQUE: A MINI REVIEW

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Polyaromatic compounds are the most harmful and persistent pollutants found in the environment. Due to their ubiquitous distribution and affect on human health their mechanism of biodegradation is fate of interest. Petroleum and its products (Poly Aromatic Hydrocarbons, NSO and Heterocyclic Compound) contaminated terrestrial ecosystem as well as marine ecosystem. Poly aromatic compounds are having 2 or more benzene ring in their structure. Some of these benzene rings may contain Nitrogen, Oxygen, or Sulphar atoms. Bioremediation is cost effective mechanism for the removal of these carcinogenic and potent toxic substances. Present review focuses on biodegradation mechanism of polyaromatic hydrocarbons.

Keywords: Anthracene; PAHs; Phenanthrene.

Introduction

Today, large numbers of ecosystems have been changed due to significant influence of human activity. As a result, many people have become aware to the need of protect different ecosystems as well as to evaluate the damage caused by contamination. During the previous years, many researchers have done extensively on bioremediation of PAHs. Most of the petroleum goes in the ecosystem via leakage of coastal oil refineries. This fact evoked the interest of scientists to investigate the oil distribution system and its fate in the environment, especially the marine environment. Approximately every year five million tons of crude oil and refined oil enter into the environment as a result of anthropogenic sources such as oil spills[26]. From previously published report, oil spills indicate that most of the oil comes from tankers, barges and other vessels as well from land pipeline spills which ultimately, extensively changes marine, as well as terrestrial ecosystems. The Nahodka oil spill, the Erica spill (1999) and the Prestige spill (2002), have recently increased the attention of environmentalists, chemists, biotechnologists and engineers[10,59] for the process of bioremediation.

The recent spill, more than 200,000 barrels of crude oil from the oil tanker Exxon Valdez in Prince William Sound Alaska[22], as well as smaller spills in Texas, Rhode Island, and the Delaware Bay[3], have refocused attention on the problem of hydrocarbon contamination in the environment. It is estimated that the annual global input of petroleum is between 1.7 and 8.8 million metric tons, the majority of which is derived from anthropogenic sources[22].

Shipping accidents have a drastic impact on the marine environment. The consequences include serious thread on flora and funna, long-term damage to marine ecosystems, terrestrial life, human health and natural resources. This can help environmentalists to predict the behavior of oil and estimate the long-term impact on the environment. It is necessary to select an appropriate clean-up method for the recovery of above mentioned problem. The recovery of oil has been studied extensively since the 1970s. Conventional remediation methods include physical removal of contaminated material which is based on use of chemicals, especially shoreline cleaners (organic solvents with or without surfactants) [52]. The shoreline cleaners with surfactants emulsify the adsorbed oil, and it entrains adjacent waters or is transported deeper into the shoreline soil. The oil solvent mixtures are collected using conventional skimming methods. Mechanical recovery of oil includes the use of the oil sorbents. Sorbents help to transform oil to a transportable form for short term storage. However, most of the used sorbents end up in the landfills. Most of the physicochemical methods use chemical agents, as well as their emulsion with oil causing toxicity, to aquatic organisms. They produce another source of pollution and also increase the oil recovery cost. However, abiotic losses due to evaporation of low molecular hydrocarbons, dispersion and photo oxidation (involves only aromatic compounds) play a major role in decontamination of the oil spilled environments[45].

Origin of Hydrocarbon: Oil and Oil contaminated environmental matrices are extremely complex mixtures that contain a vast array of aliphatic and polycyclic aromatic hydrocarbons (Wang *et al.*, 1994). Polycyclic aromatic hydrocarbons are toxic and hazardous chemicals regulated by the USEPA as priority pollutants [13,57,31]. Therefore, it is important to remove PAHs from the environment both quickly and safely following an oil spill incident. Bioremediation can be an effective option to reclaim PAHs contaminated sites due to its relatively low cost and limited impact on the environment [38].

Aromatic Hydrocarbons in the environment are originated from anthropogenic source like mineral oil spill or gas works plants and to a minor extent and also from biological production in anoxic sediments. Numerous aerobic bacteria have been isolated that can breakdown aromatic hydrocarbons as carbon and energy sources. The degradation pathways have already been elucidated [12].

They are also present as natural constituents in fossils fuels, and formed during the incomplete combustion of organic material therefore, present in relatively high concentration [46,36,8,47,62,17,63]. Petroleum refining and transport activities are major contributors to localized loadings to PAHs into the environment. Such loadings occur through discharge of industrial waste and through accidental release of raw and refined products. However, PAH released into the environment may originate from many sources including gasoline and diesel fuel combustion [39,42] . PAHs are detected in air [33,39] soil and sediment [24,60,68,35,48], surface water groundwater, and road runoff [9,27,43,50] are dispersed from the atmosphere to vegetation [61] and contaminate foods [37,19,55]. Anthropogenic and natural sources of PAHs in combination with global transport phenomena result in their worldwide distribution. Hence, the need to develop practical bioremediation strategies for heavily impacted sites is evident[28]. PAHs concentration in the environment vary widely, depending on the proximity of the contaminated site to the production source, the level of industrial development and the mode of PAH transports. Soil and sediment PAH concentration at contaminated and uncontaminated sites ranging from 1 μ/kg to over 300 g/kg have been reported[6,29,49,51,67].

Major Sources of PAHs in the Environment: Combustion of fossil fuels , Automobile engine exhausts , Atmospheric fallout of fly ash particulate , Coal tar and other coal processing wastes, Aluminium Plants, Refinery and oil storage wastes , Accidental spills from oil tankers and other ships , Tobacco and cigarette smoke , Refuse and waste incineration , Coal gasification and liquefaction process , Smoke, charcoal broiled, or pan fried foods etc.

Predominant PAH Source Profile/ Markers: The following PAH have been identified as markers for various sources in urban atmospheres: Coal combustion: Phenenthrene, fluorathene and pyrene Coke production: Anthracene, phenenthrene and benzo(a)pyrene; Incineration: Pyrene, phenenthrene and fluoranthene; Wood combustion: Benzo(a)pyrene and fluoranthene; Industrial – oil burning: Fluoranthene pyrene and chrysene; Petrol powered vehicles : Benzo perylene, indeno (123-cd)pyrene and coronene; Diesel powered vehicles : Fluoranthene and pyrene with higher ratios of benzo(b)Fluoranthene and benzo(k)fluoranthene, plus thiophene compounds. As may be noted from the markers listed above, there is much similarity and overlap between profiles from different source categories.

Chemistry of Hydrocarbons: Polycyclic aromatic hydrocarbons (PAHs) comprise a large and heterogeneous group of organic contaminants that are predominantly formed and emitted as a result of incomplete combustion of fossil fuels. But there are also natural sources, e.g. volcanic eruptions and forest fires. PAHs enter the air mostly as releases from volcanoes, forest fires, burning coal and automobile exhaust. PAHs occur in air attached to dust particles. Some PAH particles can readily evaporate into the air from soil as surface water. PAHs can break down by reacting with sunlight and chemicals present in the air, over a period of days to weeks. PAHs enter water through discharge from industrial and waste water treatment plants. Most PAH do not dissolve easily in water. They stick to solid particles and settle to the bottom of lakes as rivers. Microorganisms can breakdown PAHs in soil as in water after a period of days to weeks. In soils PAHs are most likely to stick tightly to particles while certain PAHs move through soil to contaminate underground water. PAH contents of plants and animals may be much higher than PAH contents of soil and water in which they live.

PAHs are made up of of fused benzene rings which are arranged in linear, angular or clustered arrangements (Figure 1.1). They contain by definition only carbon and hydrogen atoms. However, nitrogen, sulfur and oxygen atoms may readily substitute in the benzene rings to form heterocyclic aromatic compounds, which are commonly grouped with the PAHs. Furthermore, PAHs substituted with alkyl groups are normally found together with the PAHs in the environment. The whole group of PAHs and related compounds is sometimes referred to as polycyclic aromatic compounds (PACs). PAHs have been thoroughly studied due to their toxicity, persistency and environmental prevalence. Such studies are often limited to 16 PAHs, designated as priority pollutants by the

United States Environmental Protection Agency (US-EPA).

Interest in the biodegradation mechanism and environmental fate of polycyclic aromatic hydrocarbons is due to their ubiquitous distribution and their potentially deleterious effect on human health. Most of the PAHs are lipophilic compounds which show high affinity for organic matter. Properties such as aqueous solubility and vapor pressure range in five and twelve orders of magnitude, respectively, moving from two to six benzene rings in the PAH-molecule are shown in table 1.1. Low molecular weights (LMW) PAHs are more water soluble and volatile with comparison of high molecular weight (HMW). The high molecular weights PAHs show higher hydrophobicity than the low molecular weights compounds [41]. The difference in hydrophobic property is also reflected by the octanol-water partitioning coefficient (K_{OW}) (shown in Table 1.1). These physico-chemical properties largely determine the environmental behavior of PAHs, and indicate that transfer and turnover is more rapid for LMW PAHs than for the heavier PAHs [66]. The semi-volatile nature of the LMW PAHs means that they exist in the atmosphere partly as vapors and are therefore highly susceptible to atmospheric degradation processes. Similarly, in aqueous environments, the LMW PAHs are partly dissolved, making them readily available for various degradation particles in the atmosphere and water, and are therefore less available for degradation. Furthermore, PAHs adsorbed to particles may be transported over long distances in the atmosphere and are therefore ubiquitous in the environment [28,67].

The biodegradation of PAH molecule is also dependent upon both molecule size, i.e. the no. of aromatic rings and molecular topology at the pattern of ring linkage. Ring linkage patterns in PAHs may occur such that the tertiary carbon atoms are centers of two or three interlinked rings, as in the linear kata-annelated PAH anthracene or pericondensed PAH pyrene.

Bioremediation

Bioremediation is defined as use of biological processes to degrade, breakdown, transform, and/or essentially remove contaminants or impairments of quality from soil and water. Bioremediation is a natural process which relies on bacteria, fungi, and plants to alter contaminants as these organisms carry out their normal life functions. Metabolic processes of these organisms are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products in most cases. general process of bioremediation with in the soil environment and highlighting the biodegradation of petroleum hydrocarbons. The effect of soil conditions on rate of biodegradation of hydrocarbons is addressed. Further, limitations and potential of both ex situ and in situ bioremediation as viable alternatives to conventional remediation are explained and addressed. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and introduced to the contaminating site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. When microorganisms are introduced to a contaminated site to enhance degradation we have a process known as bioaugmentation. For

Source Distribution of the Percentage of PAHs to the Total mass of 20 PAHs

PAH*	Tunnal	Diesal Engines	Gasoline Engines	Cook Oven	Wood Combustion
2 Ring	76	8.7	55	89	11
3 Ring	16	56	18	8.9	69
4 Ring	4.3	10	12	0.97	6.9
5 Ring	3.1	18	13	0.22	13
6 Ring	0.38	5.2	0.053	0.0142	Bd
7 Ring	Bd	.18+	0.083	Bd	Bd

***2-ring**: naphthalene; **3-ring:** acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene and retene; **4-ring:** fluoranthene , pyrene, benz(a)anthracene, chrysene and triphenylene; **5-ring:** cyclopenta(c, d)pyrene, benzo (b,k) Fluoranthene, benzo (a, e)pyrene, di benzo(ghi)perylene**; 6-ring:** indeno(1,2,3,cd)pyrene and benzo(ghi)pyrene; 7-ring: coronene ; **Bd:** below the detection limit of this study.

bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. Like other technologies, bioremediation has its limitations. Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, hence it is not easy to predict the rates of clean-up for a bioremediation exercise; there are no rules to predict if a contaminant can be degraded. Bioremediation techniques are typically more economical than traditional methods such as incineration, and some pollutants can be treated on site, thus reducing exposure risks for clean-up personnel, or potentially wider exposure as a result of transportation accidents. Since bioremediation is based on natural attenuation, it becomes more acceptable than other technologies. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions (Blumer, 1976) may permit microbial organisms to degrade otherwise recalcitrant molecules.

Different techniques are employed depending on the degree of saturation and aeration of an area. In situ techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. Ex situ techniques are those that are applied to soil and groundwater which have been removed from the site via excavation (soil) or pumping (water). Bioaugmentation techniques involve the addition of microorganisms with the ability to degrade pollutants.

In situ bioremediation techniques (U.S. EPA, Seminar, 96/001, U.S. EPA 90/002) are generally the most desirable options due to lower cost and fewer disturbances since they provide the treatment in place avoiding excavation and transport of contaminants. In situ treatment is limited by the depth of the soil that can be effectively treated. In many soils effective oxygen diffusion for desirable rates of bioremediation extend to a range of only a few centimeters to about 30 cm into the soil, although depths of 60 cm and greater have

List of Bacteria capable of degrading petroleum hydrocarbons Compound Microorganism

Compound	Microorganism
Alkanes	*Pseudomonas* sp., *Bacillus* sp., *Acinetobacter calcoaceticus*, *Micrococcus* sp., *Candida Antarctica*, *Nocardia* erythroplis, *Ochrobactrum* sp., *Acinetobacter* sp., *Serratia marcescens*, *Candida tropicalis*, *Alcaligenes odorans*, *Arthrobacter* sp., *Rhodococcus* sp.
Mono-Aromatic hydrocarbons	*Brevibacillus* sp., *Pseudomonas* sp., *Bacillus* sp.,*B. stereothermophilus*, *Vibrio* sp., *Corynebacterium* sp., *Ochrobactrum*sp., *Achromobacter* sp.
Poly-aromatic hydrocarbons	*Alcaligenes odorans*, *Sphingomonas paucimobilis*, *Achromobacter* sp., *Mycobacterium* sp.,*Pseudomonas* sp., *Mycobacterium flavescens*, *Rhodococcus* sp., *Arthrobacter* sp., *Bacillus* sp., *Burkholderia cepacia*., *Xanthomonas* sp., *Alcaligenes*
Resins	*Pseudomonas* sp., Members of Vibrionaceae., Enterobacteriaceea., *Moraxella* sp**.**

been effectively treated in some cases. The most important land treatments are:

Bioventing is the most common in situ treatment and involving supply of air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing is the most common in situ treatment and involving supply of air and nutrients through wells to contaminated soil which stimulate the bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation process. Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system. bioaugmentation.

Bioremediation frequently involves the addition of microorganisms indigenous or exogenous to the contaminated sites. Two factors limit the use of added microbial cultures in a land treatment unit:

A. Non indigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and

B. Most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degrader if the land treatment unit is well managed.

Ex Situ Bioremediation: These techniques involve the excavation or removal of contaminated soil from ground. Land farming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10–35 cm of soil. Since land farming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attention as a disposal alternative.

Composting is a technique that involves combining of contaminated soil with non hazardous organic amendants such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristic of composting. Biopiles are hybrids of land farming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of land farming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms.

Bioreactors, slurry reactors or aqueous reactors are used for ex situ treatment of contaminated soil and water pumped up from a contaminated plume. A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil-bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants. In general, the rate and extent of biodegradation are greater in a bioreactor system than in situ or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages, the contaminated soil requires pre treatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction (e.g., vacuum extraction) before being placed in a bioreactor.

Microorganisms involved in Bioremediation: Most bacterial polycyclic aromatic hydrocarbon degraders have been isolated from contaminated/terrestrial environments [4,53,14,1]. Many microbial strains capable of degrading a specific compound are available commercially for bioremediation [56,11,34]. However, oil sludge is a complex mixture of alkane, aromatic NSO (Nitrogen, Sulfur, Oxygen, containing compounds), and asphaltene fractions. A single bacterial species has only limited capacity to degrade all the fractions of hydrocarbons presents [18,8,5,40].

Various class of bacteria previously isolated from PAHs contaminated soil belong to *Pseudomonas, Vibrio*[32] , *Mycobacterium*[16], *Comamonas*[20] , *Arthrobacter*[21], *Burkholderia* [30], *Flavobacterium*[54], *Sphingomonas*[7] . Other species of PAH degrading bacteria isolated from marine environment were reported as *Cycloclasticus*, *Moraxella* and Marinobacter[58].

Aerobic Biodegradation: Aerobic biodegradation is the most efficient form of PAH bioremediation. The most rapid and complete degradation of the majority of organic pollutants is brought about under aerobic conditions. Complete contaminant degradation results in the formation of CO_2 and H_2O. The initial intracellular attack organic pollutants is an oxidative process and the activation as well as incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidase. Peripheral degradation pathways convert organic pollutants step by step into intermediates of the central intermediary metabolism, for example, the tricarboxylic acid cycle. Biosynthesis of cell biomass occurs from the central precursor metabolites, for example, acetyl-CoA, succinate, pyruvate. Sugars required for various biosyntheses and growth are synthesized by gluconeogenesis. The degradation of petroleum hydrocarbons can be mediated by specific enzyme system. Other mechanisms involved are attachment of microbial cells to the substrates and production of biosurfactants. The uptake mechanism linked to the attachment of cell to oil droplet is still unknown but production of biosurfactants has been well studied.

The factors that may prevent microbial degradation and bioremediation listed as follows: 1) chemical concentrations that may be toxic to microorganisms; 2) conditions that are too acidic or alkaline; 3) lack of essential nutrients such as nitrogen, phosphorous, potassium, sulfur, and/or trace elements; 4) unfavorable moisture conditions (too wet or too dry); and 5) lack of oxygen or other electron acceptors. Typically, a lack of nutrients and/or oxygen would be a rate limiting parameter at the beginning of an *in situ* PAH bioremediation project. As time progresses, the total mass of readily degradable constituents are decreased and thus the oxygen requirements for active bioremediation are also decreased.

Anaerobic Petroleum Hydrocarbon Degradation: Although desirable for active hydrocarbon biodegradation, completely aerobic conditions are hard to implement in the field because of the low solubility of oxygen in water. It is possible that uneven distribution of water flow, nutrients and microbial populations creates a dynamic spectrum of aerobic, micro aerobic and anaerobic conditions. A basic idea for augmenting bioremediation under anaerobic conditions is to make electron acceptors available at concentrations higher than that of dissolved oxygen from air[65]. The ability of microorganism to degrade hydrocarbons under strictly anaerobic conditions is limited to a few strains and is typically much slower than aerobic degradation[25]. Since hydrocarbon metabolites are potentially toxic, the ability of indigenous microorganisms to further degrade aerobic metabolites under denitrifying conditions is also important for the ultimate success of nitrate based bioremediation[2].

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